MULTI-CHAIN CHIMERIC POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to: U.S. Patent Application No. 62/724,969, filed August 30, 2018; U.S. Patent Application No. 62/817,230, filed March 12, 2019; U.S. Patent Application Serial No. 62/725,043, filed August 30, 2018; U.S. Patent Application Serial No. 62/725,010, filed August 30, 2018; U.S. Patent Application Serial No. 62/749,007, filed October 22, 2018; U.S. Patent Application Serial No. 62/746,832, filed October 17, 2018; U.S. Patent Application Serial No. 62/749,506, filed October 23, 2018; U.S. Patent Application Serial No. 62/817,241, filed March 12, 2019; U.S. Patent Application Serial No. 62/816,683, filed March 11, 2019; and U.S. Patent Application Serial No. 62/881,088, filed July 31, 2019, each of which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

The present disclosure relates to the field of biotechnology, and more specifically, to antigen-binding molecules.

BACKGROUND

Tissue factor (TF), a 263 amino acid integral membrane glycoprotein with a molecular weight of ~46 kDa and the trigger protein of the extrinsic blood coagulation pathway, is the primary initiator of coagulation *in vivo*. Tissue factor, normally not in contact with circulating blood, initiates the coagulation cascade upon exposure to the circulating coagulation serine protease factors. Vascular damage exposes sub-endothelial cells expressing tissue factor, resulting in the formation of a calcium-dependent, high-affinity complex with pre-existing plasma factor VIIa (FVIIa). Binding of the serine protease FVIIa to tissue factor promotes rapid cleavage of FX to FXa and FIX to FIXa. The proteolytic activity of the resulting FXa and an active membrane surface then inefficiently converts a small amount of prothrombin to thrombin. The thrombin generated by FXa initiates platelet activation and activates minute amounts of the pro-cofactors factor V (FV) and factor VIII (FVIII) to become active cofactors, factor Va

(FVa) and factor VIIIa (FVIIIa). FIXa complexes with FVIIIa on the platelet surface forming the intrinsic tenase complex, which results in rapid generation of FXa. FXa complexes with FVa to form the pro-thrombinase complex on the activated platelet surface which results in rapid cleavage of prothrombin to thrombin.

In addition to the tissue factor-FVIIa complex, a recent study showed that the tissue factor-FVIIa-FXa complex can activate FVIII, which would provide additional levels of FVIIIa during the initiation phase. The extrinsic pathway is paramount in initiating coagulation via the activation of limited amounts of thrombin, whereas the intrinsic pathway maintains coagulation by dramatic amplification of the initial signal.

Much of the tissue factor expressed on a cell surface is "encrypted," which must be "decrypted" for full participation in coagulation. The mechanism of "decryption" of cell-surface tissue factor is still unclear at this time, however, exposure of anionic phospholipids plays a major role in this process. Healthy cells actively sequester anionic phospholipids such as phosphatidyl serine (PS) to the inner leaflet of the plasma membrane. Following cellular damage, activation, or increased levels of cytosolic Ca^{2+}, this bilayer asymmetry is lost, resulting in increased PS exposure on the outer leaflet, which increases the specific activity of cell-surface tissue factor-FVIIa complexes. PS exposure is known to decrease the apparent Km for activation of FIX and FX by tissue factor-FVIIa complexes, but additional mechanisms could include conformational rearrangement of tissue factor or tissue factor-FVIIa and subsequent exposure of substrate binding sites.

SUMMARY

The present disclosure is based on the discovery that soluble tissue factor can be used as a scaffold for chimeric polypeptides including an antigen-binding domain. Based on this discovery provided herein are multi-chain chimeric polypeptides that include: (a) a first chimeric polypeptide including: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the

second domain of the pair of affinity domains. Also provided herein are compositions that include any of the multi-chain chimeric polypeptides described herein, nucleic acids that encode any of the multi-chain chimeric polypeptides described herein, and cells that include any of the nucleic acids that encode any of the multi-chain chimeric polypeptides described herein. Also provided herein are methods of stimulating an immune cell and methods of treating a subject in need thereof that include the use of any of the multi-chain chimeric polypeptides described herein. Also provided herein are methods of producing any of the multi-chain chimeric polypeptides described herein.

Accordingly, provided herein is a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains. In one aspect, the present disclosure refers to a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence. In some examples, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples, the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some examples, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some examples, the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some examples, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some examples, the second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric

polypeptide. In some examples, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some examples, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some examples, the first target-binding domain and the second target-binding domain comprise the same amino acid sequence. In some examples, the first target-binding domain and the second target-binding domain bind specifically to different antigens. In some examples, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some examples, the first target-binding domain and the second target-binding domain are each antigen-binding domains. In some examples, the antigen-binding domain comprises a scFv or a single domain antibody. In some examples, one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28. In some examples, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin, a soluble cytokine protein, or a ligand protein. In some examples, the soluble interleukin, soluble cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L. In some examples, one

or both of the first target-binding domain and the second target-binding domain is a soluble interleukin, a cytokine receptor, or a soluble cell surface receptor. In some examples, the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28. In some examples, the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains. In some examples, the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains. In some examples, the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide. In some examples, at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide. In some examples, the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains. In some examples, the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide. In some examples, the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain. In some examples, at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some examples, the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity

domains in the first chimeric polypeptide. In some examples, the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some examples, the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some examples, the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some examples, the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains. In some examples, the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains. In some examples, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide. In some examples, at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide. In some examples, the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide. In some examples, at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide. In some examples, the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more

additional target-binding domains and the second target-binding domain in the second chimeric polypeptide. In some examples, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some examples, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some examples, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence. In some examples, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some examples, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some examples, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence. In some examples, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens. In some examples, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain. In some examples, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain. In some examples, antigen-binding domain comprises a scFv. In some examples, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D,

a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD3, and a receptor for CD28. In some examples, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin, a soluble cytokine protein, or a ligand protein. In some examples, the soluble interleukin, soluble cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L. In some examples, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor. In some examples, the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28. In some examples, the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide. In some examples, the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide. In some examples, the soluble tissue factor domain is a soluble human tissue factor domain. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1. In some examples, the soluble human tissue factor domain does not comprise one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that

corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples, the soluble human tissue factor domain does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some examples, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some examples, the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal. In some examples of any of the single-chain chimeric polypeptides provided herein, the human soluble tissue factor domain does not initiate blood coagulation. In some examples of any of the single-chain chimeric polypeptides provided herein, the soluble tissue factor domain comprises or consists of a soluble wildtype human tissue factor.

In some examples, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15. In some

examples, the soluble IL15 has a D8N or D8A amino acid substitution. In some examples, the human IL15Rα is a mature full-length IL15Rα. In some examples, the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some examples, the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

In one aspect, the present disclosure refers to a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain binds specifically to a ligand of TGF-β receptor II (TGF-βRII) and the second target-binding domain binds specifically to a ligand of TGF-βRII;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

In one aspect, the present disclosure refers to a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain binds specifically to a receptor for IL-18 and the second target-binding domain binds specifically to a receptor for IL-12, or the first target-binding domain binds specifically to a receptor for IL-12 and the second target-binding domain binds specifically to a receptor for IL-18;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

In one aspect, the present disclosure refers to a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain binds specifically to a receptor for IL-7 and the second target-binding domain binds specifically to a receptor for IL-21, or the first target-binding domain binds specifically to a receptor for IL-21 and the second target-binding domain binds specifically to a receptor for IL-7;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

In some examples, the multi-chain chimeric polypeptide comprises a composition. In one aspect, the present disclosure refers to a composition comprising any of the multi-chain chimeric polypeptides as disclosed herein. In some examples, the composition is a pharmaceutical composition. In some examples, the composition comprises at least one dose of the multi-chain chimeric polypeptide. In one aspect, the present disclosure refers to a kit comprising at least one dose of the composition disclosed herein.

Provided herein is a method of stimulating an immune cell, the method comprising: contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides or compositions described above. In one aspect, the present disclosure refers to an in vitro method of stimulating an immune cell, the method comprising: contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides or compositions described above. In some examples, the method comprises contacting the immune cell in vitro. In some examples, the method comprises obtaining the immune cell from a subject. In some examples, the method comprises obtaining the immune cell from the subject prior to the contacting step. In some examples, the method comprises contacting the immune cell in vivo. In some examples, the method comprises selecting the immune cell from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell. In some examples, the method comprises genetically modifying the immune cell to express a chimeric antigen receptor or a recombinant T-cell receptor. In some examples, the method comprises introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor after the contacting step. In some examples, the method

comprises administering the immune cell to a subject in need thereof. In some examples, the method comprises identifying or diagnosing the subject as having an age-related disease or condition. In some examples, the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, the method comprises identifying or diagnosing the subject as having a cancer. In some examples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some examples, the method comprises diagnosing or identifying the subject as having an infectious disease. In some examples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Also provided herein is a method of inducing or increasing proliferation of an immune cell, the method comprising: contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides or compositions described above. In one aspect, the present disclosure refers to an in vitro method of inducing or increasing proliferation of an immune cell, the method comprising: contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides or compositions described above. In some examples, the method comprises contacting the immune cell in vitro. In some examples, the method comprises obtaining the immune cell from a subject. In some examples, the method comprises obtaining the immune cell from the subject prior to the contacting step. In some examples, the method comprises contacting the immune cell in vivo. In some examples, the method comprises selecting the immune cell from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell. In some examples, the method comprises modifying the immune cell to express a chimeric antigen receptor or a recombinant T-cell receptor. In some examples, the method further comprises introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor after the contacting step. In some examples, the method further comprises administering the immune cell to a subject in need thereof. In some examples, the method comprises identifying or diagnosing the subject as having an age-related disease or condition. In some examples, the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, the method comprises

identifying or diagnosing the subject as having a cancer. In some examples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some examples, the method comprises diagnosing or identifying the subject as having an infectious disease. In some examples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Also provided herein is a method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising: contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides or compositions discussed above. In one aspect, the present disclosure refers to an in vitro method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising: contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides or the compositions described above. In some examples, the method comprises contacting the immune cell in vitro. In some examples, the method comprises obtaining the immune cell from a subject. In some examples, the method further comprises obtaining the immune cell from the subject prior to the contacting step. In some examples, the method comprises contacting the immune cell in vivo. In some examples, the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell. In some examples, the immune cell has previously been genetically modified to express a chimeric antigen

receptor or a recombinant T-cell receptor. In some examples, the method further comprises introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor after the contacting step. In some examples, the method further comprises administering the immune cell to a subject in need thereof. In some examples, the method comprises identifying or diagnosing the subject as having an age-related disease or condition. In some examples, the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, the method comprises identifying or diagnosing the subject as having a cancer. In some examples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some examples, the method comprises diagnosing or identifying the subject as having an infectious disease. In some examples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes

simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Also provided herein is a method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides or compositions discussed above. In one aspect, the present disclosure refers to use of any of the multi-chain chimeric polypeptides described above in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject having a cancer, an aging-related disease or condition, or an infectious disease. In some examples, the method comprises identifying or diagnosing the subject as having a cancer. In some examples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some examples, the method comprises identifying or diagnosing the subject as having an aging-related disease or condition. In some examples, the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, the method comprises administering to the subject a therapeutically effective amount of any of the multi-chain

chimeric polypeptides or compositions discussed above. In some examples, the method comprises identifying or diagnosing the subject as having a cancer. In some examples, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some examples, the method comprises identifying or diagnosing the subject as having an aging-related disease or condition. In some examples, the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, the method comprises diagnosing or identifying the subject as having an infectious disease. In some examples, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

In one aspect, the present disclosure refers to use of any of the multi-chain chimeric polypeptides described above in the manufacture of a medicament for treating a cancer, an aging-related disease or condition, or an infectious disease.

In one aspect, also provided herein is a nucleic acid encoding any of the multi-chain chimeric polypeptides discussed above. Some examples comprise vector containing any of the nucleic acids discussed above. In one aspect, the present disclosure refers to a vector comprising the nucleic acid discussed above. In some examples, the vector is an expression vector. Some examples comprise a cell containing any of the nucleic acids discussed above. In one aspect, the present disclosure refers to a cell comprising the nucleic acid or the vector discussed above. In one aspect, the present disclosure refers to a cell comprising the nucleic acid discussed above.

In one aspect, also provided herein is a method of producing a multi-chain chimeric polypeptide, the method comprising: culturing the cell discussed above in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium. In some examples, the method comprises producing a multi-chain chimeric polypeptide by the methods discussed above.

In some examples, the mutant soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 3. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 3. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3. In some examples, the multi-chain chimeric polypeptide of claim 140, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 3. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO:4. In some examples, the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 4.

As used herein, the term “chimeric” refers to a polypeptide that includes amino acid sequences (e.g., domains) originally derived from two different sources (e.g., two different naturally-occurring proteins, e.g., from the same or different species). For example, a chimeric polypeptide can include domains from at least two different naturally occurring human proteins. In some examples, a chimeric polypeptide can include a domain that is a synthetic sequence (e.g., an scFv) and a domain that is derived from a naturally-occurring protein (e.g., a naturally-occurring human protein). In some

examples, a chimeric polypeptide can include at least two different domains that are synthetic sequences (e.g., two different scFvs).

An "antigen-binding domain" is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigen(s). In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some examples, the antigen-binding domain can be an antibody or a fragment thereof. In some examples, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains are described herein. Additional examples of antigen-binding domains are known in the art.

A "soluble tissue factor domain" refers to a polypeptide having at least 70% identity (e.g., at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity, or 100% identical) to a segment of a wildtype mammalian tissue factor protein (e.g., a wildtype human tissue factor protein) that lacks the transmembrane domain and the intracellular domain. Non-limiting examples of soluble tissue factor domains are described herein.

The term "soluble interleukin protein" is used herein to refer to a mature and secreted interleukin protein or a biologically active fragment thereof. In some examples, a soluble interleukin protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble interleukin proteins are described herein.

The term "soluble cytokine protein" is used herein to refer to a mature and secreted cytokine protein or a biologically active fragment thereof. In some examples, a soluble cytokine protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted

mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble cytokine proteins are described herein.

The term "soluble interleukin receptor" is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble interleukin receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype interleukin receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble interleukin receptors are described herein.

The term "soluble cytokine receptor" is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble cytokine receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype cytokine receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble cytokine receptors are described herein.

The term "antibody" is used herein in its broadest sense and includes certain types of immunoglobulin molecules that include one or more antigen-binding domains that specifically bind to an antigen or epitope. An antibody specifically includes, e.g., intact antibodies (e.g., intact immunoglobulins), antibody fragments, and multi-specific antibodies. One example of an antigen-binding domain is an antigen-binding domain

formed by a VH -VL dimer. Additional examples of an antibody are described herein. Additional examples of an antibody are known in the art.

"Affinity" refers to the strength of the sum total of non-covalent interactions between an antigen-binding site and its binding partner (e.g., an antigen or epitope). Unless indicated otherwise, as used herein, "affinity" refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of an antigen-binding domain and an antigen or epitope. The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (K_D). The kinetic components that contribute to the dissociation equilibrium constant are described in more detail below. Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (e.g., BIACORE®) or biolayer interferometry (e.g., FORTEBIO®). Additional methods for determining the affinity for an antigen-binding domain and its corresponding antigen or epitope are known in the art.

A "multi-chain polypeptide" as used herein to refers to a polypeptide comprising two or more (e.g., three, four, five, six, seven, eight, nine, or ten) protein chains (e.g., at least a first chimeric polypeptide and a second polypeptide), where the two or more proteins chains associate through non-covalent bonds to form a quaternary structure.

The term "pair of affinity domains" is two different protein domain(s) that bind specifically to each other with a K_D of less than of less than 1×10^{-7} M (e.g., less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, or less than 1×10^{-11} M). In some examples, a pair of affinity domains can be a pair of naturally-occurring proteins. In some examples, a pair of affinity domains can be a pair of synthetic proteins. Non-limiting examples of pairs of affinity domains are described herein.

The term "epitope" means a portion of an antigen that specifically binds to an antigen-binding domain. Epitopes can, e.g., consist of surface-accessible amino acid residues and/or sugar side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter may be lost in the presence of denaturing solvents. An epitope may comprise amino acid residues that are directly involved in the binding, and other amino acid

residues, which are not directly involved in the binding. Methods for identifying an epitope to which an antigen-binding domain binds are known in the art.

An "immune effector cell" refers to a cell of the immune system of a mammal that is capable, directly or indirectly, of recognizing and/or causing cytostasis or cell death of a pathogenic cell (e.g., a cancer cell) in the mammal. Non-limiting examples of immune effector cells include macrophages, T-lymphocytes (e.g., cytotoxic T-lymphocytes and T-helper cells), natural killer cells, neutrophils, monocytes, and eosinophils. Additional examples of immune effector cells are known in the art.

The term "treatment" means to ameliorate at least one symptom of a disorder. In some examples, the disorder being treated is cancer and to ameliorate at least one symptom of cancer includes reducing aberrant proliferation, gene expression, signaling, translation, and/or secretion of factors. Generally, the methods of treatment include administering a therapeutically effective amount of composition that reduces at least one symptom of a disorder to a subject who is in need of, or who has been determined to be in need of such treatment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows exemplary diagrams for a multi-chain chimeric polypeptide: (i) a first chimeric polypeptide including a first target-binding domain (A), a soluble tissue factor domain, a first domain of an affinity pair of domains (soluble interleukin IL-15), and an additional target-binding domain (B); and (ii) second chimeric polypeptide

including a second domain of an affinity pair of domains (IL-15 receptor alpha sushi domain), a second target-binding domain (C), and an additional antigen-binding domain (D). The top cartoon diagram depicts the association of the first and the second chimeric polypeptides through the pair of affinity domains. The bottom schematic diagrams show the order of the domains in the first and second chimeric polypeptides.

Figure 2 shows exemplary diagrams for a multi-chain chimeric polypeptide: (i) a first chimeric polypeptide including a first target-binding domain (A), a soluble tissue factor domain including five amino acid substitutions in order to remove binding of the soluble tissue factor domain to FVIIa, a first domain of an affinity pair of domains (soluble interleukin IL-15 including a D8N or D8A amino acid substitution), and an additional target-binding domain (B); and (ii) second chimeric polypeptide including a second domain of an affinity pair of domains (IL-15 receptor alpha sushi domain), a second target-binding domain (C), and an additional antigen-binding domain (D). The top cartoon diagram depicts the association of the first and the second chimeric polypeptides through the pair of affinity domains. The bottom schematic diagrams show the order of the domains in the first and second chimeric polypeptides. In other embodiments of any of the multi-chain chimeric polypeptides described herein the soluble tissue factor domain can comprise or consists of a soluble wildtype human tissue factor domain (comprising or consisting of a contiguous sequence within wildtype human tissue factor).

Figure 3 shows a schematic diagram of an exemplary IL-12/IL-15RαSu DNA construct.

Figure 4 shows a schematic diagram of an exemplary IL-18/TF/IL-15 DNA construct.

Figure 5 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15RαSu and IL-18/TF/IL-15 DNA constructs.

Figure 6 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15RαSu and IL-18/TF/IL-15 fusion proteins resulting in IL-18/TF/IL-15:IL-12/IL-15RαSu complex (18t15-12s).

Figure 7 shows a chromatograph of 18t15-12s purification elution from an anti-TF antibody affinity column.

Figure 8 shows an exemplary chromatographic profile of anti-TF Ab / SEC-purified 18t15-12s protein following elution on an analytical size exclusion column, demonstrating separation of monomeric multiprotein 18t15-12s complexes from protein aggregates.

Figure 9 shows an example of a 4-12% SDS-PAGE of the 18t15-12s complex following disulfide bond reduction. Lane 1: SeeBlue Plus2 marker; Lane 2: an anti-tissue factor antibody affinity column-purified 18t15-12s (0.5 μg); Lane 3: an anti-tissue factor antibody affinity column-purified 18t15-12s (1 μg).

Figure 10 shows SDS PAGE analysis of deglycosylated and non-deglycosylated 18t15-12s. Lane 1: an anti-tissue factor antibody affinity column-purified 18t15-12s (0.5 μg), non-deglycosylated; Lane 2: anti-TF Ab-purified 18t15-12s (1 μg), non-deglycosylated; Lane 3: 18t15-12s (1 μg), deglycosylated, Lane 4: Mark12 unstained maker.

Figure 11 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human IL-12 detection antibody (BAF 219).

Figure 12 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human IL-15 detection antibody (BAM 247).

Figure 13 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human IL-18 detection antibody (D045-6).

Figure 14 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor (I43) capture antibody and an anti-human tissue factor detection antibody.

Figure 15 shows proliferation of IL-15-dependent 32Dβ cells mediated by the 18t15-12s complex (open squares) and recombinant IL-15 (black squares).

Figure 16 shows biological activity of IL-18 within the 18t15-12s complex (open squares), where recombinant IL-18 (black squares) and recombinant IL-12 (black circles) serve as positive and negative controls, respectively.

Figure 17 shows biological activity of IL-12 within the 18t15-12s complex (open squares), where recombinant IL-12 (black circles) and recombinant IL-18 (open squares) serve as positive and negative controls, respectively.

Figures 18A and 18B show cell-surface expression of CD25 on NK cells induced by the 18t15-12s complex and cell-surface CD69 expression of NK cells induced by the 18t15-12s complex.

Figure 19 shows a flow cytometry graph of intracellular interferon gamma expression of NK cells induced by the 18t15-12s complex.

Figure 20 shows cytotoxicity of 18t15-12s induced human NK cells against K562 cells.

Figure 21 shows a schematic diagram of an exemplary IL-12/IL-15RαSu/αCD16 DNA construct.

Figure 22 shows a schematic diagram of an exemplary IL-18/TF/IL-15 DNA construct.

Figure 23 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 DNA constructs.

Figure 24 shows a schematic diagram of an exemplary 18t15-12s/αCD16 protein complex.

Figure 25 shows a sandwich ELISA for the 18t15-12s16 complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human IL-12 or IL-18 detection antibody.

Figure 26 shows a schematic diagram of an exemplary TGFβRII/IL-15RαSu DNA construct.

Figure 27 shows a schematic diagram of an exemplary IL-21/TF/IL-15 construct.

Figure 28 shows a schematic diagram of the interaction between the exemplary IL- IL-21/TF/IL-15 and TGFβRII/IL-15RαSu constructs.

Figure 29 shows a schematic diagram of the interaction between the exemplary TGFβRII/IL-15RαSu and IL-21/TF/IL-15 fusion proteins, resulting in an IL-21/TF/IL-15/TGFβRII/IL-15RαSu complex (21t15-TGFRs).

Figure 30 shows a chromatograph of 21t15-TGFRs purification elution from an anti-TF antibody affinity column.

Figure 31 shows an exemplary 21t15-TGFRs size exclusion chromatograph showing a main protein peak and a high molecular weight peak

Figure 32 shows an example of a 4-12% SDS-PAGE of the 21t15-TGFRs complex following disulfide bond reduction. Lane 1: Mark12 unstained marker (numbers on the left side indicate molecular weights in kDa); Lane 2: 21t15-TGFRs (0.5 μg); Lane 3: 21t15-TGFRs (1 μg); Lane 4: 21t15-TGFRs, deglycosylated (1 μg), wherein the MW was the expected size of 53kDa and 39.08 kDa.

Figure 33 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human IL-21 detection antibody (13-7218-81, BioLegend).

Figure 34 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human IL-15 detection antibody (BAM 247, R&D Systems).

Figure 35 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor capture antibody and a biotinylated anti-human TGFβRII detection antibody (BAF241, R&D Systems).

Figure 36 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor (I43) capture antibody and an anti-human tissue factor detection antibody.

Figure 37 shows IL-15-dependent proliferation of 32Dβ cells mediated by the 21t15-TGFRs complex (open squares) compared to IL-15 (black squares).

Figure 38 shows biological activity of the TGFβRII domain within the 21t15-TGFRs complex (open squares). TGFβRII/Fc (black squares) served as a positive control.

Figure 39 A shows a flow cytometry graph of cell-surface CD25 expression of NK cells induced by the 21t15-TGFRs complex.

Figure 40 shows a flow cytometry graph of cell-surface CD69 expression of NK cells induced by the 21t15-TGFRs complex.

Figure 41 shows a flow cytometry graph of intracellular interferon gamma expression of NK cells induced by the 21t15-TGFRs complex.

Figure 42 shows cytotoxicity of 21t15-TGFRs-induced human NK cells against K562 cells.

Figure 43 shows a schematic diagram of an exemplary IL-7/IL-15RαSu DNA construct.

Figure 44 shows a schematic diagram of an exemplary IL-21/TF/IL-15 DNA construct.

Figure 45 shows a schematic diagram of the interaction between the exemplary IL-7/IL-15RαSu and IL-21/TF/IL-15 DNA constructs.

Figure 46 shows a schematic diagram of the interaction between the exemplary IL-7/IL-15RαSu and IL-21/TF/IL-15 fusion proteins resulting in an IL-21/TF/IL-15:IL-7/IL-15RαSu complex (21t15-7s).

Figure 47 shows the expansion of primary natural killer (NK) cells by stimulation with 21t15-7s + anti-TF IgG1 antibody.

Figure 48 shows activation of expanded primary NK cells, using CD25 MFI and CD69 MFI as markers of NK cell activation.

Figure 49 shows cytotoxic activity of expanded NK cells against K562 human tumor cells, wherein NK cells stimulated with 21t15-7s + anti-TF IgG1 antibody demonstrate greater specific lysis of K562 cells than NK cells not stimulated with 21t15-7s + anti-TF IgG1 antibody.

Figure 50 shows a schematic diagram of an exemplary IL-21/IL-15RαSu DNA construct.

Figure 51 shows a schematic diagram of an exemplary IL-7/TF/IL-15 DNA construct.

Figure 52 shows a schematic diagram of the interaction between the exemplary IL-21/IL-15RαSu and IL-7/TF/IL-15 DNA constructs.

Figure 53 shows a schematic diagram of the interaction between the exemplary IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins resulting in an IL-7/TF/IL-15:IL-21/IL-15RαSU complex (7t15-21s).

Figure 54 shows in diagrammatic form the activation and expansion of primary natural killer (NK) cells by stimulation with 21t15-TGFRs + anti-TF IgG1 antibody.

Figure 55 shows size exclusion chromatography (SEC) profiles of anti-TF IgG1 antibody, 7t15-21s and the complex containing equal amounts of anti-TF IgG1 antibody and 7t15-21s.

Figure 56 shows the oxygen consumption rate (OCR) in pmoles/min for human NK cells isolated from blood (2 x 10^6 cells/mL) of two different donors.

Figure 57 shows the extracellular acidification rate (ECAR) in mpH/minute for human NK cells isolated from blood (2 x 10^6 cells/mL) of two different donors.

Figure 58 shows a schematic of the 7t15-16s21 construct.

Figure 59 shows an additional schematic of the 7t15-16s21 construct.

Figures 60A and 60B show binding of 7t15-16s21 to CHO cells expressing human CD16b as compared to a control protein.

Figures 61A-61C are results from ELISA experiments using antibodies against IL-15, IL-21, and IL-7 in detecting 7t15-16s21.

Figure 62 shows results of the 32Dβ cell proliferation assay with 7t15-16s21 or recombinant IL-15.

Figure 63 shows the chromatographic profile of 7t15-16s21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 64 shows the analytical SEC Profile of 7t15-16s21.

Figure 65 shows a schematic of the TGFRt15-16s21 construct.

Figure 66 shows an additional schematic of the TGFRt15-16s21 construct.

Figures 67A and 67B show binding affinity of TGFRt15-16S21 and 7t15-21s with CHO cells expressing human CD16b. Figure 67A shows binding affinity of TGFRt15-16S21 with CHO cells expressing human CD16b. Figure 67B shows binding affinity of 7t15-21s with CHO cells expressing human CD16b.

Figure 68 shows results of TGFβ1 inhibition by TGFRt15-16s21 and TGFR-Fc.

Figure 69 shows results of 32Dβ cell proliferation assay with TGFRt15-16s21 or recombinant IL-15.

Figures 70A-70C show results of detecting IL-15, IL-21, and TGFβRII in TGFRt15-16s21 with corresponding antibodies using ELISA.

Figure 71 shows the chromatographic profile of TGFRt15-16s21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 72 shows results of a reduced SDS-PAGE analysis of TGFRt15-16s21.

Figure 73 shows a schematic of the 7t15-7s construct.

Figure 74 shows an additional schematic of the 7t15-7s construct.

Figure 75 shows the chromatographic profile of 7t15-7s protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 76 shows detection of TF, IL-15 and IL-7 in 7t15-7s using ELISA.

Figure 77 shows a schematic of the TGFRt15-TGFRs construct.

Figure 78 shows an additional schematic of the TGFRt15-TGFRs construct.

Figure 79 shows results of TGFβ1 inhibition by TGFRt15-TGFRs and TGFR-Fc.

Figure 80 shows results of 32Dβ cell proliferation assay with TGFRt15-TGFRs or recombinant IL-15

Figures 81A and 81B show results of detecting IL-15 and TGFβRII in TGFRt15-TGFRs with corresponding antibodies using ELISA.

Figure 82 is a line graph showing the chromatographic profile of TGFRt15-TGFRs protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 83 shows the analytical SEC profile of TGFRt15-TGFRs.

Figure 84 shows TGFRt15-TGFRs before and after deglycosylation as analyzed by reduced SDS-PAGE.

Figures 85A and 85B show spleen weight and the percentages of immune cell types in TGFRt15-TGFRs-treated and control-treated mice. Figure 85A shows spleen weight in mice treated with TGFRt15-TGFRs as compared to PBS control. Figure 85B shows the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in mice treated with TGFRt15-TGFRs as compared to PBS control.

Figure 86A and 86B show the spleen weight and immunostimulation over 92 hours in mice treated with TGFRt15-TGFRs. Figure 86A shows spleen weight of mice treated with TGFRt15-TGFRs at 16, 24, 48, 72, and 92 hours after treatment. Figure 86B

shows the percentages of immune cells in mice treated with TGFRt15-TGFRs at 16, 24, 48, 72, and 92 hours after treatment.

Figure 87A and 87B show Ki67 and Granzyme B expression in mice treated with TGFRt15-TGFRs over time.

Figure 88 shows enhancement of cytotoxicity of splenocytes by TGFRt15-TGFRs in C57BL/6 Mice.

Figure 89 shows changes in tumor size in response to PBS treatment, chemotherapy alone, TGFRt15-TGFRs alone, or chemotherapy and TGFRt15-TGFRs combination, in a pancreatic cancer mouse model.

Figure 90 shows the cytotoxicity of NK cells isolated from mice treated with TGFRt15-TGFRs.

Figure 91 shows a schematic of the 7t15-21s137L (long version) construct.

Figure 92 shows an additional schematic of the 7t15-21s137L (long version) construct.

Figure 93 is a line graph showing the chromatographic profile of 7t15-21s137L (long version) protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 94 shows the analytical SEC profile of 7t15-21s137L (long version).

Figure 95 shows binding of 7t15-21s137L (short version) to CD137L (4.1BBL)

Figures 96A-96C show detection of IL15, IL21, and IL7 in 7t15-21s137L (short version) with ELISA. Figure 96A shows detection of IL15 in 7t15-21s137L (short version) with ELISA. Figure 96B shows detection of IL21 in 7t15-21s137L (short version) with ELISA. Figure 96C shows detection of IL7 in 7t15-21s137L (short version) with ELISA.

Figure 97 shows results from a CTLL-2 cell proliferation assay.

Figure 98 shows the activity of 7t15-1s137L (short version) in promoting IL21R containing B9 cell proliferation.

Figure 99 shows a schematic of the 7t15-TGFRs construct.

Figure 100 shows an additional schematic of the 7t15-TGFRs construct.

Figure 101 shows results of TGFβ1 inhibition by 7t15-TGFRs and TGFR-Fc.

Figures 102A-102C show detection of IL-15, TGFβRII, and IL-7 in 7t15-TGFRs with ELISA.

Figure 103 shows results of a 32Dβ cell proliferation assay with 7t15-TGFRs or recombinant IL-15.

Figure 104 is a line graph showing the chromatographic profile of 7t15-TGFRs protein containing cell culture supernatant following binding and elution on anti-TF antibody affinity column.

Figure 105 shows 7t15-TGFRs before and after deglycosylation as analyzed using reduced SDS-PAGE.

Figure 106 shows ELISA detection of IL-7, IL-15 and TGFβRII in the 7t15-TGFRs protein.

Figures 107A and 107B show spleen weight and the percentages of immune cell types in 7t15-TGFRs-treated and control-treated mice. Figure 107A shows spleen weight in mice treated with 7t15-TGFRs at various dosages, as compared to PBS control. Figure 107B shows the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in mice treated with 7t15-TGFRs at various dosages, as compared to PBS control.

Figures 108A and 108B show upregulation of CD44 expression of $CD4^+$ and $CD8^+$ T cells by 7t15-TGFRs in C57BL/6 mice.

Figures 109A and 109B show upregulation of Ki67 expression and Granzyme B expression of $CD8^+$ T cells and NK Cells by 7t15-TGFRs in C57BL/6 mice.

Figure 110 shows enhancement of cytotoxicity of splenocytes by 7t15-TGFRs in C57BL/6 mice.

Figure 111 shows a schematic of the TGFRt15-21s137L construct.

Figure 112 shows an additional schematic of the TGFRt15-21s137L construct.

Figure 113 is a line graph showing the chromatographic profile of TGFRt15-21s137L protein containing cell culture supernatant following binding and elution on anti-TF antibody affinity column.

Figure 114 shows a schematic of the TGFRt15-TGFRs21 construct.

Figure 115 shows an additional schematic of the TGFRt15-TGFRs21 construct.

Figure 116 is a line graph showing the chromatographic profile of TGFRt15-TGFRs21 protein containing cell culture supernatant following binding and elution on anti-TF antibody affinity column.

Figure 117 shows TGFRt15-TGFRs21 before and after deglycosylation as analyzed by reduced SDS-PAGE.

Figures 118A and 118B show detection of components of TGFRt15-TGFRs21 using ELISA.

Figures 119A and 119B show the percentages and proliferation of $CD4^+$ T cells, $CD8^+$ T cells, and natural killer (NK) cells present in the spleen of control-treated and TGFRt15-TGFRs21-treated mice.

Figure 120 shows upregulation of Granzyme B expression of splenocytes in mice treated with TGFRt15-TGFRs21.

Figure 121 shows enhancement of cytotoxicity of splenocytes by TGFRt15-TGFRs21 in C57BL/6 Mice.

Figure 122 shows a schematic of the TGFRt15-TGFRs16 construct.

Figure 123 shows an additional schematic of the TGFRt15-TGFRs16 construct.

Figure 124 shows a schematic of the TGFRt15-TGFRs137L construct.

Figure 125 shows an additional schematic of the TGFRt15-TGFRs137L construct.

Figure 126 shows changes in the surface phenotype of lymphocyte populations after stimulation with 18t15-12s, 18t15-12s16, and 7t15-21s.

Figure 127 shows an increase in phospho-STAT4 and phospho-STAT5 levels in NK cells after stimulation with 18t15-12s.

Figures 128A-128C show *in vivo* stimulation of Tregs, NK cells, and $CD8^+$ T cells in $ApoE^{-/-}$ mice fed with a Western diet and treated with TGFRt15-TGFRs.

Figures 129A-129C show immunostimulation in C57BL/6 mice following treatment with TGFRt15-TGFRs.

Figures 130A and 130B show *in vivo* induction of proliferation of NK cells and $CD8^+$ T cells in $ApoE^{-/-}$ mice fed with a Western diet and treated with TGFRt15-TGFRs.

Figures 131A and 131B show enhancement of cytotoxicity of NK cells following treatment of NK cells with TGFRt15-TGFRs.

Figures 132A and 132B show enhancement of ADCC activity of NK cells following treatment of NK cells with TGFRt15-TGFRs.

Figures 133A-133H show antitumor activity of TGFRt15-TGFRs plus anti-TRP1 antibody (TA99) in combination with chemotherapy in a melanoma mouse model.

Figures 134A-134C show amelioration of the Western diet-induced hyperglycemia in $ApoE^{-/-}$ mice by TGFRt15-TGFRs.

Figure 135 shows cell surface staining summarizing the differentiation of NK cells into cytokine-induced memory like NK Cells (CIML-NK Cells) after stimulation with 18t15-12s and cultured in rhIL15.

Figure 136 shows upregulation shows upregulation of CD44hi memory T cells upon treatment with TGFRt15-TGFRs.

Figure 137 shows a graph of Factor X (FX) activation following treatment with single-chain or multi-chain chimeric polypeptides.

Figure 138 shows clotting time for a buffer with varying concentrations of Innovin in a prothrombin time (PT) test.

Figure 139 shows clotting time for multi-chain chimeric polypeptides in a PT Assay.

Figure 140 shows clotting time of the multi-chain chimeric polypeptides in a PT assay when mixed with 32DB cells.

Figure 141 shows clotting time of multi-chain chimeric polypeptides in a PT assay when mixed with human PBMC.

Figure 142 shows binding of 7t15-21s137L (long version) and 7t15-21s137L (short version) to CD137 (4.1BB).

Figure 143A-143D show detection of IL7, IL21, IL15, and 4.1BBL in 7t15-21s137L (long version) by the respective antibodies using ELISA.

Figure 144 shows IL-15 activity of 7t15-21s137L (long version) and 7t15-21s137L (short version) as evaluated by a IL2Rαβγ-containing CTLL2 cell proliferation assay.

DETAILED DESCRIPTION

Provided herein are multi-chain chimeric polypeptides that include: (a) a first chimeric polypeptide including: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains. Also provided herein are compositions that include any of the multi-chain chimeric polypeptides described herein, nucleic acids that encode any of the multi-chain chimeric polypeptides described herein, and cells that include any of the nucleic acids that encode any of the multi-chain chimeric polypeptides described herein. Also provided herein are methods of stimulating an immune cell and methods of treating a subject in need thereof that include the use of any of the multi-chain chimeric polypeptides described herein. Also provided herein are methods of producing any of the multi-chain chimeric polypeptides described herein.

In some examples of any of the multi-chain chimeric polypeptides described herein the total length of first chimeric polypeptide and/or the second chimeric polypeptide can each independently be about 50 amino acids to about 3000 amino acids, about 50 amino acids to about 2500 amino acids, about 50 amino acids to about 2000 amino acids, about 50 amino acids to about 1500 amino acids, about 50 amino acids to about 1000 amino acids, about 50 amino acids to about 950 amino acids, about 50 amino acids to about 900 amino acids, about 50 amino acids to about 850 amino acids, about 50 amino acids to about 800 amino acids, about 50 amino acids to about 750 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 480 amino acids, about 50 amino acids to about 460 amino acids, about 50 amino acids to about 440 amino acids, about 50 amino acids to about 420 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 380 amino acids, about 50 amino acids to about 360 amino acids, about 50 amino acids to about 340 amino acids, about 50 amino acids to about 320 amino acids, about 50 amino acids to about 300

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In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the first target-binding domains described herein) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) directly abut each other in the first chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the first target-binding domain (e.g., any of the exemplary first target-binding domains described herein) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) in the first chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) directly abut each other in the first chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains

of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) directly abut each other in the second chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) in the second chimeric polypeptide.

Non-limiting aspects of these chimeric polypeptides, nucleic acids, vectors, cells, and methods are described below, and can be used in any combination without limitation. Additional aspects of these chimeric polypeptides, nucleic acids, vectors, cells, and methods are known in the art.

Tissue Factor

Human tissue factor is a 263 amino-acid transmembrane protein containing three domains: (1) a 219-amino acid N-terminal extracellular domain (residues 1-219); (2) a 22-amino acid transmembrane domain (residues 220-242); and (3) a 21-amino acid cytoplasmic C-terminal tail (residues 242-263) ((UniProtKB Identifier Number: P13726). The cytoplasmic tail contains two phosphorylation sites at Ser253 and Ser258, and one S-palmitoylation site at Cys245. Deletion or mutation of the cytoplasmic domain was not found to affect tissue factor coagulation activity. Tissue factor has one S-palmitoylation site in the intracellular domain of the protein at Cys245. The Cys245 is located at the amino acid terminus of the intracellular domain and close to the membrane surface. The tissue factor transmembrane domain is composed of a single-spanning α-helix.

The extracellular domain of tissue factor, composed of two fibronectin type III domains, is connected to the transmembrane domain through a six-amino acid linker. This linker provides conformational flexibility to decouple the tissue factor extracellular domain from its transmembrane and cytoplasmic domains. Each tissue factor fibronectin type III module is composed of two overlapping β sheets with the top sheet domain containing three antiparallel β-strands and the bottom sheet containing four β-strands. The β-strands are connected by β-loops between strand βA and βB, βC and βD, and βE and βF, all of which are conserved in conformation in the two modules. There are three short α-helix segments connecting the β-strands. A unique feature of tissue factor is a 17-amino acid β-hairpin between strand β10 and strand β11, which is not a common element of the fibronectin superfamily. The N-terminal domain also contains a 12 amino acid loop between β6F and β7G that is not present in the C-terminal domain and is unique to tissue factor. Such a fibronectin type III domain structure is a feature of the immunoglobulin-like family of protein folds and is conserved among a wide variety of extracellular proteins.

The zymogen FVII is rapidly converted to FVIIa by limited proteolysis once it binds to tissue to form the active tissue factor-FVIIa complex. The FVIIa, which circulates as an enzyme at a concentration of approximately 0.1 nM (1% of plasma FVII), can also bind directly to tissue factor. The allosteric interaction between tissue factor and FVIIa on the tissue factor-FVIIa complex greatly increases the enzymatic activity of FVIIa: an approximate 20- to 100-fold increase in the rate of hydrolysis of small, chromogenic peptidyl substrates, and nearly a million-fold increase in the rate of activation of the natural macromolecular substrates FIX and FX. In concert with allosteric activation of the active site of FVIIa upon binding to tissue factor, the formation of tissue factor-FVIIa complex on phospholipid bilayer (i.e., upon exposure of phosphatidyl-L-serine on membrane surfaces) increases the rate of FIX or FX activation, in a Ca^{2+}-dependent manner, an additional 1,000-fold. The roughly million-fold overall increase in FX activation by tissue factor-FVIIa-phospholipid complex relative to free FVIIa is a critical regulatory point for the coagulation cascade.

FVII is a ~50 kDa, single-chain polypeptide consisting of 406 amino acid residues, with an N-terminal γ-carboxyglutamate-rich (GLA) domain, two epidermal

growth factor-like domains (EGF1 and EFG2), and a C-terminal serine protease domain. FVII is activated to FVIIa by a specific proteolytic cleavage of the Ile-^{154}-Arg^{152} bond in the short linker region between the EGF2 and the protease domain. This cleavage results in the light and heavy chains being held together by a single disulfide bond of Cys^{135} and Cys^{262}. FVIIa binds phospholipid membrane in a Ca^{2+}-dependent manner through its N-terminal GLA-domain. Immediately C-terminal to the GLA domain is an aromatic stack and two EGF domains. The aromatic stack connects the GLA to EGF1 domain which binds a single Ca^{2+} ion. Occupancy of this Ca^{2+}-binding site increases FVIIa amidolytic activity and tissue factor association. The catalytic triad consist of His^{193}, Asp^{242}, and Ser^{344}, and binding of a single Ca^{2+} ion within the FVIIa protease domain is critical for its catalytic activity. Proteolytic activation of FVII to FVIIa frees the newly formed amino terminus at Ile^{153} to fold back and be inserted into the activation pocket forming a salt bridge with the carboxylate of Asp^{343} to generate the oxyanion hole. Formation of this salt bridge is critical for FVIIa activity. However, oxyanion hole formation does not occur in free FVIIa upon proteolytic activation. As a result, FVIIa circulates in a zymogen-like state that is poorly recognized by plasma protease inhibitors, allowing it to circulate with a half-life of approximately 90 minutes.

Tissue factor-mediated positioning of the FVIIa active site above the membrane surface is important for FVIIa towards cognate substrates. Free FVIIa adopts a stable, extended structure when bound to the membrane with its active site positioned ~80Å above the membrane surface. Upon FVIIa binding to tissue factor, the FVa active site is repositioned ~6Å closer to the membrane. This modulation may aid in a proper alignment of the FVIIa catalytic triad with the target substrate cleavage site. Using GLA-domainless FVIIa, it has been shown that the active site was still positioned a similar distance above the membrane, demonstrating that tissue factor is able to fully support FVIIa active site positioning even in the absence of FVIIa-membrane interaction. Additional data showed that tissue factor supported full FVIIa proteolytic activity as long as the tissue factor extracellular domain was tethered in some way to the membrane surface. However, raising the active site of FVIIa greater than 80Å above the membrane surface greatly reduced the ability of the tissue factor-FVIIa complex to activate FX but did not diminish tissue factor-FVIIa amidolytic activity.

Alanine scanning mutagenesis has been used to assess the role of specific amino acid side chains in the tissue factor extracellular domain for interaction with FVIIa (Gibbs et al., *Biochemistry* 33(47): 14003-14010, 1994; Schullek et al., *J Biol Chem* 269(30): 19399-19403, 1994). Alanine substitution identified a limited number of residue positions at which alanine replacements cause 5- to 10-fold lower affinity for FVIIa binding. Most of these residue side chains were found to be well-exposed to solvent in the crystal structure, concordant with macromolecular ligand interaction. The FVIIa ligand-binding site is located over an extensive region at the boundary between the two modules. In the C-module, residues Arg^{135} and Phe^{140} located on the protruding B-C loop provide an independent contact with FVIIa. Leu^{133} is located at the base of the fingerlike structure and packed into the cleft between the two modules. This provides continuity to a major cluster of important binding residues consisting of Lys^{20}, Thr^{60}, Asp^{58}, and Ile^{22}. Thr^{60} is only partially solvent-exposed and may play a local structural role rather than making a significant contact with ligand. The binding site extends onto the concave side of the intermodule angle involving Glu^{24} and Gln^{110}, and potentially the more distant residue Val^{207}. The binding region extends from Asp58 onto a convex surface area formed by Lys^{48}, Lys^{46}, Gln^{37}, Asp^{44}, and Trp^{45}. Trp^{45} and Asp^{44} do not interact independently with FVIIa, indicating that the mutational effect at the Trp^{45} position may reflect a structural importance of this side chain for the local packing of the adjacent Asp^{44} and Gln^{37} side chain. The interactive area further includes two surface-exposed aromatic residues, Phe^{76} and Tyr^{78}, which form part of the hydrophobic cluster in the N-module.

The known physiologic substrates of tissue factor-FVIIa are FVII, FIX, and FX and certain proteinase-activated receptors. Mutational analysis has identified a number of residues that, when mutated, support full FVIIa amidolytic activity towards small peptidyl substrates but are deficient in their ability to support macromolecular substrate (i.e., FVII, FIX, and FX) activation (Ruf et al., *J Biol Chem* 267(31): 22206-22210, 1992; Ruf et al., *J Biol Chem* 267(9): 6375-6381, 1992; Huang et al., *J Biol Chem* 271(36): 21752-21757, 1996; Kirchhofer et al., *Biochemistry* 39(25): 7380-7387, 2000). The tissue factor loop region at residues 159-165, and residues in or adjacent to this flexible loop have been shown to be critical for the proteolytic activity of the tissue factor-FVIIa

complex. This defines the proposed substrate-binding exosite region of tissue factor that is quite distant from the FVIIa active site. A substitution of the glycine residue by a marginally bulkier residue alanine, significantly impairs tissue factor-FVIIa proteolytic activity. This suggests that the flexibility afforded by glycine is critical for the loop of residues 159-165 for tissue factor macromolecular substrate recognition.

The residues Lys^{165} and Lys^{166} have also been demonstrated to be important for substrate recognition and binding. Mutation of either of these residues to alanine results in a significant decrease in the tissue factor co-factor function. Lys^{165} and Lys^{166} face away from each other, with Lys^{165} pointing towards FVIIa in most tissue factor-FVIIa structures, and Lys^{166} pointing into the substrate binding exosite region in the crystal structure. Putative salt bridge formation between Lys^{165} of and Gla^{35} of FVIIa would support the notion that tissue factor interaction with the GLA domain of FVIIa modulates substrate recognition. These results suggest that the C-terminal portion of the tissue factor ectodomain directly interacts with the GLA-domain, the possible adjacent EGF1 domains, of FIX and FX, and that the presence of the FVIIa GLA-domain may modulate these interactions either directly or indirectly.

Soluble Tissue Factor Domain

In some embodiments of any of the polypeptides, compositions, or methods described herein, the soluble tissue factor domain can be a wildtype tissue factor polypeptide lacking the signal sequence, the transmembrane domain, and the intracellular domain. In some examples, the soluble tissue factor domain can be a tissue factor mutant, wherein a wildtype tissue factor polypeptide lacking the signal sequence, the transmembrane domain, and the intracellular domain, and has been further modified at selected amino acids. In some examples, the soluble tissue factor domain can be a soluble human tissue factor domain. In some examples, the soluble tissue factor domain can be a soluble mouse tissue factor domain. In some examples, the soluble tissue factor domain can be a soluble rat tissue factor domain. Non-limiting examples of soluble human tissue factor domains, a mouse soluble tissue factor domain, a rat soluble tissue factor domain, and mutant soluble tissue factor domains are shown below.

Exemplary Soluble Human Tissue Factor Domain (SEQ ID NO: 1)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQ
PTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGK
KTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRE

Exemplary Nucleic Acid Encoding Soluble Human Tissue Factor Domain (SEQ ID NO: 2)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACC
GTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCAC
CGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACC
TACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTC
CGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGA
CCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGT
GAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTT
CTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGG
AAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTT
TAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATC
CCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGG
GCCAAGAAAAGGGCGAGTTCCGGGAG

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 3)

SGTTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDWKSKCFYTT
DTECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNL
GQPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLYYWKSSSS
GKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEF
RE

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 4)

SGTTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDAKSKCFYTTD
TECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLAENSPEFTPYLETNLG

QPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

Exemplary Soluble Mouse Tissue Factor Domain (SEQ ID NO: 5)

agipekafnltwistdfktilewqpkptnytytvqisdrsrnwknkcfstt
dtecdltdeivkdvtwayeakvlsvprrnsvhgdgdqlvihgeeppftnap
kflpyrdtnlgqpviqqfeqdgrklnvvvkdsltlvrkngtfltlrqvfgk
dlgyiityrkgsstgkktnitntnefsidveegvsycffvqamifsrktnq
nspgsstvcteqwksflge

Exemplary Soluble Rat Tissue Factor Domain (SEQ ID NO: 6)

Agtppgkafnltwistdfktilewqpkptnytytvqisdrsrnwkykctgt
tdtecdltdeivkdvnwtyearvlsvpwrnsthgketlfgthgeeppftna
rkflpyrdtkigqpviqkyeqggtklkvtvkdsftlvrkngtfltlrqvfg
ndlgyiltyrkdsstgrktntthtneflidvekgvsycffaqavifsrktn
hkspesitkcteqwksvlge

In some embodiments, a soluble tissue factor domain can include a sequence that is at least 70% identical, at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 1, 3, 4, 5, or 6. In some embodiments, a soluble tissue factor domain can include a sequence of SEQ ID NO: 1, 3, 4, 5, or 6, with one to twenty amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from its N-terminus and/or one to twenty amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from its C-terminus.

As can be appreciated in the art, one skilled in the art would understand that mutation of amino acids that are conserved between different mammalian species is more likely to decrease the activity and/or structural stability of the protein, while mutation of

amino acids that are not conserved between different mammalian species is less likely to decrease the activity and/or structural stability of the protein.

In some examples of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some examples of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

In some examples, the soluble tissue factor domain can be a soluble human tissue factor domain. In some embodiments, the soluble tissue factor domain can be a soluble mouse tissue factor domain. In some embodiments, the soluble tissue factor domain can be a soluble rat tissue factor domain.

In some examples, the soluble tissue factor domain does not include one or more (e.g., two, three, four, five, six, or seven) of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some embodiments, the mutant soluble tissue factor possesses the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

In some examples, the soluble tissue factor domain can be encoded by a nucleic acid including a sequence that is at least 70% identical, at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96%

identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 2.

In some embodiments, the soluble tissue factor domain can have a total length of about 20 amino acids to about 220 amino acids, about 20 amino acids to about 215 amino acids, about 20 amino acids to about 210 amino acids, about 20 amino acids to about 205 amino acids, about 20 amino acids to about 200 amino acids, about 20 amino acids to about 195 amino acids, about 20 amino acids to about 190 amino acids, about 20 amino acids to about 185 amino acids, about 20 amino acids to about 180 amino acids, about 20 amino acids to about 175 amino acids, about 20 amino acids to about 170 amino acids, about 20 amino acids to about 165 amino acids, about 20 amino acids to about 160 amino acids, about 20 amino acids to about 155 amino acids, about 20 amino acids to about 150 amino acids, about 20 amino acids to about 145 amino acids, about 20 amino acids to about 140 amino acids, about 20 amino acids to about 135 amino acids, about 20 amino acids to about 130 amino acids, about 20 amino acids to about 125 amino acids, about 20 amino acids to about 120 amino acids, about 20 amino acids to about 115 amino acids, about 20 amino acids to about 110 amino acids, about 20 amino acids to about 105 amino acids, about 20 amino acids to about 100 amino acids, about 20 amino acids to about 95 amino acids, about 20 amino acids to about 90 amino acids, about 20 amino acids to about 85 amino acids, about 20 amino acids to about 80 amino acids, about 20 amino acids to about 75 amino acids, about 20 amino acids to about 70 amino acids, about 20 amino acids to about 60 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 30 amino acids, about 30 amino acids to about 220 amino acids, about 30 amino acids to about 215 amino acids, about 30 amino acids to about 210 amino acids, about 30 amino acids to about 205 amino acids, about 30 amino acids to about 200 amino acids, about 30 amino acids to about 195 amino acids, about 30 amino acids to about 190 amino acids, about 30 amino acids to about 185 amino acids, about 30 amino acids to about 180 amino acids, about 30 amino acids to about 175 amino acids, about 30 amino acids to about 170 amino acids, about 30 amino acids to about 165 amino acids, about 30 amino acids to about 160 amino acids, about 30 amino acids to about 155 amino acids, about 30 amino acids to about 150 amino acids, about 30 amino acids to about 145 amino acids, about 30 amino

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Linker Sequences

In some embodiments, the linker sequence can be a flexible linker sequence. Non-limiting examples of linker sequences that can be used are described in Klein et al., *Protein Engineering, Design & Selection* 27(10):325–330, 2014; Priyanka et al., *Protein Sci.* 22(2):153–167, 2013. In some examples, the linker sequence is a synthetic linker sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art). In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art).

In some embodiments, a linker sequence can have a total length of 1 amino acid to about 100 amino acids, 1 amino acid to about 90 amino acids, 1 amino acid to about 80 amino acids, 1 amino acid to about 70 amino acids, 1 amino acid to about 60 amino acids, 1 amino acid to about 50 amino acids, 1 amino acid to about 45 amino acids, 1 amino acid to about 40 amino acids, 1 amino acid to about 35 amino acids, 1 amino acid to about 30 amino acids, 1 amino acid to about 25 amino acids, 1 amino acid to about 24 amino acids, 1 amino acid to about 22 amino acids, 1 amino acid to about 20 amino acids, 1 amino acid to about 18 amino acids, 1 amino acid to about 16 amino acids, 1 amino acid to about 14 amino acids, 1 amino acid to about 12 amino acids, 1 amino acid to about 10 amino acids, 1 amino acid to about 8 amino acids, 1 amino acid to about 6 amino acids, 1 amino acid to about 4 amino acids, about 2 amino acids to about 100 amino acids, about 2 amino acids to about 90 amino acids, about 2 amino acids to about 80 amino acids, about 2 amino acids to about 70 amino acids, about 2 amino acids to about 60 amino acids, about 2 amino acids to about 50 amino acids, about 2 amino acids to about 45 amino acids, about 2 amino acids to about 40 amino acids, about 2 amino acids to about 35 amino acids, about 2 amino acids to about 30 amino acids, about 2 amino acids to about 25 amino acids, about 2 amino acids to about 24 amino acids, about 2 amino acids to about 22 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 18 amino acids, about 2 amino acids to about 16 amino acids, about 2 amino acids to about 14 amino acids, about 2 amino acids to about 12 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 6 amino acids, about 2 amino acids to about 4 amino acids, about 4 amino acids to about 100 amino acids, about 4 amino acids to about 90 amino acids, about 4 amino acids to about 80 amino acids, about 4 amino acids to about 70 amino acids, about 4 amino acids to about 60 amino acids, about 4 amino acids to about 50 amino acids, about 4 amino acids to about 45 amino acids, about 4 amino acids to about 40 amino acids, about 4 amino acids to about 35 amino acids, about 4 amino acids to about 30 amino acids, about 4 amino acids to about 25 amino acids, about 4 amino acids to about 24 amino acids, about 4 amino acids to about 22 amino acids, about 4 amino acids to about 20 amino acids, about 4 amino acids to about 18 amino acids, about 4 amino acids to about 16 amino acids, about 4 amino acids to about 14

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In some embodiments, the linker is rich in glycine (Gly or G) residues. In some embodiments, the linker is rich in serine (Ser or S) residues. In some embodiments, the linker is rich in glycine and serine residues. In some embodiments, the linker has one or more glycine-serine residue pairs (GS), e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GS pairs. In some embodiments, the linker has one or more Gly-Gly-Gly-Ser (GGGS) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGS sequences. In some embodiments, the linker has one or more Gly-Gly-Gly-Gly-Ser (GGGGS) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGGS sequences. In some embodiments, the linker has one or more Gly-Gly-Ser-Gly (GGSG) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGSG sequences.

In some embodiments, the linker sequence can comprise or consist of GGGGSGGGGSGGGGS (SEQ ID NO: 7). In some embodiments, the linker sequence can be encoded by a nucleic acid comprising or consisting of: GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT (SEQ ID

NO: 8). In some embodiments, the linker sequence can comprise or consist of: GGGSGGGS (SEQ ID NO: 9).

Target-Binding Domains

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and/or the additional one or more target-binding domains can be an antigen-binding domain (e.g., any of the exemplary antigen-binding domains described herein or known in the art), a soluble interleukin or cytokine protein (e.g., any of the exemplary soluble interleukin proteins or soluble cytokine proteins described herein), and a soluble interleukin or cytokine receptor (e.g., any of the exemplary soluble interleukin receptors or soluble cytokine receptors described herein).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary first target binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary second target binding domains described herein or known in the art), and the one or more additional target binding domains can each, independently, bind specifically to a target selected from the group of: bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3 (e.g., one or more of CD3α, CD3β, CD3δ, CD3ε, and CD3γ), CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein (e.g., ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6), HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF- β receptor II (TGF- β RII), a ligand of TGF- β RIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell

factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and/or the one or more additional target-binding domains can each independent have a total number of amino acids of about 5 amino acids to about 1000 amino acids, about 5 amino acids to about 950 amino acids, about 5 amino acids to about 900 amino acids, about 5 amino acids to about 850 amino acids, about 5 amino acids to about 800 amino acids, about 5 amino acids to about 750 amino acids, about 5 amino acids to about 700 amino acids, about 5 amino acids to about 650 amino acids, about 5 amino acids to about 600 amino acids, about 5 amino acids to about 550 amino acids, about 5 amino acids to about 500 amino acids, about 5 amino acids to about 450 amino acids, about 5 amino acids to about 400 amino acids, about 5 amino acids to about 350 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 280 amino acids, about 5 amino acids to about 260 amino acids, about 5 amino acids to about 240 amino acids, about 5 amino acids to about 220 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 195 amino acids, about 5 amino acids to about 190 amino acids, about 5 amino acids to about 185 amino acids, about 5 amino acids to about 180 amino acids, about 5 amino acids to about 175 amino acids, about 5 amino acids to about 170 amino acids, about 5 amino acids to about 165 amino acids, about 5 amino acids to about 160 amino acids, about 5 amino acids to about 155 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 145 amino acids, about 5 amino acids to about 140 amino acids, about 5 amino acids to about 135 amino acids, about 5 amino acids to about 130 amino acids, about 5 amino acids to about 125 amino acids, about 5 amino acids to about 120 amino acids, about 5 amino acids to about 115 amino acids, about 5 amino acids to about 110 amino acids, about 5 amino acids to about 105 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 95 amino acids, about 5 amino acids to about 90 amino acids, about 5 amino acids to about 85 amino acids, about 5 amino acids to about 80 amino acids, about 5 amino acids to about 75 amino acids, about 5 amino acids to about 70 amino acids, about 5

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Any of the target-binding domains described herein can bind to its target with a dissociation equilibrium constant (K_D) of less than 1 x 10^{-7} M, less than 1 x 10^{-8} M, less than 1 x 10^{-9} M, less than 1 x 10^{-10} M, less than 1 x 10^{-11} M, less than 1 x 10^{-12} M, or less than 1 x 10^{-13} M. In some embodiments, the antigen-binding protein construct provided herein can bind to an identifying antigen with a K_D of about 1 x 10^{-3} M to about 1 x 10^{-5} M, about 1 x 10^{-4} M to about 1 x 10^{-6} M, about 1 x 10^{-5} M to about 1 x 10^{-7} M, about 1 x 10^{-6} M to about 1 x 10^{-8} M, about 1 x 10^{-7} M to about 1 x 10^{-9} M, about 1 x 10^{-8} M to about 1 x 10^{-10} M, or about 1 x 10^{-9} M to about 1 x 10^{-11} M (inclusive).

Any of the target-binding domains described herein can bind to its target with a K_D of between about 1 pM to about 30 nM (e.g., about 1 pM to about 25 nM, about 1 pM to about 20 nM, about 1 pM to about 15 nM, about 1 pM to about 10 nM, about 1 pM to about 5 nM, about 1 pM to about 2 nM, about 1 pM to about 1 nM, about 1 pM to about 950 pM, about 1 pM to about 900 pM, about 1 pM to about 850 pM, about 1 pM to about 800 pM, about 1 pM to about 750 pM, about 1 pM to about 700 pM, about 1 pM to about 650 pM, about 1 pM to about 600 pM, about 1 pM to about 550 pM, about 1 pM to about 500 pM, about 1 pM to about 450 pM, about 1 pM to about 400 pM, about 1 pM to about 350 pM, about 1 pM to about 300 pM, about 1 pM to about 250 pM, about 1 pM to about 200 pM, about 1 pM to about 150 pM, about 1 pM to about 100 pM, about 1 pM to about 90 pM, about 1 pM to about 80 pM, about 1 pM to about 70 pM, about 1 pM to about 60 pM, about 1 pM to about 50 pM, about 1 pM to about 40 pM, about 1 pM to about 30 pM, about 1 pM to about 20 pM, about 1 pM to about 10 pM, about 1 pM to about 5 pM, about 1 pM to about 4 pM, about 1 pM to about 3 pM, about 1 pM to about 2 pM, about 2 pM to about 30 nM, about 2 pM to about 25 nM, about 2 pM to about 20 nM, about 2 pM to about 15 nM, about 2 pM to about 10 nM, about 2 pM to about 5 nM, about 2 pM to about 2 nM, about 2 pM to about 1 nM, about 2 pM to about 950 pM, about 2 pM to about 900 pM, about 2 pM to about 850 pM, about 2 pM to about 800 pM, about 2 pM to about 750 pM, about 2 pM to about 700 pM, about 2 pM to about 650 pM, about 2 pM to about 600 pM, about 2 pM to about 550 pM, about 2 pM to about 500 pM, about 2 pM to about 450 pM, about 2 pM to about 400 pM, about 2 pM to about 350 pM, about 2 pM to about 300 pM, about 2 pM to about 250 pM, about 2 pM to about 200 pM, about 2 pM to about 150 pM, about 2 pM to about 100 pM, about 2 pM to about 90 pM, about 2 pM to

about 80 pM, about 2 pM to about 70 pM, about 2 pM to about 60 pM, about 2 pM to about 50 pM, about 2 pM to about 40 pM, about 2 pM to about 30 pM, about 2 pM to about 20 pM, about 2 pM to about 10 pM, about 2 pM to about 5 pM, about 2 pM to about 4 pM, about 2 pM to about 3 pM, about 5 pM to about 30 nM, about 5 pM to about 25 nM, about 5 pM to about 20 nM, about 5 pM to about 15 nM, about 5 pM to about 10 nM, about 5 pM to about 5 nM, about 5 pM to about 2 nM, about 5 pM to about 1 nM, about 5 pM to about 950 pM, about 5 pM to about 900 pM, about 5 pM to about 850 pM, about 5 pM to about 800 pM, about 5 pM to about 750 pM, about 5 pM to about 700 pM, about 5 pM to about 650 pM, about 5 pM to about 600 pM, about 5 pM to about 550 pM, about 5 pM to about 500 pM, about 5 pM to about 450 pM, about 5 pM to about 400 pM, about 5 pM to about 350 pM, about 5 pM to about 300 pM, about 5 pM to about 250 pM, about 5 pM to about 200 pM, about 5 pM to about 150 pM, about 5 pM to about 100 pM, about 5 pM to about 90 pM, about 5 pM to about 80 pM, about 5 pM to about 70 pM, about 5 pM to about 60 pM, about 5 pM to about 50 pM, about 5 pM to about 40 pM, about 5 pM to about 30 pM, about 5 pM to about 20 pM, about 5 pM to about 10 pM, about 10 pM to about 30 nM, about 10 pM to about 25 nM, about 10 pM to about 20 nM, about 10 pM to about 15 nM, about 10 pM to about 10 nM, about 10 pM to about 5 nM, about 10 pM to about 2 nM, about 10 pM to about 1 nM, about 10 pM to about 950 pM, about 10 pM to about 900 pM, about 10 pM to about 850 pM, about 10 pM to about 800 pM, about 10 pM to about 750 pM, about 10 pM to about 700 pM, about 10 pM to about 650 pM, about 10 pM to about 600 pM, about 10 pM to about 550 pM, about 10 pM to about 500 pM, about 10 pM to about 450 pM, about 10 pM to about 400 pM, about 10 pM to about 350 pM, about 10 pM to about 300 pM, about 10 pM to about 250 pM, about 10 pM to about 200 pM, about 10 pM to about 150 pM, about 10 pM to about 100 pM, about 10 pM to about 90 pM, about 10 pM to about 80 pM, about 10 pM to about 70 pM, about 10 pM to about 60 pM, about 10 pM to about 50 pM, about 10 pM to about 40 pM, about 10 pM to about 30 pM, about 10 pM to about 20 pM, about 15 pM to about 30 nM, about 15 pM to about 25 nM, about 15 pM to about 20 nM, about 15 pM to about 15 nM, about 15 pM to about 10 nM, about 15 pM to about 5 nM, about 15 pM to about 2 nM, about 15 pM to about 1 nM, about 15 pM to about 950 pM, about 15 pM to about 900 pM, about 15 pM to about 850 pM, about 15 pM to about 800 pM, about 15 pM to about

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15 nM, about 400 pM to about 10 nM, about 400 pM to about 5 nM, about 400 pM to about 2 nM, about 400 pM to about 1 nM, about 400 pM to about 950 pM, about 400 pM to about 900 pM, about 400 pM to about 850 pM, about 400 pM to about 800 pM, about 400 pM to about 750 pM, about 400 pM to about 700 pM, about 400 pM to about 650 pM, about 400 pM to about 600 pM, about 400 pM to about 550 pM, about 400 pM to about 500 pM, about 500 pM to about 30 nM, about 500 pM to about 25 nM, about 500 pM to about 30 nM, about 500 pM to about 15 nM, about 500 pM to about 10 nM, about 500 pM to about 5 nM, about 500 pM to about 2 nM, about 500 pM to about 1 nM, about 500 pM to about 950 pM, about 500 pM to about 900 pM, about 500 pM to about 850 pM, about 500 pM to about 800 pM, about 500 pM to about 750 pM, about 500 pM to about 700 pM, about 500 pM to about 650 pM, about 500 pM to about 600 pM, about 500 pM to about 550 pM, about 600 pM to about 30 nM, about 600 pM to about 25 nM, about 600 pM to about 30 nM, about 600 pM to about 15 nM, about 600 pM to about 10 nM, about 600 pM to about 5 nM, about 600 pM to about 2 nM, about 600 pM to about 1 nM, about 600 pM to about 950 pM, about 600 pM to about 900 pM, about 600 pM to about 850 pM, about 600 pM to about 800 pM, about 600 pM to about 750 pM, about 600 pM to about 700 pM, about 600 pM to about 650 pM, about 700 pM to about 30 nM, about 700 pM to about 25 nM, about 700 pM to about 30 nM, about 700 pM to about 15 nM, about 700 pM to about 10 nM, about 700 pM to about 5 nM, about 700 pM to about 2 nM, about 700 pM to about 1 nM, about 700 pM to about 950 pM, about 700 pM to about 900 pM, about 700 pM to about 850 pM, about 700 pM to about 800 pM, about 700 pM to about 750 pM, about 800 pM to about 30 nM, about 800 pM to about 25 nM, about 800 pM to about 30 nM, about 800 pM to about 15 nM, about 800 pM to about 10 nM, about 800 pM to about 5 nM, about 800 pM to about 2 nM, about 800 pM to about 1 nM, about 800 pM to about 950 pM, about 800 pM to about 900 pM, about 800 pM to about 850 pM, about 900 pM to about 30 nM, about 900 pM to about 25 nM, about 900 pM to about 30 nM, about 900 pM to about 15 nM, about 900 pM to about 10 nM, about 900 pM to about 5 nM, about 900 pM to about 2 nM, about 900 pM to about 1 nM, about 900 pM to about 950 pM, about 1 nM to about 30 nM, about 1 nM to about 25 nM, about 1 nM to about 20 nM, about 1 nM to about 15 nM, about 1 nM to about 10 nM, about 1 nM to about 5 nM, about 2 nM to about 30 nM, about 2 nM to about 25 nM, about 2 nM

to about 20 nM, about 2 nM to about 15 nM, about 2 nM to about 10 nM, about 2 nM to about 5 nM, about 4 nM to about 30 nM, about 4 nM to about 25 nM, about 4 nM to about 20 nM, about 4 nM to about 15 nM, about 4 nM to about 10 nM, about 4 nM to about 5 nM, about 5 nM to about 30 nM, about 5 nM to about 25 nM, about 5 nM to about 20 nM, about 5 nM to about 15 nM, about 5 nM to about 10 nM, about 10 nM to about 30 nM, about 10 nM to about 25 nM, about 10 nM to about 20 nM, about 10 nM to about 15 nM, about 15 nM to about 30 nM, about 15 nM to about 25 nM, about 15 nM to about 20 nM, about 20 nM to about 30 nM, and about 20 nM to about 25 nM).

Any of the target-binding domains described herein can bind to its target with a K_D of between about 1 nM to about 10 nM (e.g., about 1 nM to about 9 nM, about 1 nM to about 8 nM, about 1 nM to about 7 nM, about 1 nM to about 6 nM, about 1 nM to about 5 nM, about 1 nM to about 4 nM, about 1 nM to about 3 nM, about 1 nM to about 2 nM, about 2 nM to about 10 nM, about 2 nM to about 9 nM, about 2 nM to about 8 nM, about 2 nM to about 7 nM, about 2 nM to about 6 nM, about 2 nM to about 5 nM, about 2 nM to about 4 nM, about 2 nM to about 3 nM, about 3 nM to about 10 nM, about 3 nM to about 9 nM, about 3 nM to about 8 nM, about 3 nM to about 7 nM, about 3 nM to about 6 nM, about 3 nM to about 5 nM, about 3 nM to about 4 nM, about 4 nM to about 10 nM, about 4 nM to about 9 nM, about 4 nM to about 8 nM, about 4 nM to about 7 nM, about 4 nM to about 6 nM, about 4 nM to about 5 nM, about 5 nM to about 10 nM, about 5 nM to about 9 nM, about 5 nM to about 8 nM, about 5 nM to about 7 nM, about 5 nM to about 6 nM, about 6 nM to about 10 nM, about 6 nM to about 9 nM, about 6 nM to about 8 nM, about 6 nM to about 7 nM, about 7 nM to about 10 nM, about 7 nM to about 9 nM, about 7 nM to about 8 nM, about 8 nM to about 10 nM, about 8 nM to about 9 nM, and about 9 nM to about 10 nM).

A variety of different methods known in the art can be used to determine the K_D values of any of the antigen-binding protein constructs described herein (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

Antigen-Binding Domains

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the antigen-binding domain includes or is a scFv or a single domain antibody (e.g., a VHH or a VNAR domain).

In some examples, an antigen-binding domain (e.g., any of the antigen-binding domains described herein) can bind specifically to any one of CD16a (see, e.g., those described in U.S. Patent No. 9,035,026), CD28 (see, e.g., those described in U.S. Patent No. 7,723,482), CD3 (see, e.g., those described in U.S. Patent No. 9,226,962), CD33 (see, e.g., those described in U.S. Patent No. 8,759,494), CD20 (see, e.g., those described in WO 2014/026054), CD19 (see, e.g., those described in U.S. Patent No. 9,701,758), CD22 (see, e.g., those described in WO 2003/104425), CD123 (see, e.g., those described in WO 2014/130635), IL-1R (see, e.g., those described in U.S. Patent No. 8,741,604), IL-1 (see, e.g., those described in WO 2014/095808), VEGF (see, e.g., those described in U.S. Patent No. 9,090,684), IL-6R (see, e.g., those described in U.S. Patent No. 7,482,436), IL-4 (see, e.g., those described in U.S. Patent Application Publication No. 2012/0171197), IL-10 (see, e.g., those described in U.S. Patent Application Publication

No. 2016/0340413), PDL-1 (see, e.g., those described in Drees et al., *Protein Express. Purif.* 94:60-66, 2014), TIGIT (see, e.g., those described in U.S. Patent Application Publication No. 2017/0198042), PD-1 (see, e.g., those described in U.S. Patent No. 7,488,802), TIM3 (see, e.g., those described in U.S. Patent No. 8,552,156), CTLA4 (see, e.g., those described in WO 2012/120125), MICA (see, e.g., those described in WO 2016/154585), MICB (see, e.g., those described in U.S. Patent No. 8,753,640), IL-6 (see, e.g., those described in Gejima et al., *Human Antibodies* 11(4):121-129, 2002), IL-8 (see, e.g., those described in U.S. Patent No. 6,117,980), TNFα (see, e.g., those described in Geng et al., *Immunol. Res.* 62(3):377-385, 2015), CD26a (see, e.g., those described in WO 2017/189526), CD36 (see, e.g., those described in U.S. Patent Application Publication No. 2015/0259429), ULBP2 (see, e.g., those described in U.S. Patent No. 9,273,136), CD30 (see, e.g., those described in Homach et al., *Scand. J. Immunol.* 48(5):497-501, 1998), CD200 (see, e.g., those described in U.S. Patent No. 9,085,623), IGF-1R (see, e.g., those described in U.S. Patent Application Publication No. 2017/0051063), MUC4AC (see, e.g., those described in WO 2012/170470), MUC5AC (see, e.g., those described in U.S. Patent No. 9,238,084), Trop-2 (see, e.g., those described in WO 2013/068946), CMET (see, e.g., those described in Edwardraja et al., *Biotechnol. Bioeng.* 106(3):367-375, 2010), EGFR (see, e.g., those described in Akbari et al., *Protein Expr. Purif.* 127:8-15, 2016), HER1 (see, e.g., those described in U.S. Patent Application Publication No. 2013/0274446), HER2 (see, e.g., those described in Cao et al., *Biotechnol. Lett.* 37(7):1347-1354, 2015), HER3 (see, e.g., those described in U.S. Patent No. 9,505,843), PSMA (see, e.g., those described in Parker et al., *Protein Expr. Purif.* 89(2):136-145, 2013), CEA (see, e.g., those described in WO 1995/015341), B7H3 (see, e.g., those described in U.S. Patent No. 9,371,395), EPCAM (see, e.g., those described in WO 2014/159531), BCMA (see, e.g., those described in Smith et al., *Mol. Ther.* 26(6):1447-1456, 2018), P-cadherin (see, e.g., those described in U.S. Patent No. 7,452,537), CEACAM5 (see, e.g., those described in U.S. Patent No. 9,617,345), a UL16-binding protein (see, e.g., those described in WO 2017/083612), HLA-DR (see, e.g., Pistillo et al., *Exp. Clin. Immunogenet.* 14(2):123-130, 1997), DLL4 (see, e.g., those described in WO 2014/007513), TYRO3 (see, e.g., those described in WO 2016/166348), AXL (see, e.g., those described in WO 2012/175692), MER (see, e.g., those described in

WO 2016/106221), CD122 (see, e.g., those described in U.S. Patent Application Publication No. 2016/0367664), CD155 (see, e.g., those described in WO 2017/149538), or PDGF-DD (see, e.g., those described in U.S. Patent No. 9,441,034).

The antigen-binding domains present in any of the multi-chain chimeric polypeptides described herein are each independently selected from the group consisting of: a VHH domain, a VNAR domain, and a scFv. In some embodiments, any of the antigen-binding domains described herein is a BiTe, a $(scFv)_2$, a nanobody, a nanobody-HSA, a DART, a TandAb, a scDiabody, a scDiabody-CH3, scFv-CH-CL-scFv, a HSAbody, scDiabody-HAS, or a tandem-scFv. Additional examples of antigen-binding domains that can be used in any of the multi-chain chimeric polypeptide are known in the art.

A VHH domain is a single monomeric variable antibody domain that can be found in camelids. A VNAR domain is a single monomeric variable antibody domain that can be found in cartilaginous fish. Non-limiting aspects of VHH domains and V_{NAR} domains are described in, e.g., Cromie et al., *Curr. Top. Med. Chem.* 15:2543-2557, 2016; De Genst et al., *Dev. Comp. Immunol.* 30:187-198, 2006; De Meyer et al., *Trends Biotechnol.* 32:263-270, 2014; Kijanka et al., *Nanomedicine* 10:161-174, 2015; Kovaleva et al., *Expert. Opin. Biol. Ther.* 14:1527-1539, 2014; Krah et al., *Immunopharmacol. Immunotoxicol.* 38:21-28, 2016; Mujic-Delic et al., *Trends Pharmacol. Sci.* 35:247-255, 2014; Muyldermans, *J. Biotechnol.* 74:277-302, 2001; Muyldermans et al., *Trends Biochem. Sci.* 26:230-235, 2001; Muyldermans, *Ann. Rev. Biochem.* 82:775-797, 2013; Rahbarizadeh et al., *Immunol. Invest.* 40:299-338, 2011; Van Audenhove et al., *EBioMedicine* 8:40-48, 2016; Van Bockstaele et al., *Curr. Opin. Investig. Drugs* 10:1212-1224, 2009; Vincke et al., *Methods Mol. Biol.* 911:15-26, 2012; and Wesolowski et al., *Med. Microbiol. Immunol.* 198:157-174, 2009.

In some embodiments, each of the antigen-binding domains in the multi-chain chimeric polypeptides described herein are both VHH domains, or at least one antigen-binding domain is a VHH domain. In some embodiments, each of the antigen-binding domains in the multi-chain chimeric polypeptides described herein are both VNAR domains, or at least one antigen-binding domain is a VNAR domain. In some embodiments, each of the antigen-binding domains in the multi-chain chimeric

polypeptides described herein are both scFv domains, or at least one antigen-binding domain is a scFv domain.

In some embodiments, two or more of polypeptides present in the multi-chain chimeric polypeptide can assemble (e.g., non-covalently assemble) to form any of the antigen-binding domains described herein, e.g., an antigen-binding fragment of an antibody (e.g., any of the antigen-binding fragments of an antibody described herein), a VHH-scAb, a VHH-Fab, a Dual scFab, a F(ab')2, a diabody, a crossMab, a DAF (two-in-one), a DAF (four-in-one), a DutaMab, a DT-IgG, a knobs-in-holes common light chain, a knobs-in-holes assembly, a charge pair, a Fab-arm exchange, a SEEDbody, a LUZ-Y, a Fcab, a κλ-body, an orthogonal Fab, a DVD-IgG, a IgG(H)-scFv, a scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, DVI-IgG, Diabody-CH3, a triple body, a miniantibody, a minibody, a TriBi minibody, scFv-CH3 KIH, Fab-scFv, a F(ab')2-scFv2, a scFv-KIH, a Fab-scFv-Fc, a tetravalent HCAb, a scDiabody-Fc, a Diabody-Fc, a tandem scFv-Fc, an Intrabody, a dock and lock, a lmmTAC, an IgG-IgG conjugate, a Cov-X-Body, and a scFv1-PEG-scFv2. See, e.g., Spiess et al., *Mol. Immunol.* 67:95-106, 2015, incorporated in its entirety herewith, for a description of these elements. Non-limiting examples of an antigen-binding fragment of an antibody include an Fv fragment, a Fab fragment, a $F(ab')_2$ fragment, and a Fab' fragment. Additional examples of an antigen-binding fragment of an antibody is an antigen-binding fragment of an IgG (e.g., an antigen-binding fragment of IgG1, IgG2, IgG3, or IgG4) (e.g., an antigen-binding fragment of a human or humanized IgG, e.g., human or humanized IgG1, IgG2, IgG3, or IgG4); an antigen-binding fragment of an IgA (e.g., an antigen-binding fragment of IgA1 or IgA2) (e.g., an antigen-binding fragment of a human or humanized IgA, e.g., a human or humanized IgA1 or IgA2); an antigen-binding fragment of an IgD (e.g., an antigen-binding fragment of a human or humanized IgD); an antigen-binding fragment of an IgE (e.g., an antigen-binding fragment of a human or humanized IgE); or an antigen-binding fragment of an IgM (e.g., an antigen-binding fragment of a human or humanized IgM).

An "Fv" fragment includes a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

A "Fab" fragment includes, the constant domain of the light chain and the first constant domain (C_{H1}) of the heavy chain, in addition to the heavy and light chain variable domains of the Fv fragment.

A "F(ab')$_2$" fragment includes two Fab fragments joined, near the hinge region, by disulfide bonds.

A "dual variable domain immunoglobulin" or "DVD-Ig" refers to multivalent and multispecific binding proteins as described, e.g., in DiGiammarino et al., *Methods Mol. Biol.* 899:145-156, 2012; Jakob et al., *MABs* 5:358-363, 2013; and U.S. Patent Nos. 7,612,181; 8,258,268; 8,586,714; 8,716,450; 8,722,855; 8,735,546; and 8,822,645, each of which is incorporated by reference in its entirety.

DARTs are described in, e.g., Garber, *Nature Reviews Drug Discovery* 13:799-801, 2014.

In some embodiments of any of the antigen-binding domains described herein can bind to an antigen selected from the group consisting of: a protein, a carbohydrate, a lipid, and a combination thereof.

Additional examples and aspects of antigen-binding domains are known in the art.

Soluble Interleukin or Cytokine Protein

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain can be a soluble interleukin protein or soluble cytokine protein. In some embodiments, the soluble interleukin or soluble cytokine protein is selected from the group of: IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L. Non-limiting examples of soluble IL-2, IL-3, IL-7, IL-8, IL-10, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3Lare provided below.

Human Soluble IL-2 (SEQ ID NO: 9)

aptssstkkt qlqlehllld lqmilnginn yknpkltrml tfkfympkka
telkhlqcle eelkpleevl nlaqsknfhl rprdlisnin vivlelkgse
ttfmceyade tativeflnr witfcqsiis tlt

Human Soluble IL-3 (SEQ ID NO: 10)

apmtqttplkt swvncsnmid eiithlkqpp lplldfnnln gedqdilmen nlrrpnleaf nravkslqna saiesilknl lpclplataa ptrhpihikd gdwnefrrkl tfylktlena qaqqttlsla if

Human Soluble IL-7 (SEQ ID NO: 11)

dcdiegkdgkqyesv lmvsidqlld smkeigsncl nnefnffkrh icdankegmf lfraarklrq flkmnstgdf dlhllkvseg ttillnctgq vkgrkpaalg eaqptkslee nkslkeqkkl ndlcflkrll qeiktcwnki lmgtkeh

Human Soluble IL-8 (SEQ ID NO: 12)

egavlprsak elrcqcikty skpfhpkfik elrviesgph canteiivkl sdgrelcldp kenwvqrvve kflkraens

Human Soluble IL-10 (SEQ ID NO: 13)

spgqgtqsensc thfpgnlpnm lrdlrdafsr vktffqmkdq ldnlllkesl ledfkgylgc qalsemiqfy leevmpqaen qdpdikahvn slgenlktlr lrlrrchrfl pcenkskave qvknafnklq ekgiykamse fdifinyiea ymtmkirn

Human Soluble IL-15 (SEQ ID NO: 14)

Nwvnvisdlkki edliqsmhid atlytesdvh psckvtamkc fllelqvisl esgdasihdt venliilann slssngnvte sgckeceele eknikeflqs fvhivqmfin ts

Human Soluble IL-17 (SEQ ID NO: 15)

gitiprn pgcpnsedkn fprtvmvnln ihnrntntnp krssdyynrs tspwnlhrne dperypsviw eakcrhlgci nadgnvdyhm nsvpiqqeil vlrrepphcp nsfrlekilv svgctcvtpi vhhva

Human Soluble IL-18 (SEQ ID NO: 16)

yfgklesklsvirn lndqvlfidq gnrplfedmt dsdcrdnapr tifiismykd
sqprgmavti svkcekistl scenkiisfk emnppdnikd tksdiiffqr
svpghdnkmq fesssyegyf lacekerdlf klilkkedel gdrsimftvq ned

Human Soluble PDGF-DD (SEQ ID NO: 17)

rdtsatpqsasi kalrnanlrr desnhltdly rrdetiqvkg ngyvqsprfp
nsyprnlllt wrlhsqentr iqlvfdnqfg leeaendicr ydfvevedis
etstiirgrw cghkevppri ksrtnqikit fksddyfvak pgfkiyysll
edfqpaaase tnwesvtssi sgvsynspsv tdptliadal dkkiaefdtv
edllkyfnpe swqedlenmy ldtpryrgrs yhdrkskvdl drlnddakry
sctprnysvn ireelklanv vffprcllvq rcggncgcgt vnwrsctcns
gktvkkyhev lqfepghikr rgraktmalv diqldhherc dcicssrppr

Human Soluble SCF (SEQ ID NO: 18)

egicrnrvtnnvkdv tklvanlpkd ymitlkyvpg mdvlpshcwi semvvqlsds
ltdlldkfsn iseglsnysi idklvnivdd lvecvkenss kdlkksfksp
eprlftpeef frifnrsida fkdfvvaset sdcvvsstls pekdsrvsvt
kpfmlppvaa sslrndssss nrkaknppgd sslhwaamal palfsliigf
afgalywkkr qpsltraven iqineednei smlqekeref qev

Human Soluble FLT3L (SEQ ID NO: 19)

tqdcsfqhspissd favkirelsd yllqdypvtv asnlqdeelc gglwrlvlaq
rwmerlktva gskmqgller vnteihfvtk cafqpppscl rfvqtnisrl
lqetseqlva lkpwitrqnf srclelqcqp dsstlpppws prpleatapt
apqpplllll llpvglllla aawclhwqrt rrrtprpgeq vppvpspqdl
llveh

Non-limiting examples of soluble MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6 are provided below.

Human Soluble MICA (SEQ ID NO: 20)

ephslry nltvlswdgs vqsgfltevh ldgqpflrcd rqkcrakpqg
qwaedvlgnk twdretrdlt gngkdlrmtl ahikdqkegl hslqeirvce
ihednstrss qhfyydgelf lsqnletkew tmpqssraqt lamnvrnflk
edamktkthy hamhadclqe lrrylksgvv lrrtvppmvn vtrseasegn
itvtcrasgf ypwnitlswr qdgvslshdt qqwgdvlpdg ngtyqtwvat
ricqgeeqrf tcymehsgnh sthpvpsgkv lvlqshwqtf hvsavaaaai
fviiifyvrc ckkktsaaeg pelvslqvld qhpvgtsdhr datqlgfqpl
msdlgstgst ega

Human Soluble MICB (SEQ ID NO: 21)

aephslry nlmvlsqdes vqsgflaegh ldgqpflryd rqkrrakpqg
qwaedvlgak twdtetedlt engqdlrrtl thikdqkggl hslqeirvce
ihedsstrgs rhfyydgelf lsqnletqes tvpqssraqt lamnvtnfwk
edamktkthy ramqadclqk lqrylksgva irrtvppmvn vtcsevsegn
itvtcrassf yprnitltwr qdgvslshnt qqwgdvlpdg ngtyqtwvat
rirqgeeqrf tcymehsgnh gthpvpsgkv lvlqsqrtdf pyvsaampcf
viiiilcvpc ckkktsaaeg pelvslqvld qhpvgtgdhr
daaqlgfqpl msatgstgst ega

Human Soluble ULBP1 (SEQ ID NO: 22)

wvdthclcydfiit pksrpepqwc evqglvderp flhydcvnhk akafaslgkk
vnvtktweeq tetlrdvvdf lkgqlldiqv enlipieplt lqarmscehe
ahghgrgswq flfngqkfll fdsnnrkwta lhpgakkmte kweknrdvtm
ffqkislgdc kmwleeflmy weqmldptkp pslapg

Human Soluble ULBP2 (SEQ ID NO: 23)

gradphslcyditvi pkfrpgprwc avqgqvdekt flhydcgnkt vtpvsplgkk
lnvttawkaq npvlrevvdi lteqlrdiql enytpkeplt lqarmsceqk
aeghssgswq fsfdgqifll fdsekrmwtt vhpgarkmke kwendkvvam
sfhyfsmgdc igwledflmg mdstlepsag aplams

Human Soluble ULBP3 (SEQ ID NO: 24)

dahslwynfti ihlprhgqqw cevqsqvdqk nflsydcgsd kvlsmghlee
qlyatdawgk qlemlrevgq rlrleladte ledftpsgpl tlqvrmscec
eadgyirgsw qfsfdgrkfl lfdsnnrkwt vvhagarrmk ekwekdsglt
tffkmvsmrd ckswlrdflm hrkkrlepta pptmapg

Human Soluble ULBP4 (SEQ ID NO: 25)

hslcfnftik slsrpgqpwc eaqvflnknl flqynsdnnm vkplgllgkk
vyatstwgel tqtlgevgrd lrmllcdikp qiktsdpstl qvemfcqrea
erctgaswqf atngeksllf damnmtwtvi nheaskiket wkkdrgleky
frklskgdcd hwlreflghw eampeptvsp vnasdihwss sslpdrwiil
gafillvlmg ivlicvwwqn gewqaglwpl rts

Human Soluble ULBP5 (SEQ ID NO: 26)

gladp hslcyditvi pkfrpgprwc avqgqvdekt flhydcgskt
vtpvsplgkk lnvttawkaq npvlrevvdi lteqlldiql enyipkeplt
lqarmsceqk aeghgsgswq lsfdgqifll fdsenrmwtt vhpgarkmke
kwendkdmtm sfhyismgdc tgwledflmg mdstlepsag apptmssg

Human Soluble ULBP6 (SEQ ID NO: 27)

rrddp hslcyditvi pkfrpgprwc avqgqvdekt flhydcgnkt
vtpvsplgkk lnvtmawkaq npvlrevvdi lteqlldiql enytpkeplt
lqarmsceqk aeghssgswq fsidgqtfll fdsekrmwtt vhpgarkmke
kwendkdvam sfhyismgdc igwledflmg mdstlepsag aplamssg

Additional examples of soluble interleukin proteins and soluble cytokine proteins are known in the art.

Soluble Receptor

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding

domain is a soluble interleukin receptor, a soluble cytokine receptor or a ligand receptor. In some embodiments, the soluble receptor is a soluble TGF-β receptor II (TGF-β RII) (see, e.g., those described in Yung et al., *Am. J. Resp. Crit. Care Med.* 194(9):1140-1151, 2016), a soluble TGF-βRIII (see, e.g., those described in Heng et al., *Placenta* 57:320, 2017), a soluble NKG2D (see, e.g., Cosman et al., *Immunity* 14(2):123-133, 2001; Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble NKp30 (see, e.g., Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble NKp44 (see, e.g., those described in Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble NKp46 (see, e.g., Mandelboim et al., *Nature* 409:1055-1060, 2001; Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble DNAM-1 (see, e.g., those described in Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a scMHCI (see, e.g., those described in Washburn et al., *PLoS One* 6(3):e18439, 2011), a scMHCII (see, e.g., those described in Bishwajit et al., *Cellular Immunol.* 170(1):25-33, 1996), a scTCR (see, e.g., those described in Weber et al., *Nature* 356(6372):793-796, 1992), a soluble CD155 (see, e.g., those described in Tahara-Hanaoka et al., *Int. Immunol.* 16(4):533-538, 2004), or a soluble CD28 (see, e.g., Hebbar et al., *Clin. Exp. Immunol.* 136:388-392, 2004).

Additional examples of soluble interleukin receptors and soluble cytokine receptors are known in the art.

Additional Antigen-Binding Domains

In some embodiments of any of the multi-chain chimeric polypeptides, the first chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described

herein). In some embodiments, the first chimeric polypeptide can further include a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the at least one of the one or more additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art), and/or a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein). In some embodiments, the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein

or known in the art) between the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the linker sequences described herein or known in the art) disposed between the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first

target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) disposed between the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the first domains described herein or any of the exemplary pairs of affinity domains described herein), directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) disposed (i) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein), and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the N-terminal end and/or the C-terminal end of the second chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the second domain of the pair of affinity domains (e.g., any of the second domains described herein of any of the exemplary pairs of affinity domains described herein) in the second chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the second target-binding domain (e.g., any of the target-binding domains described herein or known in the art) in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between at least one of the one or more additional target-binding domains (e.g., any of the exemplary target binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target binding domains described herein or known in the art) in the second chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more (e.g., three or

more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the same amino acid sequence. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens. In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain (e.g., a scFv or a single-domain antibody).

Pairs of Affinity Domains

In some embodiments, a multi-chain chimeric polypeptide includes: 1) a first chimeric polypeptide that includes a first domain of a pair of affinity domains, and 2) a second chimeric polypeptide that includes a second domain of a pair of affinity domains such that the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity

domains. In some embodiments, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15. A sushi domain, also known as a short consensus repeat or type 1 glycoprotein motif, is a common motif in protein-protein interaction. Sushi domains have been identified on a number of protein-binding molecules, including complement components C1r, C1s, factor H, and C2m, as well as the nonimmunologic molecules factor XIII and β2-glycoprotein. A typical Sushi domain has approximately 60 amino acid residues and contains four cysteines (Ranganathan, Pac. Symp Biocomput. 2000:155-67). The first cysteine can form a disulfide bond with the third cysteine, and the second cysteine can form a disulfide bridge with the fourth cysteine. In some embodiments in which one member of the pair of affinity domains is a soluble IL-15, the soluble IL15 has a D8N or D8A amino acid substitution. In some embodiments in which one member of the pair of affinity domains is an alpha chain of human IL-15 receptor (IL15Rα), the human IL15Rα is a mature full-length IL15Rα. In some embodiments, the pair of affinity domains is barnase and barnstar. In some embodiments, the pair of affinity domains is a PKA and an AKAP. In some embodiments, the pair of affinity domains is an adapter/docking tag module based on mutated RNase I fragments (Rossi, *Proc Natl Acad Sci USA*. 103:6841-6846, 2006; Sharkey et al., *Cancer Res*. 68:5282-5290, 2008; Rossi et al., *Trends Pharmacol Sci*. 33:474-481, 2012) or SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25 (Deyev et al., *Nat Biotechnol*. 1486-1492, 2003).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a dissociation equilibrium constant (K_D) of less than 1×10^{-7} M, less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, less than 1×10^{-11} M, less than 1×10^{-12} M, or less than 1×10^{-13} M. In some embodiments, the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a K_D of about 1×10^{-4} M to about 1×10^{-6} M, about 1×10^{-5} M to about 1×10^{-7} M, about 1×10^{-6} M to about 1×10^{-8}

M, about 1 x 10^{-7} M to about 1 x 10^{-9} M, about 1 x 10^{-8} M to about 1 x 10^{-10} M, about 1 x 10^{-9} M to about 1 x 10^{-11} M, about 1 x 10^{-10} M to about 1 x 10^{-12} M, about 1 x 10^{-11} M to about 1 x 10^{-13} M, about 1 x 10^{-4} M to about 1 x 10^{-5} M, about 1 x 10^{-5} M to about 1 x 10^{-6} M, about 1 x 10^{-6} M to about 1 x 10^{-7} M, about 1 x 10^{-7} M to about 1 x 10^{-8} M, about 1 x 10^{-8} M to about 1 x 10^{-9} M, about 1 x 10^{-9} M to about 1 x 10^{-10} M, about 1 x 10^{-10} M to about 1 x 10^{-11} M, about 1 x 10^{-11} M to about 1 x 10^{-12} M, or about 1 x 10^{-12} M to about 1 x 10^{-13} M (inclusive). Any of a variety of different methods known in the art can be used to determine the K_D value of the binding of the first domain of the pair of affinity domains and the second domain of the pair of affinity domains (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains, the second domain of the pair of affinity domains, or both is about 10 to 100 amino acids in length. For example, a first domain of a pair of affinity domains, a second domain of a pair of affinity domains, or both can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about

10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a first domain of a pair of affinity domains, a second domain of a pair of affinity domains, or both is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the first and/or second domains of a pair of affinity domains disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the first and/or second domains of a pair of affinity domains remains intact. For example, a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a soluble IL-15. Additionally or alternatively, a soluble IL-15 can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα).

A non-limiting example of a sushi domain from an alpha chain of IL-15 receptor alpha (IL15Rα) can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH WTTPSLKCIR (SEQ ID NO: 28). In some embodiments, a sushi domain from an alpha chain of IL15Rα can be encoded by a nucleic acid including ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAG CTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGA AGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGT GGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 29).

In some embodiments, a soluble IL-15 can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINT S (SEQ ID NO: 14). In some embodiments, a soluble IL-15 can be encoded by a nucleic acid including the sequence of AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTC CATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAA GGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAG CGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATA ACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGA AGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTG TCCAGATGTTCATCAATACCTCC (SEQ ID NO: 30).

Signal Sequence

In some embodiments, a multi-chain chimeric polypeptide includes a first chimeric polypeptide that includes a signal sequence at its N-terminal end. In some embodiments, a multi-chain chimeric polypeptide includes a second chimeric polypeptide that includes a signal sequence at its N-terminal end. In some embodiments, both the first chimeric polypeptide of a multi-chain chimeric polypeptide and a second chimeric polypeptide of the multi-chain chimeric polypeptide include a signal sequence. As will be understood by those of ordinary skill in the art, a signal sequence is an amino acid sequence that is present at the N-terminus of a number of endogenously produced proteins that directs the protein to the secretory pathway (e.g., the protein is directed to reside in certain intracellular organelles, to reside in the cell membrane, or to be secreted from the cell). Signal sequences are heterogeneous and differ greatly in their primary amino acid sequences. However, signal sequences are typically 16 to 30 amino acids in length and include a hydrophilic, usually positively charged N-terminal region, a central hydrophobic domain, and a C-terminal region that contains the cleavage site for signal peptidase.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MKWVTFISLLFLFSSAYS (SEQ ID NO: 31). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence encoded by the nucleic acid sequence ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC (SEQ ID NO: 32), ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCTACAGC (SEQ ID NO: 33), or ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC (SEQ ID NO: 34).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 35). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MGQIVTMFEALPHIIDEVINIVIIVLIIITSIKAVYNFATCGILALVSFLFLAGRSCG (SEQ ID NO: 36). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MPNHQSGSPTGSSDLLLSGKKQRPHLALRRKRRREMRKINRKVRRMNLAPIKEK TAWQHLQALISEAEEVLKTSQTPQNSLTLFLALLSVLGPPVTG (SEQ ID NO: 37). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS (SEQ ID NO: 38). Those of ordinary skill in the art will be aware of other appropriate signal sequences for use in a first

chimeric polypeptide and/or a second chimeric polypeptide of multi-chain chimeric polypeptides described herein.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence that is about 10 to 100 amino acids in length. For example, a signal sequence can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a signal sequence is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the signal sequences disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the signal sequence remains

intact. For example, a signal sequence having the amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 35) can include one or more additional amino acids at the N-terminus or C-terminus, while still retaining the ability to direct a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both to the secretory pathway.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence that directs the multi-chain chimeric polypeptide into the extracellular space. Such embodiments are useful in producing multi-chain chimeric polypeptides that are relatively easy to be isolated and/or purified.

Peptide Tags

In some embodiments, a multi-chain chimeric polypeptide includes a first chimeric polypeptide that includes a peptide tag (e.g., at the N-terminal end or the C-terminal end of the first chimeric polypeptide). In some embodiments, a multi-chain chimeric polypeptide includes a second chimeric polypeptide that includes a peptide tag (e.g., at the N-terminal end or the C-terminal end of the second chimeric polypeptide). In some embodiments, both the first chimeric polypeptide of a multi-chain chimeric polypeptide and a second chimeric polypeptide of the multi-chain chimeric polypeptide include a peptide tag. In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both include two or more peptide tags.

Exemplary peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both include, without limitation, AviTag (GLNDIFEAQKIEWHE; SEQ ID NO: 39), a calmodulin-tag (KRRWKKNFIAVSAANRFKKISSSGAL; SEQ ID NO: 40), a polyglutamate tag (EEEEEE; SEQ ID NO: 41), an E-tag (GAPVPYPDPLEPR; SEQ ID NO: 42), a FLAG-tag (DYKDDDDK; SEQ ID NO: 43), an HA-tag, a peptide from hemagglutinin (YPYDVPDYA; SEQ ID NO: 44), a his-tag (HHHHH (SEQ ID NO: 45); HHHHHH (SEQ ID NO: 46); HHHHHHH (SEQ ID NO: 47); HHHHHHHH (SEQ ID NO: 48);

HHHHHHHHH (SEQ ID NO: 49); or HHHHHHHHHH (SEQ ID NO: 50)), a myc-tag (EQKLISEEDL; SEQ ID NO: 51), NE-tag (TKENPRSNQEESYDDNES; SEQ ID NO: 52), S-tag, (KETAAAKFERQHMDS; SEQ ID NO: 53), SBP-tag (MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP; SEQ ID NO: 54), Softag 1 (SLAELLNAGLGGS; SEQ ID NO: 55), Softag 3 (TQDPSRVG; SEQ ID NO: 56), Spot-tag (PDRVRAVSHWSS; SEQ ID NO: 57), Strep-tag (WSHPQFEK; SEQ ID NO: 58), TC tag (CCPGCC; SEQ ID NO: 59), Ty tag (EVHTNQDPLD; SEQ ID NO: 60), V5 tag (GKPIPNPLLGLDST; SEQ ID NO: 61), VSV-tag (YTDIEMNRLGK; SEQ ID NO: 62), and Xpress tag (DLYDDDDK; SEQ ID NO: 63). In some embodiments, tissue factor protein is a peptide tag.

Peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both can be used in any of a variety of applications related to the multi-chain chimeric polypeptide. For example, a peptide tag can be used in the purification of a multi-chain chimeric polypeptide. As one non-limiting example, a first chimeric polypeptide of a multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both can include a myc tag; the multi-chain chimeric polypeptide that includes the myc-tagged first chimeric polypeptide, the myc-tagged second chimeric polypeptide, or both can be purified using an antibody that recognizes the myc tag(s). One non-limiting example of an antibody that recognizes a myc tag is 9E10, available from the non-commercial Developmental Studies Hybridoma Bank. As another non-limiting example, a first chimeric polypeptide of a multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both can include a histidine tag; the multi-chain chimeric polypeptide that includes the histidine-tagged first chimeric polypeptide, the histidine-tagged second chimeric polypeptide, or both can be purified using a nickel or cobalt chelate. Those of ordinary skill in the art will be aware of other suitable tags and agent that bind those tags for use in purifying multi-chain chimeric polypeptide. In some embodiments, a peptide tag is

removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the multi-chain chimeric polypeptide after purification. In some embodiments, a peptide tag is not removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the multi-chain chimeric polypeptide after purification.

Peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both can be used, for example, in immunoprecipitation of the multi-chain chimeric polypeptide, imaging of the multi-chain chimeric polypeptide (e.g., via Western blotting, ELISA, flow cytometry, and/or immunocytochemistry), and/or solubilization of the multi-chain chimeric polypeptide.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a peptide tag that is about 10 to 100 amino acids in length. For example, a peptide tag can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in

length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a peptide tag is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

Peptide tags included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both can be of any suitable length. For example, peptide tags can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids in length. In embodiments in which a multi-chain chimeric polypeptide includes two or more peptide tags, the two or more peptide tags can be of the same or different lengths. In some embodiments, any of the peptide tags disclosed herein may include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at the N-terminus and/or C-terminus, so long as the function of the peptide tag remains intact. For example, a myc tag having the amino acid sequence EQKLISEEDL (SEQ ID NO: 64) can include one or more additional amino acids (e.g., at the N-terminus and/or the C- terminus of the peptide tag), while still retaining the ability to be bound by an antibody (e.g., 9E10).

Exemplary Multi-Chain Chimeric Polypeptides- Type A

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-18 or a receptor of IL-12. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-

chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-18 or a receptor of IL-12. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-12, and the second target-binding domain binds specifically to a receptor for IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a

receptor for IL-18, and the second target-binding domain bind specifically to a receptor for IL-12.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-18 (e.g., a soluble human IL-18).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 16).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT (SEQ ID NO: 65).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble IL-12 (e.g., a soluble human IL-12). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-15 includes a sequence of soluble human IL-12β (p40) and a sequence of soluble human IL-12α (p35). In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-15 human IL-15 further includes a linker sequence (e.g., any of the exemplary linker sequences described herein) between the sequence of soluble IL-12β (p40) and the

sequence of soluble human IL-12α (p35). In some examples of these multi-chain chimeric polypeptides, the linker sequence comprises GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 66).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12β (p40) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCC (SEQ ID NO: 67).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKD
KTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKM
YQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPD
FYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 68).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTACACCA
CAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAGGCAG
ACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGATATCA
CCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGACAAA
GAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGGCTCTT
GTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTCCATCT
ACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCCAAGCT
GCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGGCTGTG
ATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCTCAGA
AGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGTGCAT
TTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATGAGCT
ATTTAAACGCCAGC (SEQ ID NO: 69).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ
PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM
QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDSGTTNTVAAYN
LTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKD
VKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGT

KVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEF
LIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLK
KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLI
ILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:
70).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 71).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSD
CRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKD
TKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM
FTVQNEDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 72).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCTACAGC
TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT

ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 73).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPV
ATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTV
EACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEF
KTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTK
IKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHADIWVKSYSLYSRERYICN
SGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 74).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG

ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 75).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGI
TWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIW
STDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYE
NYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQV
QGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGG
GSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYP
CTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM

ALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNS
ETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHA
DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
(SEQ ID NO: 76).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC
ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA

GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 77).

Exemplary Multi-Chain Chimeric Polypeptides- Type B

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-21 or to TGF-β. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 (e.g., a soluble human IL-21 polypeptide) or a soluble TGF-β receptor (e.g., a soluble TGFRβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble TGF-β receptor (e.g., a soluble TGFRβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain bind specifically to a receptor for IL-21.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-21 (e.g., a soluble human IL-21). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble TGF-β receptor (e.g., a soluble TGFRβRII receptor (e.g., a soluble human TGFRβRII receptor)). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCCA TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCA TCTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGA GAATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGAT TTCATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGA AGCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGA CAATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical,

at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments of these multi-chain chimeric polypeptides, the human TGFβRII receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER

FKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQ
VYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVEST
GSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLS
LRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECE
ELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 86).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCTCCGGCACCACCAAT
ACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGACAATTC
TGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGATCTCCAC
CAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACCGAGTGT
GATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGGCTCGGG
TCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGCGAGCCT
CTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTTAGGCCA
GCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTCACCGTC
GAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCCTCCGGG
ATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCCAGCTCC
TCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTGACGTGG
ACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTCTCGTAC
CGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAAGAAAA
GGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATC
GAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGA
CGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGC
AAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAA
TTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGT
CCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTC
TGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 87).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTW
KSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQ
TYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVN
VTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDV
DKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIED
LIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAN
NSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 88).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCTCCGGCACCACCAA
TACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGACAATT
CTGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGATCTCCA
CCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACCGAGT
GTGATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGGCTCG
GGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGCGAGC
CTCTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTTAGGC
CAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTCACCG
TCGAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCCTCCG
GGATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCCAGCT
CCTCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTGACGT
GGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTCTCGT

ACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAAGAAA
AGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGAT
CGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCG
ACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTG
CAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGA
ATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAG
TCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTT
CTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 89).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT
ECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 90).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCATCTGTGAGAAGCCTC

AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACATCACGTGTCCTCCTCCTATGTCC
GTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGGGAGC
GGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGAC
GGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAACCCCCAGT
CTCAAATGTATTAGA (SEQ ID NO: 91).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRER
YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 92).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCATCTGTGAGAAGCCT

CAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGG
AAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGA
CGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGAC
CTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTA
GCGAGGAATACAATACCAGCAACCCCGACATCACGTGTCCTCCTCCTATGTC
CGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGGGAG
CGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGAC
GGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAACCCCCAGT
CTCAAATGTATTAGA (SEQ ID NO: 93).

Exemplary Multi-Chain Chimeric Polypeptides- Type C

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7 or a receptor of IL-21. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 (e.g., a soluble human IL-21 polypeptide) or a soluble IL-7 (e.g., a soluble human IL-7 polypeptide). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a receptor of IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to a receptor for IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to a receptor for IL-21.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-21 (e.g., a soluble human IL-21).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 94).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-7 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO:
11).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC (SEQ ID NO: 95).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQ
VYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVEST
GSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLS
LRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECE
ELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 96).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG

CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCCTCAGGCACTACAAATAC
TGTGGCAGCATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTGG
AGTGGGAACCCAAACCCGTCAATCAAGTCTACACTGTTCAAATAAGCACTAA
GTCAGGAGATTGGAAAAGCAAATGCTTTTACACAACAGACACAGAGTGTGAC
CTCACCGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTCT
TCTCCTACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCT
GTATGAGAACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAG
CCAACAATTCAGAGTTTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAG
AAGATGAACGGACTTTAGTCAGAAGGAACAACACTTTCCTAAGCCTCCGGGA
TGTTTTTGGCAAGGACTTAATTTATACACTTTATTATTGGAAATCTTCAAGTTC
AGGAAAGAAAACAGCCAAAACAAACACTAATGAGTTTTTGATTGATGTGGAT
AAAGGAGAAAACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCCCGAACAG
TTAACCGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAG
GGGAATTCAGAGAAAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCG
AAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGAC
GTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCA
AGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAAT
TTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTC
CGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCT
GCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 97).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MGVKVLFALICIAVAEAQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTW
KSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQ
TYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVN
VTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDV
DKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIED
LIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAN
NSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 98).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGGCCCA
AGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTGATC
AGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCAGAA
GATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGGCCCA
ACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATCAATT
AAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACAGAAA
CACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCCAAAG
AATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGCATCTG
TCCTCTAGAACACACGGAAGTGAAGATTCCTCAGGCACTACAAATACTGTGG
CAGCATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTGGAGTGG
GAACCCAAACCCGTCAATCAAGTCTACACTGTTCAAATAAGCACTAAGTCAG
GAGATTGGAAAAGCAAATGCTTTTACACAACAGACACAGAGTGTGACCTCAC
CGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTCTTCTCC
TACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATG
AGAACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAAC
AATTCAGAGTTTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGAT
GAACGGACTTTAGTCAGAAGGAACAACACTTTCCTAAGCCTCCGGGATGTTT
TTGGCAAGGACTTAATTTATACACTTTATTATTGGAAATCTTCAAGTTCAGGA
AAGAAAACAGCCAAAACAAACACTAATGAGTTTTTGATTGATGTGGATAAAG
GAGAAAACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCCCGAACAGTTAAC
CGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGGGAA
TTCAGAGAAAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATT
TAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCAC
CCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTAT
CTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATC
ATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCT
GCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAAT
CCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 99)

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
(SEQ ID NO: 100)

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACACATCACGTGCCCTC
CCCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTA
CTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACG
TCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGA
CAACCCCCAGTCTCAAATGCATTAGA (SEQ ID NO: 101).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MGVKVLFALICIAVAEADCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK
ATNVAHWTTPSLKCIR (SEQ ID NO: 102).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGGCCGA
TTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGGTC
AGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCTGA
ATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAAGGT
ATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAATAG
CACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAATAC
TGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGGTGA
AGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACAGAA
AAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAAACT
TGTTGGAATAAAATTTTGATGGGCACTAAAGAACACATCACGTGCCCTCCCC
CCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTC
CAGGGAGCGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCC
AGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAA
CCCCCAGTCTCAAATGCATTAGA (SEQ ID NO: 103).

Exemplary Multi-Chain Chimeric Polypeptides- Type D

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7 or a receptor of IL-21. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 (e.g., a soluble human IL-21 polypeptide) or a soluble IL-7 (e.g., a soluble human IL-7 polypeptide). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a receptor of IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to a receptor for IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to a receptor for IL-21.

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82%

identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 94).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-7 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 11).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC (SEQ ID NO: 95).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 104).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA
GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC
(SEQ ID NO: 105).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 106).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA

CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 107).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 108).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTC
CCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAG
CCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAG
CAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACA
ACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 109).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ
ID NO: 110).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 111).

Exemplary Multi-Chain Chimeric Polypeptides- Type E

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-18 (e.g., a soluble human IL-18), a receptor for IL-12 (e.g., a soluble human IL-12), or CD16 (e.g., an anti-CD16 scFv). In some embodiments of these multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 or a receptor for IL-12.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional antigen-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional antigen-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-18 or a receptor of IL-12. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-12, and the second target-binding domain binds specifically to a receptor for IL-18. In some embodiments of these multi-

chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-18, and the second target-binding domain bind specifically to a receptor for IL-12. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to CD16, and the second target-binding domain binds specifically to a receptor for IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-18, and the second target-binding domain bind specifically to CD16.

In some embodiments of these multi-chain chimeric polypeptides, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-18 (e.g., a soluble human IL-18).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ
PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM
QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 16).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC

TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
(SEQ ID NO: 65).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble IL-12 (e.g., a soluble human IL-12). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-15 includes a sequence of soluble human IL-12β (p40) and a sequence of soluble human IL-12α (p35). In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-15 (e.g., soluble human IL-15) further includes a linker sequence (e.g., any of the exemplary linker sequences described herein) between the sequence of soluble IL-12β (p40) and the sequence of soluble human IL-12α (p35). In some examples of these multi-chain chimeric polypeptides, the linker sequence comprises GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 66).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12β (p40) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCC (SEQ ID NO: 67).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKD
KTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKM
YQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPD
FYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 68).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTACACCA
CAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAGGCAG
ACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGATATCA
CCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGACAAA
GAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGGCTCTT
GTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTCCATCT

ACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCCAAGCT
GCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGGCTGTG
ATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCTCAGA
AGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGTGCAT
TTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATGAGCT
ATTTAAACGCCAGC (SEQ ID NO: 69).

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain includes an scFv that specifically binds to CD16 (e.g., an anti-CD16 scFv).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH
(SEQ ID NO: 112).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCAT (SEQ ID NO: 113).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical,

at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 114).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 115).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ
PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM
QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDSGTTNTVAAYN
LTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKD
VKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGT
KVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEF
LIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLK
KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLI
ILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 116).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%

identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 117).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSD
CRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKD

TKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM
FTVQNEDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 118).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCTACAGC
TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA

GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 119).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPV
ATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTV
EACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEF
KTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTK
IKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHADIWVKSYSLYSRERYICN
SGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRSELTQDPAVSVALGQTVRIT
CQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITG
AQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQL
VESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGG
STGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQ
GTLVTVSR (SEQ ID NO: 120).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG

ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGTCCGAGCTGACCCAGGACCCTGCTGTGT
CCGTGGCTCTGGGCCAGACCGTGAGGATCACCTGCCAGGGCGACTCCCTGAG
GTCCTACTACGCCTCCTGGTACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTG
GTGATCTACGGCAAGAACAACAGGCCCTCCGGCATCCCTGACAGGTTCTCCG
GATCCTCCTCCGGCAACACCGCCTCCCTGACCATCACAGGCGCTCAGGCCGA
GGACGAGGCTGACTACTACTGCAACTCCAGGGACTCCTCCGGCAACCATGTG
GTGTTCGGCGGCGGCACCAAGCTGACCGTGGGCCATGGCGGCGGCGGCTCCG
GAGGCGGCGGCAGCGGCGGAGGAGGATCCGAGGTGCAGCTGGTGGAGTCCG
GAGGAGGAGTGGTGAGGCCTGGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTC
CGGCTTCACCTTCGACGACTACGGCATGTCCTGGGTGAGGCAGGCTCCTGGA
AAGGGCCTGGAGTGGGTGTCCGGCATCAACTGGAACGGCGGATCCACCGGCT
ACGCCGATTCCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAGA
ACTCCCTGTACCTGCAGATGAACTCCCTGAGGGCCGAGGACACCGCCGTGTA
CTACTGCGCCAGGGGCAGGTCCCTGCTGTTCGACTACTGGGGACAGGGCACC
CTGGTGACCGTGTCCAGG (SEQ ID NO: 121).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGI
TWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIW
STDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYE
NYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQV
QGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGG
GSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYP
CTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNS
ETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHA
DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRSE
LTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGI
PDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHGG
GGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQA
PGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAV
YYCARGRSLLFDYWGQGTLVTVSR (SEQ ID NO: 122).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC
ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC

TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGTCCGAGCTGACCCAGGACCCTGCTGTGT
CCGTGGCTCTGGGCCAGACCGTGAGGATCACCTGCCAGGGCGACTCCCTGAG
GTCCTACTACGCCTCCTGGTACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTG
GTGATCTACGGCAAGAACAACAGGCCCTCCGGCATCCCTGACAGGTTCTCCG
GATCCTCCTCCGGCAACACCGCCTCCCTGACCATCACAGGCGCTCAGGCCGA
GGACGAGGCTGACTACTACTGCAACTCCAGGGACTCCTCCGGCAACCATGTG
GTGTTCGGCGGCGGCACCAAGCTGACCGTGGGCCATGGCGGCGGCGGCTCCG
GAGGCGGCGGCAGCGGCGGAGGAGGATCCGAGGTGCAGCTGGTGGAGTCCG
GAGGAGGAGTGGTGAGGCCTGGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTC
CGGCTTCACCTTCGACGACTACGGCATGTCCTGGGTGAGGCAGGCTCCTGGA
AAGGGCCTGGAGTGGGTGTCCGGCATCAACTGGAACGGCGGATCCACCGGCT
ACGCCGATTCCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAGA
ACTCCCTGTACCTGCAGATGAACTCCCTGAGGGCCGAGGACACCGCCGTGTA
CTACTGCGCCAGGGGCAGGTCCCTGCTGTTCGACTACTGGGGACAGGGCACC
CTGGTGACCGTGTCCAGG (SEQ ID NO: 123).

Exemplary Multi-Chain Chimeric Polypeptides- Type F

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7 (e.g., a soluble human IL-7), CD16 (e.g., an anti-CD16 scFv), or a receptor for IL-21 (e.g., a soluble human IL-21). In some embodiments of these multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some

embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 or a receptor for IL-21.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second

domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional antigen-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional antigen-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain binds specifically to a receptor IL-7 and the second target-binding domain binds specifically to CD16 or a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain includes a soluble IL-7 protein. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-7 protein is a soluble human IL-7. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second antigen-binding domain includes a target-binding domain that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes an scFv that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain

binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-21 is a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the additional target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-21 is a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to CD16.

In some embodiments of these multi-chain chimeric polypeptides, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-7 (e.g., a soluble human IL-7).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 11).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC (SEQ ID NO: 95).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 124).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-21 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 94).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79)

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain includes an scFv that specifically binds to CD16 (e.g., an anti-CD16 scFv).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical,

at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH (SEQ ID NO: 112).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA CCGTGGGCCAT (SEQ ID NO: 113).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY WGQGTLVTVSR (SEQ ID NO: 114).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 115).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 125).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT

CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA
GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC
(SEQ ID NO: 126).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 127).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 128).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHG
GGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQ
APGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEHADIWVKSYSLYSRERYIC
NSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIVD

QLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKK
LKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTH
GSEDS (SEQ ID NO: 129).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACATG
CCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGC
CTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCG
GCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCA
CTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACATG
ATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTGA
ACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACTG
CGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAAC
ACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCGG
AAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTGC
CCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGGT
TCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCCA
CGGCTCCGAGGACTCC (SEQ ID NO: 130).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQ
KPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSS
GNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSC
AASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNA
KNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQ
GQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQL
KSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERF
KSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 131).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACC
AGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAG
GCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCT
CCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAA
CTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTG
ACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGA
GGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGA
GGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGG
CATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGC
ATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGT
TCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTC
CCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTG
CTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACAT
GCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAG
CCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCC
GGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTC
ACTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACAT
GATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTG
AACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACT
GCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAA
CACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCG
GAAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTG
CCCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGG
TTCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCC
ACGGCTCCGAGGACTCC (SEQ ID NO: 132).

Exemplary Multi-Chain Chimeric Polypeptides- Type G

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGFβ (e.g., a human TGFβRII receptor), CD16 (e.g., an anti-CD16 scFv), or a receptor for IL-21 (e.g., a soluble human IL-21). In some embodiments of these multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 or a receptor for IL-21.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional antigen-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional antigen-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, CD16, or a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first

target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to CD16 or a receptor of IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain is a soluble TGF-β receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, soluble TGF-β receptor is a soluble TGFβRII receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second antigen-binding domain includes an antigen-binding domain that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second antigen-binding domain includes an scFv that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the additional target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-21 is a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to CD16.

In some embodiments of these multi-chain chimeric polypeptides, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more of the first target-binding domain, the

second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a TGFβRII receptor (e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82)

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at

least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-21 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC

AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 94).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH
(SEQ ID NO: 112).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG

CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCAT (SEQ ID NO: 113).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 114).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 115).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK

NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKS
GDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYEN
SPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLI
YTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPV
ECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKC
FLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL
QSFVHIVQMFINTS (SEQ ID NO: 133).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA

ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 134).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYP
AGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLV
RRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSV
QAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATL
YTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT
ESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 135).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC

GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 136).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHG
GGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQ
APGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEHADIWVKSYSLYSRERYIC
NSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIVD
QLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKK

LKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTH GSEDS (SEQ ID NO: 137).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACATG
CCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGC
CTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCG
GCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCA
CTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACATG
ATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTGA
ACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACTG
CGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAAC
ACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCGG
AAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTGC
CCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGGT
TCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCCA
CGGCTCCGAGGACTCC (SEQ ID NO: 138).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQ
KPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSS
GNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSC
AASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNA
KNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQ
GQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQL
KSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERF
KSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 139).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACC
AGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAG
GCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCT
CCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAA
CTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTG
ACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGA
GGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGA
GGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGG
CATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGC
ATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGT
TCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTC
CCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTG
CTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACAT
GCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAG
CCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCC
GGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTC
ACTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACAT
GATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTG
AACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACT
GCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAA
CACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCG
GAAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTG
CCCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGG
TTCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCC
ACGGCTCCGAGGACTCC (SEQ ID NO: 140).

Exemplary Multi-Chain Chimeric Polypeptides- Type H

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor for IL-7. In some embodiments of these multi-chain chimeric

polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include a soluble IL-7 (e.g., a soluble human IL-7). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 11).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 95).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 141).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA

GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA
GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC
(SEQ ID NO: 142).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 143).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA

TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 144).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
(SEQ ID NO: 145).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA

TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACACATCACGTGCCCTC
CCCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTA
CTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACG
TCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGA
CAACCCCCAGTCTCAAATGCATTAGA (SEQ ID NO: 146).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK
ATNVAHWTTPSLKCIR (SEQ ID NO: 147).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATATTACATGC
CCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCC
TCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGG
CACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCAC
TGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 148).

Exemplary Multi-Chain Chimeric Polypeptides- Type I

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain each independently bind specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides,

the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to: IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to: c (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical,

at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKS
GDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYEN
SPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLI
YTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPV
ECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKC
FLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL
QSFVHIVQMFINTS (SEQ ID NO: 149).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 150).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYP
AGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLV
RRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSV
QAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATL
YTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT
ESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 151).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG

CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 152).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT
ECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 153).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
GGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
CGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
TTAAAGTGCATCCGG (SEQ ID NO: 154).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRER
YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 155).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
GGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
CGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
TTAAAGTGCATCCGG (SEQ ID NO: 156).

Exemplary Multi-Chain Chimeric Polypeptides- Type J

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7, a receptor of IL-21, or a receptor of CD137L. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor for IL-21 (e.g., a soluble IL-21, e.g., a soluble human IL-21) or a receptor for CD137L (e.g., a soluble CD137L, e.g., a soluble human CD137L).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary

linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g.,

any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments, the second chimeric polypeptide can include an additional target-binding domain. In some embodiments, the additional target-binding domain and the

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional target-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional target-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L. In some embodiments, the additional target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble IL-7 (e.g., a soluble human IL-7). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 11).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 124).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain binds specifically to a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain is a soluble IL-21 (e.g., a soluble human IL-21).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain binds specifically to a receptor for CD137L. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for CD137L. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain and/or the additional target-binding domain is a soluble CD137L (e.g., a soluble human CD137L).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 157).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG

GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 158).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLP
PASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEI (SEQ ID NO: 159).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGG
CCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGG
CCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAA
GGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGC
TGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCA
CCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGG
ACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGC
CGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGA
GGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 160).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ

PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID
NO: 161).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA

GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 162).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 163).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA

TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 164).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSREGPELS
PDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTK
ELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVD
LPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLF
RVTPEIPAGLPSPRSE (SEQ ID NO: 165).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC

CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTC
CCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAG
CCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAG
CAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACA
ACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCT
CCGGCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGC
CGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAAT
GTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAG
GCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGG
TGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGC
GTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGC
CACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCA
CCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCT
GCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGG
GCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCC
GGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ
ID NO: 166).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPG
LAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHL
QPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEAR
ARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 167).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC

GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGC
TCCGGCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCG
CCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAA
TGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCA
GGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTG
GTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCG
CGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAG
CCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCC
ACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGC
TGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAG
GGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTC
CGGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 168).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSDPAGLL
DLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKA
GVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEA
RNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEI (SEQ ID NO: 169).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTC
CCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAG
CCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAG
CAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACA
ACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCT
CCGGCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCAT
GTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCT
GGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTA
CAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTC
TTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCG
TTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCC
CTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGC
CTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGC
GTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGG
GCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 170).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTG
GLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAG
AAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQL
TQGATVLGLFRVTPEI (SEQ ID NO: 171).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least

86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGC
TCCGGCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCA
TGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGC
TGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCT
ACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATG
TCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTC
CGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCG
CCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCG
GCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGG
GCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCA
GGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 172).

Exemplary Multi-Chain Chimeric Polypeptides- Type K

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7 or TGF-β. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to a receptor for IL-7.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-7 protein (e.g., a soluble human IL-7 protein). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 protein includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 11).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 124).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain comprises a target-binding domain that binds specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at

least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG

CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAYN
LTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKD
VKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTK
VNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLI
DVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIE
DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 173).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATGG
TGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCCTC
AACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGAGG
GCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATGAA
CTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCACC
ATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCTGG
GAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGAGC
AGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATCAA
GACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACAACC
AACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCAT
CCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCA
CCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGC
GATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGT
GTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTT
TATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAG

CCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGG
AGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGA
TGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTC
CGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGAT
AAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGT
GAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGC
GAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAG
ATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTA
TCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATC
ATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTG
CAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCC
TTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 174).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSK
CFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWK
SSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEK
GEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS (SEQ ID NO: 175).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATGG
TGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCCTC
AACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGAGG
GCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATGAA

CTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCACC
ATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCTGG
GAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGAGC
AGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATCAA
GACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACAACC
AACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCAT
CCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCA
CCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGC
GATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGT
GTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTT
TATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAG
CCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGG
AGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGA
TGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTC
CGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGAT
AAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGT
GAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGC
GAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAG
ATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTA
TCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATC
ATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTG
CAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCC
TTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 176).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIR (SEQ ID NO: 177).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
G (SEQ ID NO: 178).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 179).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
G (SEQ ID NO: 180).

Exemplary Multi-Chain Chimeric Polypeptides- Type L

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, a receptor of IL-21, or a receptor of CD137L. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor for IL-21 (e.g., a soluble IL-21, e.g., a soluble human IL-21) or a receptor for CD137L (e.g., a soluble CD137L, e.g., a soluble human CD137L).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the

first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g.,

any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional target-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional target-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β and the second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82)

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG

AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO:
83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain binds specifically to a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain includes a soluble IL-21(e.g., a soluble human IL-21).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain binds specifically to a receptor for CD137L. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain and/or the additional target-binding domain includes a soluble CD137L (e.g., a soluble human CD137L).

In some embodiments of these multi-chain chimeric polypeptides, a soluble CD137L includes a sequence that is at least 80% identical (e.g., at least 82% identical, at

least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 157).

In some embodiments of these multi-chain chimeric polypeptides, a soluble CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG
GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 158).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLP
PASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEI (SEQ ID NO: 159).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGG
CCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGG
CCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAA
GGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGC
TGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCA
CCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGG
ACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGC
CGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGA
GGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 160).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 181).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC

CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 182).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE

PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 183).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA

ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 184).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSREGPELSP
DDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKE
LVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPP
ASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVT
PEIPAGLPSPRSE (SEQ ID NO: 185).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGG

CCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTC
TGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGT
GTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGT
GGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGG
TGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACT
GCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCG
CCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCAC
CTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCAC
GCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTG
ACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 186).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPG
LAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQ
PLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA
RHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 187).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC

CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGG
CCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTC
TGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGT
GTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGT
GGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGG
TGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACT
GCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCG
CCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCAC
CTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCAC
GCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTG
ACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 188).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSDPAGLLD
LRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGV
YYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNS
AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEI (SEQ ID NO: 189).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA

GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTT
GCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTA
CAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAA
GAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCT
TTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTC
ACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGG
CTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTC
GGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCC
ATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCC
ACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 190).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGG
LSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQG
ATVLGLFRVTPEI (SEQ ID NO: 191).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG

ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTT
GCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTA
CAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAA
GAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCT
TTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTC
ACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGG
CTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTC
GGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCC
ATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCC
ACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 192).

Exemplary Multi-Chain Chimeric Polypeptides- Type M

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or a receptor of IL-21. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor for IL-21 (e.g., a soluble IL-21, e.g., a soluble human IL-21) or a TGF-β (e.g., a soluble TGF-β receptor, e.g., a soluble TGFβRII receptor).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to TGF-β or a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain binds specifically to a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble IL-21 (e.g., a human soluble IL-21). In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 78).

In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 79).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 193).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 194).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 195).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA

AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 196).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAP
EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHR
LTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO:197).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT

GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 198).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIV
DQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIK
KLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRT
HGSEDS (SEQ ID NO: 199).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 200).

Exemplary Multi-Chain Chimeric Polypeptides- Type N

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or CD16. In some embodiments of these multi-

chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 (e.g., an anti-CD16 scFv) or a TGF- β (e.g., a soluble TGF-β receptor, e.g., a soluble TGFβRII receptor).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-

chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to TGF-β or CD16. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT

TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC

TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain binds specifically to CD16. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes an anti-CD16 scFv. In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH (SEQ ID NO: 112).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA CCGTGGGCCAT (SEQ ID NO: 113).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92%

identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 114).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 115).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 201).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 202).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 203).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA

TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 204).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIRSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQ
QKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDS
SGNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLS
CAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDN
AKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSR (SEQ ID NO: 205).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least

86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 206).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRSELTQDPAVSVALGQTVR
ITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTIT
GAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQ
LVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGG
STGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQG
TLVTVSR (SEQ ID NO: 207).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG

AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 208).

Exemplary Multi-Chain Chimeric Polypeptides- Type O

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or a receptor of CD137L. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor to TGF- β (e.g., a soluble TGF-β receptor, e.g., a soluble TGFβRII receptor) or CD137L.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor

domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds

specifically to CD137L. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain or the additional target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 7).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94%

identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 82).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 85).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 84).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble CD137L protein (e.g., a soluble human CD137L protein). In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 157).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG
GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 158).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLP
PASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEI (SEQ ID NO: 159).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGG
CCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGG
CCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAA
GGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGC
TGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCA
CCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGG
ACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGC
CGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGA
GGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 160).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 209).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT

GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 210).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 211).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 212).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSREGPELSPDDPAGLLDLRQ
GMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYV
FFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFG
FQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRS
E (SEQ ID NO: 213).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GGGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCTCGCGA
GGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAG

GGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCT
GAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTG
AGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACT
ATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGC
TCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGC
CGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACT
CGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTG
GGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCA
GGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATCCCAGCCGGA
CTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 214).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSRE
GPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYK
EDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALAL
TVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVL
GLFRVTPEIPAGLPSPRSE (SEQ ID NO: 215).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC

ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GGGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCTCGCGA
GGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAG
GGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCT
GAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTG
AGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACT
ATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGC
TCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGC
CGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACT
CGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTG
GGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCA
GGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATCCCAGCCGGA
CTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 216).

Compositions/Kits

Also provided herein are compositions (e.g., pharmaceutical compositions) that include at least one of any multi-chain chimeric polypeptides, any of the cells, or any of the nucleic acids described herein. In some embodiments, the compositions include at least one of any of the multi-chain chimeric polypeptides described herein. In some embodiments, the compositions include any of the immune cells (e.g., any of the immune cells described herein, e.g., any of the immune cells produced using any of the methods described herein).

In some embodiments, the pharmaceutical compositions are formulated for different routes of administration (e.g., intravenous, subcutaneous). In some embodiments, the pharmaceutical compositions can include a pharmaceutically acceptable carrier (e.g., phosphate buffered saline).

Single or multiple administrations of pharmaceutical compositions can be given to a subject in need thereof depending on for example: the dosage and frequency as required

and tolerated by the subject. The formulation should provide a sufficient quantity of active agent to effectively treat, prevent or ameliorate conditions, diseases or symptoms.

Also provided herein are kits that include any of the multi-chain chimeric polypeptides, compositions, nucleic acids, or cells (e.g., immune cells) described herein. In some embodiments, the kits can include instructions for performing any of the methods described herein. In some embodiments, the kits can include at least one dose of any of the pharmaceutical compositions described herein.

Nucleic Acids/Vectors

Also provided herein are nucleic acids that encode any of the multi-chain chimeric polypeptides described herein. In some embodiments, a first nucleic acid can encode the first chimeric polypeptide and a second nucleic acid can encode the second chimeric polypeptide. In some embodiments, a single nucleic acid can encode both the first chimeric polypeptide and the second chimeric polypeptide.

Also provided herein are vectors that include any of the nucleic acids encoding any of the multi-chain chimeric polypeptides described herein. In some embodiments, a first vector can include a nucleic acid encoding the first chimeric polypeptide and a second vector can include a nucleic acid encoding the second chimeric polypeptide. In some embodiments, a single vector can include a first nucleic acid encoding the first chimeric polypeptide and a second nucleic acid encoding the second chimeric polypeptide.

Any of the vectors described herein can be an expression vector. For example, an expression vector can include a promoter sequence operably linked to the sequence encoding the first chimeric polypeptide and the second chimeric polypeptide.

Non-limiting examples of vectors include plasmids, transposons, cosmids, and viral vectors (e.g., any adenoviral vectors (e.g., pSV or pCMV vectors), adeno-associated virus (AAV) vectors, lentivirus vectors, and retroviral vectors), and any Gateway® vectors. A vector can, e.g., include sufficient cis-acting elements for expression; other elements for expression can be supplied by the host mammalian cell or in an in vitro expression system. Skilled practitioners will be capable of selecting suitable vectors and

mammalian cells for making any of the multi-chain chimeric polypeptides described herein.

Cells

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) comprising any of the nucleic acids described herein that encode any of the multi-chain chimeric polypeptides described herein (e.g., encoding both the first and second chimeric polypeptides). Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) comprising any of the nucleic acids described herein that encode any of the first chimeric polypeptides described herein. Also provided are cells (e.g., any of the exemplary cells described herein or known in the art) comprising any of the nucleic acids described herein that encode any of the second chimeric polypeptides described herein.

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include any of the vectors described herein that encode any of the multi-chain chimeric polypeptides described herein (e.g., encoding both the first and second chimeric polypeptides). Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include any of the vectors described herein that encode any of the first chimeric polypeptides described herein. Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include any of the vectors described herein that encode any of the second chimeric polypeptides described herein).

In some embodiments of any of the methods described herein, the cell can be a eukaryotic cell. As used herein, the term "eukaryotic cell" refers to a cell having a distinct, membrane-bound nucleus. Such cells may include, for example, mammalian (e.g., rodent, non-human primate, or human), insect, fungal, or plant cells. In some embodiments, the eukaryotic cell is a yeast cell, such as Saccharomyces cerevisiae. In some embodiments, the eukaryotic cell is a higher eukaryote, such as mammalian, avian, plant, or insect cells. Non-limiting examples of mammalian cells include Chinese hamster ovary cells and human embryonic kidney cells (e.g., HEK293 cells).

Methods of introducing nucleic acids and expression vectors into a cell (e.g., a eukaryotic cell) are known in the art. Non-limiting examples of methods that can be used to introduce a nucleic acid into a cell include lipofection, transfection, electroporation, microinjection, calcium phosphate transfection, dendrimer-based transfection, cationic polymer transfection, cell squeezing, sonoporation, optical transfection, impalefection, hydrodynamic delivery, magnetofection, viral transduction (e.g., adenoviral and lentiviral transduction), and nanoparticle transfection.

Methods of Producing Multi-Chain Chimeric Polypeptides

Also provided herein are methods of producing any of the multi-chain chimeric polypeptides described herein that include culturing any of the cells described herein in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Also provided herein are method of producing any of the multi-chain chimeric polypeptides described herein that include: culturing any of cells described herein in a first culture medium under conditions sufficient to result in the production of the first chimeric polypeptide; recovering the first chimeric polypeptide from the cell and/or the first culture medium; culturing any of the cells described herein in a second culture medium under conditions sufficient to result in the production of the second chimeric polypeptide; recovering the second chimeric polypeptide from the cell and/or the second culture medium; and combining (e.g., mixing) the recovered first chimeric polypeptide and the recovered second chimeric polypeptide to form the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein).

The recovery of the multi-chain chimeric polypeptide, the first chimeric polypeptide, or the second chimeric polypeptide from a cell (e.g., a eukaryotic cell) can be performed using techniques well-known in the art (e.g., ammonium sulfate precipitation, polyethylene glycol precipitation, ion-exchange chromatography (anion or cation), chromatography based on hydrophobic interaction, metal-affinity chromatography, ligand-affinity chromatography, and size exclusion chromatography).

Methods of culturing cells are well known in the art. Cells can be maintained *in vitro* under conditions that favor proliferation, differentiation and growth. Briefly, cells can be cultured by contacting a cell (e.g., any cell) with a cell culture medium that includes the necessary growth factors and supplements to support cell viability and growth.

Also provided herein are multi-chain chimeric polypeptides (e.g., any of the multi-chain chimeric polypeptides described herein), first chimeric polypeptides (e.g., any of the first chimeric polypeptides), or second chimeric polypeptides (e.g., any of the second chimeric polypeptides described herein) produced by any of the methods described herein.

Methods of Stimulating an Immune Cell

Also provided herein are methods of stimulating an immune cell (e.g., any of the exemplary immune cells described herein or known in the art) that include contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides described herein or any of the compositions (e.g., pharmaceutical compositions) described herein. In some examples, the immune cell is contacted *in vitro* (e.g., in a suitable liquid culture medium under conditions sufficient to result in stimulation of the immune cell).

In some examples, the immune cell has been previously obtained from a subject (e.g., a mammal, e.g., a human). Some embodiments of these methods further include obtaining the immune cell from the subject prior to the contacting step.

In some examples, the immune cell is contacted *in vivo*. In such embodiments, the multi-chain chimeric polypeptide is administered to a subject (e.g., a mammal, e.g., a human) in an amount sufficient to result in stimulation of an immune cell in the subject.

In some examples of any of the methods described herein, the immune cell can be an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a

macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, or a natural killer cell, or a combination thereof.

In some examples, the immune cell has previously been genetically-modified to express a chimeric antigen receptor or a recombinant T-cell receptor. In some examples, the immune cell (e.g., any of the immune cells described herein) has previously been genetically-modified to express a co-stimulatory molecule (e.g., CD28).

Some embodiments of these methods can further include, after the contacting step, introducing into the immune cell (e.g., any of the immune cells described herein) a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods can further include, after the contacting step, introducing into the immune cell (e.g., any of the immune cells described herein) a nucleic acid encoding a co-stimulatory molecule (e.g., CD28).

Some embodiments of these methods can further include administering a therapeutically effective amount of the immune cell to a subject in need thereof (e.g., any of the exemplary subjects described herein).

In some examples, the subject can be a subject identified or diagnosed as having an age-related disease or condition. Non-limiting examples of age-related diseases or disorders include: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples, the subject can be a subject that has been identified or diagnosed as having a cancer. Non-limiting examples of cancers include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma,

B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples, the subject can be a subject that has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, or influenza virus.

Activation of an immune cell can be determined using methods known in the art. For example, activation of an immune cell can be determined by detecting the levels of cytokines and chemokines that are secreted or cytotoxicity granules and regulatory molecules that are upregulated upon activation of an immune cell. Non-limiting examples of cytokines, chemokines, cytotoxicity granules, and regulatory molecules that are secreted or upregulated upon activation of an immune cell include: IL-2, IFN-γ, IL-1, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, IL-22, IL-33, leukotriene B4, CCL5, TNFα, granzymes, perforin, TGFβ, STAT3, STAT4, STAT5, RORγT, FOXP3, STAT6, and GATA3. The detection of these cytokines, chemokines, cytotoxicity granules, or regulatory molecules can be performed using an immunoassay (e.g., an enzyme-linked immunosorbent assay) and quantitative PCR. For example, activation of an immune cell can result in an increase of about 1% to about 800% (e.g., about 1% to about 750%, about 1% to about 700%, about 1% to about 650%, about 1% to about 600%, about 1% to about 550%, about 1% to about 500%, about 1% to about 450%, about 1% to about 400%, about 1% to about 350%, about 1% to about 300%, about 1% to about 280%, about 1% to about 260%, about 1% to about 240%, about 1% to about 220%, about 1% to about 200%, about 1% to about 180%, about 1% to about 160%, about 1% to about 140%, about 1% to about 120%, about 1% to about 100%,

about 1% to about 90%, about 1% to about 80%, about 1% to about 70%, about 1% to about 60%, about 1% to about 50%, about 1% to about 45%, about 1% to about 40%, about 1% to about 35%, about 1% to about 30%, about 1% to about 25%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 5%, about 5% to about 800%, about 5% to about 750%, about 5% to about 700%, about 5% to about 650%, about 5% to about 600%, about 5% to about 550%, about 5% to about 500%, about 5% to about 450%, about 5% to about 400%, about 5% to about 350%, about 5% to about 300%, about 5% to about 280%, about 5% to about 260%, about 5% to about 240%, about 5% to about 220%, about 5% to about 200%, about 5% to about 180%, about 5% to about 160%, about 5% to about 140%, about 5% to about 120%, about 5% to about 100%, about 5% to about 90%, about 5% to about 80%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 5% to about 10%, about 10% to about 800%, about 10% to about 750%, about 10% to about 700%, about 10% to about 650%, about 10% to about 600%, about 10% to about 550%, about 10% to about 500%, about 10% to about 450%, about 10% to about 400%, about 10% to about 350%, about 10% to about 300%, about 10% to about 280%, about 10% to about 260%, about 10% to about 240%, about 10% to about 220%, about 10% to about 200%, about 10% to about 180%, about 10% to about 160%, about 10% to about 140%, about 10% to about 120%, about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 10% to about 20%, about 10% to about 15%, about 15% to about 800%, about 15% to about 750%, about 15% to about 700%, about 15% to about 650%, about 15% to about 600%, about 15% to about 550%, about 15% to about 500%, about 15% to about 450%, about 15% to about 400%, about 15% to about 350%, about 15% to about 300%, about 15% to about 280%, about 15% to about 260%, about 15% to about 240%, about 15% to about 220%, about 15% to about 200%, about 15% to about 180%, about 15% to about 160%, about 15% to about 140%, about 15% to about 120%, about 15% to about 100%, about 15% to about 90%, about 15% to about

80%, about 15% to about 70%, about 15% to about 60%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 15% to about 20%, about 20% to about 800%, about 20% to about 750%, about 20% to about 700%, about 20% to about 650%, about 20% to about 600%, about 20% to about 550%, about 20% to about 500%, about 20% to about 450%, about 20% to about 400%, about 20% to about 350%, about 20% to about 300%, about 20% to about 280%, about 20% to about 260%, about 20% to about 240%, about 20% to about 220%, about 20% to about 200%, about 20% to about 180%, about 20% to about 160%, about 20% to about 140%, about 20% to about 120%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 70%, about 20% to about 60%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 800%, about 25% to about 750%, about 25% to about 700%, about 25% to about 650%, about 25% to about 600%, about 25% to about 550%, about 25% to about 500%, about 25% to about 450%, about 25% to about 400%, about 25% to about 350%, about 25% to about 300%, about 25% to about 280%, about 25% to about 260%, about 25% to about 240%, about 25% to about 220%, about 25% to about 200%, about 25% to about 180%, about 25% to about 160%, about 25% to about 140%, about 25% to about 120%, about 25% to about 100%, about 25% to about 90%, about 25% to about 80%, about 25% to about 70%, about 25% to about 60%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 35% to about 800%, about 35% to about 750%, about 35% to about 700%, about 35% to about 650%, about 35% to about 600%, about 35% to about 550%, about 35% to about 500%, about 35% to about 450%, about 35% to about 400%, about 35% to about 350%, about 35% to about 300%, about 35% to about 280%, about 35% to about 260%, about 35% to about 240%, about 35% to about 220%, about 35% to about 200%, about 35% to about 180%, about 35% to about 160%, about 35% to about 140%, about 35% to about 120%, about 35% to about 100%, about 35% to about 90%, about 35% to about 80%, about 35% to about 70%, about 35% to about 60%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 800%, about 40% to about 750%, about 40% to about 700%, about 40% to about 650%, about 40% to about

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RORγT, FOXP3, and GATA3) (e.g., as compared to the level of the one or more cytokines, chemokines, cytotoxicity granules, and regulatory molecules in a control not contacted with any of the multi-chain chimeric polypeptides described herein).

Methods of Inducing or Increasing Proliferation of an Immune Cell

Also provided herein are methods of inducing or increasing proliferation of an immune cell (e.g., any of the exemplary immune cells described herein or known in the art) that include contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides described herein or any of the compositions (e.g., pharmaceutical compositions) described herein. In some examples, the immune cell is contacted *in vitro* (e.g., in a suitable liquid culture medium under conditions sufficient to result in stimulation of the immune cell).

In some examples, the immune cell has been previously obtained from a subject (e.g., a mammal, e.g., a human). Some embodiments of these methods further include obtaining the immune cell from the subject prior to the contacting step.

In some examples, the immune cell is contacted *in vivo*. In such embodiments, the multi-chain chimeric polypeptide is administered to a subject (e.g., a mammal, e.g., a human) in an amount sufficient to result in stimulation of an immune cell in the subject.

In some examples of any of the methods described herein, the immune cell can be an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, or a natural killer cell, or a combination thereof.

In some examples, the immune cell has previously been genetically-modified to express a chimeric antigen receptor or a recombinant T-cell receptor. In some examples, the immune cell (e.g., any of the immune cells described herein) has previously been genetically-modified to express a co-stimulatory molecule (e.g., CD28).

Some embodiments of these methods can further include, after the contacting step, introducing into the immune cell (e.g., any of the immune cells described herein) a

nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods can further include, after the contacting step, introducing into the immune cell (e.g., any of the immune cells described herein) a nucleic acid encoding a co-stimulatory molecule (e.g., CD28).

Some embodiments of these methods can further include administering a therapeutically effective amount of the immune cell to a subject in need thereof (e.g., any of the exemplary subjects described herein).

In some examples, the subject can be a subject identified or diagnosed as having an age-related disease or condition. Non-limiting examples of age-related diseases or disorders include: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples, the subject can be a subject that has been identified or diagnosed as having a cancer. Non-limiting examples of cancers include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples, the subject can be a subject that has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, or influenza virus.

Detection of the proliferation of an immune cell can be performed using methods known in the art, e.g., cytometry (e.g., fluorescence-assisted flow cytometry), microscopy, and immunofluorescence microscopy, e.g., by comparing the rate of increase in the concentration of the immune cell in a sample not contacted with a multi-chain chimeric polypeptide to the rate of increase in the concentration of the immune cell in a similar sample contacted with any of the multi-chain chimeric polypeptides described herein).

In other examples, the proliferation of an immune cell can be indirectly detected by detecting an increase in the level of one or more cytokines or chemokines or cytotoxicity granules or regulatory molecules secreted or upregulated by proliferating immune cells (e.g., one or more of IL-2, IFN-γ, IL-1, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, IL-22, IL-33, leukotriene B4, CCL5, TNFα, granzymes, perforin, TGFβ, STAT3, STAT4, STAT5, RORγT, FOXP3, and GATA3) (e.g., as compared to the level of the one or more cytokines, chemokines, cytotoxicity granules, and regulatory molecules in a control not contacted with any of the multi-chain chimeric polypeptides described herein).

In some embodiments, the methods provided herein can result in an increase (e.g., about 1% to about 800% increase, or any of the subranges of this range described herein) in the rate of increase in the concentration of the immune cell in a sample contacted with any of the multi-chain chimeric polypeptides described herein as compared to the rate of increase in a similar control sample not contacted with any of the multi-chain chimeric polypeptides described herein.

Methods of Inducing Differentiation of an Immune Cell

Also provided herein are method of inducing differentiation of an immune cell (e.g., any of the exemplary immune cells described herein or known in the art) into a

memory or memory-like immune cell that include contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides described herein or any of the compositions (e.g., pharmaceutical compositions) described herein. In some examples, the immune cell is contacted in vitro (e.g., in a suitable liquid culture medium under conditions sufficient to result in stimulation of the immune cell).

In some examples, the immune cell has been previously obtained from a subject (e.g., a mammal, e.g., a human). Some embodiments of these methods further include obtaining the immune cell from the subject prior to the contacting step.

In some examples, the immune cell is contacted in vivo. In such embodiments, the multi-chain chimeric polypeptide is administered to a subject (e.g., a mammal, e.g., a human) in an amount sufficient to result in stimulation of an immune cell in the subject.

In some examples of any of the methods described herein, the immune cell can be an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, or a natural killer cell, or a combination thereof.

In some examples, the immune cell has previously been genetically-modified to express a chimeric antigen receptor or a recombinant T-cell receptor. In some examples, the immune cell (e.g., any of the immune cells described herein) has previously been genetically-modified to express a co-stimulatory molecule (e.g., CD28).

In some examples, an effective amount of any of the multi-chain chimeric polypeptides described herein or any of the compositions (e.g., pharmaceutical compositions) described herein is combined with an anti-TF IgG1 antibody to create a memory or memory like immune cell.

Some embodiments of these methods can further include, after the contacting step, introducing into the immune cell (e.g., any of the immune cells described herein) a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods can further include, after the contacting step,

introducing into the immune cell (e.g., any of the immune cells described herein) a nucleic acid encoding a co-stimulatory molecule (e.g., CD28).

Some embodiments of these methods can further include administering a therapeutically effective amount of the immune cell to a subject in need thereof (e.g., any of the exemplary subjects described herein).

In some examples, the subject can be a subject identified or diagnosed as having an age-related disease or condition. Non-limiting examples of age-related diseases or disorders include: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples, the subject can be a subject that has been identified or diagnosed as having a cancer. Non-limiting examples of cancers include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples, the subject can be a subject that has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease

include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, or influenza virus.

In some examples, the immune cell is a NK cell, and the detection of a memory NK cell can include, e.g., the detection of the level of one or more of IL-12, IL-18, IL-33, CD25, CD69, CD62L, STAT4, Zbtb32, DNAM-1, NKp30, NKp44, NKp46, BIM, Noxa, SOCS1, BNIP3, BNIP3L, IFN-γ, CXCL16, CXCR6, NKG2D, TRAIL, CD49, Ly49D, CD49b, and Ly79H. A description of NK memory cells and methods of detecting the same is described in O'Sullivan et al., *Immunity* 43:634-645, 2015.

In some examples, the immune cell is a T cell, and the detection of memory T cells can include, e.g., the detection of the level of expression of one or more of CD45RO, CCR7, L-selectin (CD62L), CD44, CD45RA, integrin αeβ7, CD43, CD27, CD28, IL-7Rα, CD95, IL-2Rβ, CXCR3, and LFA-1. In some examples, the immune cell is a B cell and the detection of memory B cells can include, e.g., the detection of the level of expression of CD27. Other types and markers of memory or memory-like immune cells are known in the art.

Methods of Treatment

Also provided herein are methods of treating a subject in need thereof (e.g., any of the exemplary subjects described herein or known in the art) that include administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides described herein or any of the compositions (e.g., pharmaceutical compositions) described herein.

In some embodiments of these methods, the subject has been identified or diagnosed as having a cancer. Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung

cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodiments, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the cancer in the subject (e.g., as compared to the number, severity, or frequency of the one or more symptoms of the cancer in the subject prior to treatment). In some embodiments, these methods can result in a reduction (e.g., about 1% reduction to about 99% reduction, about 1% reduction to about 95% reduction, about 1% reduction to about 90% reduction, about 1% reduction to about 85% reduction, about 1% reduction to about 80% reduction, about 1% reduction to about 75% reduction, about 1% reduction to about 70% reduction, about 1% reduction to about 65% reduction, about 1% reduction to about 60% reduction, about 1% reduction to about 55% reduction, about 1% reduction to about 50% reduction, about 1% reduction to about 45% reduction, about 1% reduction to about 40% reduction, about 1% reduction to about 35% reduction, about 1% reduction to about 30% reduction, about 1% reduction to about 25% reduction, about 1% reduction to about 20% reduction, about 1% reduction to about 15% reduction, about 1% reduction to about 10% reduction, about 1% reduction to about 5% reduction, about 5% reduction to about 99% reduction, about 5% reduction to about 95% reduction, about 5% reduction to about 90% reduction, about 5% reduction to about 85% reduction, about 5% reduction to about 80% reduction, about 5% reduction to about 75% reduction, about 5% reduction to about 70% reduction, about 5% reduction to about 65% reduction, about 5% reduction to about 60% reduction, about 5% reduction to about 55% reduction, about 5% reduction to about 50% reduction, about 5% reduction to about 45% reduction, about 5% reduction to about 40% reduction, about 5% reduction to about 35% reduction, about 5% reduction to about 30% reduction, about 5% reduction to about 25% reduction, about 5% reduction to about 20% reduction, about 5% reduction to about 15% reduction, about 5% reduction to about 10% reduction, about 10% reduction to about 99% reduction, about 10% reduction to about 95% reduction, about 10% reduction to about 90% reduction, about 10% reduction to about 85% reduction, about 10% reduction to about 80% reduction, about 10% reduction to about 75% reduction, about 10% reduction to about 70% reduction, about 10% reduction

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(e.g., as compared to the risk of developing a metastasis or developing one or more additional metastasis in a subject prior to treatment or in a similar subject or a population of subjects administered a different treatment).

In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. Non-limiting examples of aging-related diseases and conditions include Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the aging-related disease or condition in the subject (e.g., as compared to the number, severity, or frequency of the one or more symptoms of the aging-related disease or condition in the subject prior to treatment). In some examples, the methods can result in a decrease (e.g., about 1% decrease to about 99% decrease, an about 1% decrease to about 95% decrease, about 1% decrease to about 90% decrease, about 1% decrease to about 85% decrease, about 1% decrease to about 80% decrease, about 1% decrease to about 75% decrease, about 1% to about 70% decrease, about 1% decrease to about 65% decrease, about 1% decrease to about 60% decrease, about 1% decrease to about 55% decrease, about 1% decrease to about 50% decrease, about 1% decrease to about 45% decrease, about 1% decrease to about 40% decrease, about 1% decrease to about 35% decrease, about 1% decrease to about 30% decrease, about 1% decrease to about 25% decrease, about 1% decrease to about 20% decrease, about 1% decrease to about 15% decrease, about 1% decrease to about 10% decrease, about 1% decrease to about 5% decrease, about 5% decrease to about 99% decrease, an about 5% decrease to about 95% decrease, about 5% decrease to about 90% decrease, about 5% decrease to about 85% decrease, about 5%

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decrease) in the number of senescent cells in the subject (e.g., a decrease in the number of senescent cells in one or more specific tissues involved and/or implicated in the aging-related disease or disorder in the subject), e.g., as compared to the number of senescent cells in the subject prior to treatment.

In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus. In some embodiments, these methods can result in a decrease in the infectious titer (e.g., viral titer) in a subject (e.g., as compared to the infectious titer in the subject prior to treatment). In some embodiments, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the infectious disease (e.g., viral infection) in the subject (e.g., as compared to the number, severity, or frequency of the one or more symptoms of the infectious disease in the subject prior to treatment).

The term "subject" refers to any mammal. In some embodiments, the subject or "subject in need of treatment" may be a canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), ovine, bovine, porcine, caprine, primate, e.g., a simian (e.g., a monkey (e.g., marmoset, baboon), or an ape (e.g., a gorilla, chimpanzee, orangutan, or gibbon) or a human; or rodent (e.g., a mouse, a guinea pig, a hamster, or a rat). In some embodiments, the subject or "subject in need of treatment" may be a non-human mammal, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g., murine, lapine, porcine, canine or primate animals) may be employed.

Methods of Killing a Cancer Cell, an Infected Cell, or a Senescent Cell

Also provided herein are methods of killing a cancer cell (e.g., any of the exemplary types of cancer described herein or known in the art), an infected cell (e.g., a cell infected with any of the exemplary viruses described herein or known in the art), or a senescent cell (e.g., a senescent cancer cell, a senescent fibroblast, or a senescent endothelial cell) in a subject in need thereof (e.g., any of the exemplary subjects

described herein or known in the art) that include administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides described herein or any of the compositions (e.g., pharmaceutical compositions) described herein.

In some embodiments of these methods, the subject has been identified or diagnosed as having a cancer. Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. Non-limiting examples of aging-related diseases and conditions include Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. Non-limiting examples of an infectious disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus,

coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Senescent Cells

Senescence is a form of irreversible growth arrest accompanied by phenotypic changes, resistance to apoptosis and activation of damage-sensing signaling pathways. Cellular senescence was first described in cultured human fibroblast cells that lost their ability to proliferate, reaching permanent arrest after about 50 population doublings (referred to as the Hayflick limit). Senescence is considered a stress response that can be induced by a wide range of intrinsic and extrinsic insults, including oxidative and genotoxic stress, DNA damage, telomere attrition, oncogenic activation, mitochondrial dysfunction, or chemotherapeutic agents.

Senescent cells remain metabolically active and can influence the tissue hemostasis, disease and aging through their secretory phenotype. Senescence is considered as a physiologic process and is important in promoting wound healing, tissue homeostasis, regeneration, and fibrosis regulation. For instance, transient induction of senescent cells is observed during would healing and contributes to wound resolution. Perhaps one of the most important roles of senescence is its role in tumor suppression. However, the accumulation of senescent cells also drives aging- and aging-related diseases and conditions. The senescent phenotype also can trigger chronic inflammatory responses and consequently augment chronic inflammatory conditions to promote tumor growth. The connection between senescence and aging was initially based on observations that senescent cells accumulate in aged tissue. The use of transgenic models has enabled the detection of senescent cells systematically in many age-related pathologies. Strategies to selectively eliminate senescent cells has demonstrated that senescent cells can indeed play a causal role in aging and related pathologies.

Senescent cells display important and unique properties which include changes in morphology, chromatin organization, gene expression, and metabolism. There are several biochemical and functional properties associated with cellular senescence, such as (i) increased expression of p16 and p21, inhibitors of cyclin-dependent kinases, (ii) presence of senescence-associated β-galactosidase, a marker of lysosomal activity, (iii)

appearance of senescence-associated heterochromatin foci and downregulation of lamin B1 levels, (iv) resistance to apoptosis caused by an increased expression of anti-apoptotic BCL-family protein, and (v) upregulation of CD26 (DPP4), CD36 (Scavenger receptor), forkhead box 4 (FOXO4), and secretory carrier membrane protein 4 (SCAMP4). Senescent cells also express an inflammatory signature, the so-called senescence-associated secretory phenotype (SASP). Through SASP, the senescent cells produce a wide range of inflammatory cytokines (IL-6, IL-8), growth factors (TGF-β), chemokines (CCL-2), and matrix metalloproteinases (MMP-3, MMP-9) that operate in a cell-autonomous manner to reinforce senescence (autocrine effects) and communicate with and modify the microenvironment (paracrine effects). SASP factors can contribute to tumor suppression by triggering senescence surveillance, an immune-mediated clearance of senescent cells. However, chronic inflammation is also a known driver of tumorigenesis, and accumulating evidence indicates that chronic SASP can also boost cancer and aging-related diseases.

The secretion profile of senescent cells is context dependent. For instance, the mitochondrial dysfunction-associated senescence (MiDAS), induced by different mitochondrial dysfunction in human fibroblasts, led to the appearance of a SASP that was deficient in IL-1-dependent inflammatory factors. A decrease in the NAD+/NADH ratio activated AMPK signaling which induced MiDAS through the activation of p53. As a result, p53 inhibited NF-κB signaling which is a crucial inducer of pro-inflammatory SASP. In contrast, the cellular senescence caused by persistent DNA damage in human cells induced an inflammatory SASP, which was dependent on the activation of ataxia-telangiectasia mutated (ATM) kinase but not on that of p53. In particular, the expression and secretion levels of IL-6 and IL-8 were increased. It was also demonstrated that cellular senescence caused by the ectopic expression p16INK4a and p21CIP1 induced the senescent phenotype in human fibroblasts without an inflammatory SASP indicating that the growth arrest itself did not stimulate SASP.

One of the most defining characteristics of senescence is stable growth arrest. This is achieved by two important pathways, the p16/Rb and the p53/p21, both of which are central in tumor suppression. DNA damage results in: (1) high deposition of γH2Ax (histone coding gene) and 53BP1 (involved in DNA damage response) in chromatin: this

leads to activation of a kinase cascade eventually resulting in p53 activation, and (2) activation of p16INK4a and ARF (both encoded by CDKN2A) and P15INK4b (encoded by CDKN2B): p53 induces transcription of cyclin-dependent kinase inhibitor (p21) and along with both p16INK4a and p15INK4b block genes for cell cycle progression (CDK4 and CDK6). This eventually leads to hypophosphorylation of Retinoblastoma protein (Rb) and cell cycle arrest at the G1 phase.

Selectively killing senescent cells has been shown to significantly improve the health span of mice in the context of normal aging and ameliorates the consequences of age-related disease or cancer therapy (Ovadya, *J Clin Invest.* 128(4):1247-1254, 2018). In nature, the senescent cells are normally removed by the innate immune cells. Induction of senescence not only prevents the potential proliferation and transformation of damaged/altered cells, but also favors tissue repair through the production of SASP factors that function as chemoattractants mainly for Natural Killer (NK) cells (such as IL-15 and CCL2) and macrophages (such as CFS-1 and CCL2). These innate immune cells mediate the immunosurveillance mechanism for eliminating stressed cells. Senescent cells usually up-regulate the NK-cell activating receptor NKG2D and DNAM-1 ligands, which belong to a family of stress-inducible ligands: an important component of the frontline immune defense against infectious diseases and malignancies. Upon receptor activation, NK cells can then specifically induce the death of senescent cells through their cytolytic machinery. A role for NK cells in the immune surveillance of senescent cells has been pointed out in liver fibrosis (Sagiv, *Oncogene* 32(15): 1971-1977, 2013), hepatocellular carcinoma (Iannello, *J Exp Med* 210(10): 2057-2069, 2013), multiple myeloma (Soriani, *Blood* 113(15): 3503-3511, 2009), and glioma cells stressed by dysfunction of the mevalonate pathway (Ciaglia, *Int J Cancer* 142(1): 176-190, 2018). Endometrial cells undergo acute cellular senescence and do not differentiate into decidual cells. The differentiated decidual cells secrete IL-15 and thereby recruit uterine NK cells to target and eliminate the undifferentiated senescent cells thus helping to re-model and rejuvenate the endometrium (Brighton, *Elife* 6: e31274, 2017). With a similar mechanism, during liver fibrosis, p53-expressing senescent liver satellite cells skewed the polarization of resident Kupfer macrophages and freshly infiltrated macrophages toward the pro-inflammatory M1 phenotype, which display senolytic activity. F4/80+

macrophages have been shown to play a key role in the clearance of mouse uterine senescent cells to maintain postpartum uterine function.

Senescent cells recruit NK cells by mainly upregulating ligands to NKG2D (expressed on NK cells), chemokines, and other SASP factors. In vivo models of liver fibrosis have shown effective clearance of senescent cells by activated NK cells (Krizhanovsky, *Cell* 134(4): 657-667, 2008). Studies have described various models to study senescence including liver fibrosis (Krizhanovsky, Cell 134(4): 657-667, 2008), osteoarthritis (Xu, *J Gerontol A Biol Sci Med Sci* 72(6): 780-785, 2017), and Parkinson's disease (Chinta, *Cell Rep* 22(4): 930-940, 2018). Animal models for studying senescent cells are described in: Krizhanovsky, Cell 134(4): 657-667, 2008; Baker, *Nature* 479(7372): 232-236, 2011; Farr, *Nat Med* 23(9): 1072-1079, 2017; Bourgeois, *FEBS Lett* 592(12): 2083-2097, 2018; Xu, *Nat Med* 24(8): 1246-1256, 2018).

Additional Therapeutic Agents

Some embodiments of any of the methods described herein can further include administering to a subject (e.g., any of the subjects described herein) a therapeutically effective amount of one or more additional therapeutic agents. The one or more additional therapeutic agents can be administered to the subject at substantially the same time as the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein) or immune cell (e.g., administered as a single formulation or two or more formulations to the subject). In some embodiments, one or more additional therapeutic agents can be administered to the subject prior to administration of the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein) or immune cell. In some embodiments, one or more additional therapeutic agents can be administered to the subject after administration of the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein) or immune cell to the subject.

Non-limiting examples of additional therapeutic agents include: anti-cancer drugs, activating receptor agonists, immune checkpoint inhibitors, agents for blocking HLA-specific inhibitory receptors, Glucogen Synthase Kinase (GSK) 3 inhibitors, and antibodies.

Non-limiting examples of anticancer drugs include antimetabolic drugs (e.g., 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine, cytarabine, floxuridine, fludarabine, gemcitabine, hydroxycarbamide, methotrexate, 6-thioguanine, cladribine, nelarabine, pentostatin, or pemetrexed), plant alkaloids (e.g., vinblastine, vincristine, vindesine, camptothecin, 9-methoxycamptothecin, coronaridine, taxol, naucleaorals, diprenylated indole alkaloid, montamine, schischkiniin, protoberberine, berberine, sanguinarine, chelerythrine, chelidonine, liriodenine, clivorine, β-carboline, antofine, tylophorine, cryptolepine, neocryptolepine, corynoline, sampangine, carbazole, crinamine, montanine, ellipticine, paclitaxel, docetaxel, etoposide, tenisopide, irinotecan, topotecan, or acridone alkaloids), proteasome inhibitors (e.g., lactacystin, disulfiram, epigallocatechin-3-gallate, marizomib (salinosporamide A), oprozomib (ONX-0912), delanzomib (CEP-18770), epoxomicin, MG132, beta-hydroxy beta-methylbutyrate, bortezomib, carfilzomib, or ixazomib), antitumor antibiotics (e.g., doxorubicin, daunorubicin, epirubicin, mitoxantrone, idarubicin, actinomycin, plicamycin, mitomycin, or bleomycin), histone deacetylase inhibitors (e.g., vorinostat, panobinostat, belinostat, givinostat, abexinostat, depsipeptide, entinostat, phenyl butyrate, valproic acid, trichostatin A, dacinostat, mocetinostat, pracinostat, nicotinamide, cambinol, tenovin 1, tenovin 6, sirtinol, ricolinostat, tefinostat, kevetrin, quisinostat, resminostat, tacedinaline, chidamide, or selisistat), tyrosine kinase inhibitors (e.g., axitinib, dasatinib, encorafinib, erlotinib, imatinib, nilotinib, pazopanib, and sunitinib), and chemotherapeutic agents (e.g., all-trans retinoic acid, azacitidine, azathioprine, doxifluridine, epothilone, hydroxyurea, imatinib, teniposide, tioguanine, valrubicin, vemurafenib, and lenalidomide). Additional examples of chemotherapeutic agents include alkylating agents, e.g., mechlorethamine, cyclophosphamide, chlorambucil, melphalan, ifosfamide, thiotepa, hexamethylmelamine, busulfan, altretamine, procarbazine, dacarbazine, temozolomide, carmustine, lumustine, streptozocin, carboplatin, cisplatin, and oxaliplatin.

Non-limiting examples of activating receptor agonists include any agonists for activating receptors which activate and enhance the cytotoxicity of NK cells, including anti-CD16 antibodies (e.g., anti-CD16/CD30 bispecific monoclonal antibody (BiMAb)) and Fc-based fusion proteins. Non-limiting examples of checkpoint inhibitors include

anti-PD-1 antibodies (e.g., MEDI0680), anti-PD-L1 antibodies (e.g., BCD-135, BGB-A333, CBT-502, CK-301, CS1001, FAZ053, KN035, MDX-1105, MSB2311, SHR-1316, anti-PD-L1/CTLA-4 bispecific antibody KN046, anti-PD-L1/TGFβRII fusion protein M7824, anti-PD-L1/TIM-3 bispecific antibody LY3415244, atezolizumab, or avelumab), anti-TIM3 antibodies (e.g., TSR-022, Sym023, or MBG453) and anti-CTLA-4 antibodies (e.g., AGEN1884, MK-1308, or an anti-CTLA-4/OX40 bispecific antibody ATOR-1015). Non-limiting examples of agents for blocking HLA-specific inhibitory receptors include monalizumab (e.g., an anti-HLA-E NKG2A inhibitory receptor monoclonal antibody). Non-limiting examples of GSK3 inhibitor include tideglusib or CHIR99021. Non-limiting examples of antibodies that can be used as additional therapeutic agents include anti-CD26 antibodies (e.g., YS110), anti-CD36 antibodies, and any other antibody or antibody construct that can bind to and activate an Fc receptor (e.g., CD16) on a NK cell. In some embodiments, an additional therapeutic agent can be insulin or metformin.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1. Construction of exemplary multi-chain chimeric polypeptides and evaluation of properties thereof

Two multi-chain chimeric polypeptides were generated and their properties were evaluated. Each of the two multi-chain chimeric polypeptides includes a first chimeric polypeptide that includes a soluble tissue factor domain covalently linked a first target-binding domain and a first domain of an affinity pair of domains. The second chimeric polypeptide in each of the two multi-chain chimeric polypeptides includes a second domain of the affinity pair of domains, and a second target-binding domain.

Description of logic underlying construction of multi-chain chimeric polypeptides

Tissue Factor (TF) is a stable, transmembrane protein containing 236 amino acid residues. The truncated, recombinant 219-amino-acid extracellular domain of tissue

factor is soluble and is known to be expressed at high levels in bacteria or mammalian cells. Without wishing to be bound to a particular theory, the applicants speculated that the 219-aa tissue factor could be used as a connector linker for creation of unique multi-chain chimeric polypeptides.

First chimeric polypeptides including soluble tissue factor domain were produced at high levels by CHO cells grown in fermentation broth. These first chimeric polypeptides were purified by an anti-tissue factor monoclonal antibody (mAb) coupled on a solid matrix. Notably, tissue factor contains binding sites for FVIIa and FX. The catalytic activity of the tissue factor-FVIIa complex for FX is approximately 1 million-fold lower when tissue factor is not anchored to a phospholipid bilayer. Thus, without wishing to be bound to a particular theory, applicants speculated that using the 219-aa extracellular domain of tissue factor without the transmembrane in construction of the first chimeric polypeptides may eliminate the pro-coagulation activity of tissue factor in the first chimeric polypeptides. In an effort to further reduce or eliminate the pro-coagulation activity of the 219-aa tissue factor, select mutations in tissue factor can be made, specifically at seven amino acid residues that are known to contribute to binding energy of the FVIIa binding site.

Characterization of binding interactions for described chimeric polypeptides

To determine if the first and second chimeric polypeptides bind to each other to form multi-chain chimeric polypeptides, *in vitro* binding assays were performed. To determine if the first chimeric polypeptide comprising soluble tissue factor domain are recognized and bound by anti-TF mAb, *in vitro* binding assays were performed. Notably, the data indicated that the mutated tissue factor proteins are still recognized and selectively bound by the anti-TF mAb which is known to bind to the FX binding site on tissue factor. To determine if the first chimeric polypeptides comprising soluble tissue factor domain covalently linked to scFvs or cytokines (see Figure 1 and Figure 2) possess functional scFvs or cytokines, *in vitro* binding assays were performed. The data from the aforementioned assays were consistent with the purified first chimeric polypeptides having the expected biological activities (e.g. scFvs selectively bind expected target antigens or cytokines selectively bind expected receptors or binding proteins).

In addition, experiments performed using the two multi-chain chimeric polypeptides including a first and second chimeric polypeptide bound to each other demonstrate the expected target binding activity (e.g., the multi-chain chimeric polypeptide binds specifically to the target specifically recognized by the first target-binding domain and the target specifically recognized by the second target-binding domain).

Based on the aforementioned results, applicants concluded that the soluble tissue factor connecter linker provided or enabled appropriate display of the polypeptides encoding either scFvs, interleukins, cytokines, interleukin receptors, or cytokine receptors in three-dimensional space relative to soluble tissue factor domain and relative to one another such that each retained expected biological properties and activities.

When both the first and second chimeric polypeptides were co-expressed, the heterodimeric complexes were secreted into the fermentation broths at high levels. The complexes were captured and readily purified by anti-TF mAb conjugated to a solid matrix using affinity chromatography. The first and second target-binding domains of these multi-chain chimeric polypeptides retained their expected biological activities as assayed by *in vitro* binding assays. Thus, the assembly of the multi-chain chimeric polypeptides provides the appropriate spatial display and folding of the domains for biological activities. Importantly, the spatial arrangement of the multi-chain chimeric polypeptides does not interfere with the FX binding site on tissue factor which enables the use of anti-TF mAb for affinity purification.

Characterization of stability for described chimeric polypeptides

Both purified multi-chain chimeric polypeptides are stable. These multi-chain chimeric polypeptides are structurally intact and fully biologically active when they are incubated in human serum at 37 ºC for 72 hours.

Characterization of propensity of described chimeric polypeptides to aggregate

Both purified multi-chain chimeric polypeptides developed do not form aggregates when stored at 4 °C in PBS.

Characterization of viscosity of described chimeric polypeptides

There is no viscosity issue when the multi-chain chimeric polypeptides are formulated at a concentration as high as 50 mg/mL in PBS.

Addditional applications of the multi-chain chimeric polypeptide platform

The data from these studies show that the platform technologies described herein can be utilized to create molecules that could be fused to target-binding domains derived from antibodies, in any of the formats as described herein including, without limitation, adhesion molecules, receptors, cytokines, ligands, and chemokines. With the appropriate target-binding domain, the resulting multi-chain chimeric polypeptides could promote conjugation of various immune effector cells and mediate destruction of target cells, including cancer cells, virally-infected cells, or senescent cells. Other domains in the multi-chain chimeric polypeptides stimulate, activate, and attract the immune system for enhancing cytotoxicity of effector cells for the targeted cells.

Example 2: Creation of an IL-12/IL-15RαSu DNA construct

In a non-limiting example, an IL-12/IL-15RαSu DNA construct was created (Figure 3). The human IL-12 subunit sequences, human IL-15RαSu sequence, human IL-15 sequence, human tissue factor 219 sequence, and human IL-18 sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-12 subunit beta (p40) to IL-12 subunit alpha (p35) with a GS (3) linker to generate a single chain version of IL-12 and then directly linking the IL-12 sequence to the IL-15RαSu sequence. The final IL-12/IL-15RαSu DNA construct sequence was synthesized by Genewiz.

The nucleic acid sequence of the IL12/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 77):

(*Signal peptide*)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCT
ACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTAT
CCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAA
GACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGC
CACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGG
AAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGA
ATAAGACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGT
TGGTGGCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCG
GGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCT
GAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAA
GAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGG
TGGACGCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATC
CGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCA
AAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCAC
ACCCCACAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCA
AGCGGGAGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCA
TCTGTCGGAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCC
AGCAGCTGGTCCGAGTGGGCCAGCGTGCCTTGTTCC

(Linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human IL-12 subunit alpha (p35))

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTAC
ACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAG
GCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT
ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGA
CAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGG
CTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTC
CATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCC
AAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGG
CTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCT

CAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGT
GCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATG
AGCTATTTAAACGCCAGC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

Example 3: Creation of an IL-18/TF/IL-15 DNA construct

In a non-limiting example, an IL-18/TF/IL-15 construct was made (Figure 4) linking the IL-18 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-18/TF construct with the N-terminus coding region of IL-15. The nucleic acid sequence of the IL-18/TF/IL-15 construct (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 73):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCT
ACAGC

(Human IL-18)

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAAC
GACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGAC
CGACTCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGT
ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGA
GAAAATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATG
AACCCCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGC
GGTCCGTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGA
GGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCA
AGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGA
GGAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 4: Secretion of IL-12/IL-15RαSu and IL-18/TF/IL-15 fusion proteins

The IL-12/IL-15RαSu and IL-18/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of a soluble IL-18/TF/IL-15:IL-12/IL-15RαSu protein complex (referred to as 18t15-12s; Figure 5 and Figure 6). The 18t15-12s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody affinity

chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-12/IL-15RαSu and IL-18/TF/IL-15 fusion proteins.

The amino acid sequence of the IL12/IL-15RαSu fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 76):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-12 subunit beta (p40))

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS
GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV
RGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD
PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF
TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

(*Linker*)

GGGGSGGGGSGGGGS

(*Human IL-12 subunit alpha (p35))*

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHE
DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS
LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

The amino acid sequence of the IL-18/TF/IL-15 fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 72):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISM YKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPG HDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

In some cases, the leader (signal sequence) peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

Example 5: Purification of 18t15-12s by immunoaffinity chromatography

An anti-TF antibody affinity column was connected to a GE Healthcare™ AKTA Avant protein purification system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Cell culture harvest of 18t15-12s was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After loading the sample, the column was washed with 5 column volumes PBS, followed by elution with 6 column volumes 0.1M acetic acid, pH 2.9. Absorbance at 280 nm was collected and then the sample was neutralized to pH 7.5-8.0 by adding 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon® centrifugal filters with a 30 KDa molecular weight cutoff. Figure 7 shows that the 18t15-12s complex binds the anti-TF antibody affinity column, wherein TF is an 18t15-12s binding partner. The buffer-exchanged protein sample is stored at 2-8°C for further biochemical analysis and biological activity testing.

After each elution, the anti-TF antibody affinity column was then stripped using 6 column volumes 0.1M glycine, pH 2.5. The column was then neutralized using 10 column volumes PBS, 0.05% sodium azide and stored at 2-8°C.

Example 6: Size exclusion chromatography of 18t15-12s

A GE Healthcare Superdex® 200 Increase 10/300 GL gel filtration column was connected to a GE Healthcare AKTA™ Avant protein purification system. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.8 mL/min. A capillary loop was used to inject 200µL of 1 mg/mL of 18t15-12s complex onto the column. The injection was chased with 1.25 column volumes of PBS. The SEC chromatograph is shown in Figure 8. There is a main 18t15-12s protein peak with a minor high molecular weight peak, likely due to differing degrees of glycosylation of 18t15-12s dimers or aggregates.

Example 7: SDS-PAGE of 18t15-12s

To determine the purity and protein molecular weight, the purified 18t15-12s protein sample was analyzed using 4-12% NuPage Bis-Tris protein gel SDS-PAGE. The gel was stained with InstantBlue™ for about 30 min, followed by destaining overnight in purified water. Figure 9 shows an example SDS gel of anti-TF antibody affinity purified 18t15-12s, with bands at the expected molecular weights (66 kDa and 56 kDa).

Example 8: Glycosylation of 18t15-12s in CHO-K1 cells

Glycosylation of 18t15-12s in CHO-K1 cells was confirmed using the Protein Deglycosylation Mix II kit (New England Biolabs), according to the manufacturer's instructions. Figure 10 shows an example SDS PAGE of deglycosylated and non-deglycosylated 18t15-12s. Deglycosylation reduces the molecular weight of 18t15-12s as seen in Figure 10, lane 4.

Example 9: Recombinant protein quantitation of 18t15-12s complexes

The 18t15-12s complex was detected and quantified using standard sandwich ELISA methods (Figures 11-14). Anti-human tissue factor antibody served as the

capture antibody and biotinylated anti-human IL-12, IL-15, or IL-18 antibody (BAF 219, BAM 247, D045-6, all R&D Systems) served as the detection antibody. Tissue factor in purified 18t15-12s protein complexes was also detected using an anti-human tissue factor capture antibody (I43), and anti-human tissue factor antibody detection antibody. The I43/ anti-TF antibody ELISA was compared to purified tissue factor at similar concentrations.

Example 10: Immunostimulatory capacity of the 18t15-12s complex

To assess the IL-15 immunostimulatory activity of the 18t15-12s complex, increasing concentrations of 18t15-12s was added to 32Dβ cells (104 cell/well) in 200 µL IMDM:10% FBS media. The 32Dβ cells were incubated for 3 days at 37°C. On the fourth day, WST-1 proliferation reagent (10 µL/well) was added and after 4 hours, absorbance was measured at 450 nm to determine cell proliferation based on cleavage of WST-1 to a soluble formazan dye. Bioactivity of human recombinant IL-15 was assessed as a positive control. As shown in Figure 15, 18t15-12s demonstrated IL-15-dependent cell proliferation of 32Dβ cells. The 18t15-12s complex demonstrated reduced activity compared to human recombinant IL-15, possibly due to the linkage of IL-18 and tissue factor to the IL-15 domain.

In order to assess the individual activities of IL-12 and IL-18 in the 18t15-12s complex, 18t15-12s was added to HEK-Blue IL-12 and HEK-Blue IL-18 reporter cells ($5x10^4$ cell/well; hkb-il12 and hkb-hmil18, InvivoGen) in 200 µL IMDM:10% heat-inactivated FBS media. Cells were incubated for overnight at 37°C. 20 µl of induced HEK-Blue IL-12 and HEK-Blue IL-18 reporter cell supernatant was added to 180 µl of QUANTI-Blue (InvivoGen), and incubated for 1-3 hours at 37°C. IL-12 or IL-18 activity was assessed by measuring absorbance at 620 nm. Human recombinant IL-12 or IL-18 was assessed as a positive or negative control. As shown in Figure 16 and Figure 17, each of the cytokine domains of the 18t15-12s complex retain specific biological activity. The activity of 18t15-12s was reduced compared to that of human recombinant IL-18 or IL-12, possibly due to linkage of IL-15 and tissue factor to the IL-18 domain and linkage of IL-12 to the IL-15Rα sushi domain.

Example 11: Induction of cytokine-induced memory-like NK cells by the 18t15-12s complex

Cytokine-induced memory-like NK cells can be induced *ex vivo* following overnight stimulation of purified NK cells with saturating amounts of IL-12 (10 ng/mL), IL-15 (50 ng/mL), and IL-18 (50 ng/mL). These memory-like properties have been measured through expression of IL-2 receptor α (IL-2Rα, CD25), CD69 (and other activation markers), and increased IFN-γ production. To evaluate the ability of 18t15-12s complexes to promote generation of cytokine-induced memory-like NK cells, purified human NK cells (>95% CD56+) were stimulated for 14-18 hours with 0.01nM to 10000 nM of the 18t15-12s complex or a combination of individual cytokines (recombinant IL-12 (10 ng/mL), IL-18 (50 ng/mL), and IL-15 (50 ng/mL)). Cell-surface CD25 and CD 69 expression and intracellular IFN-γ levels were assessed by antibody-staining and flow cytometry.

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with antibodies specific to CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 0.2 x 10^6/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either a mixture of cytokines hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL) (R&D Systems) and hIL-15 (50 ng/mL) (NCI) or with 0.01 nM to 10000nM of the 18t15-12s at 37°C, 5% CO_2 for 14-18 hrs. The cells were then harvested and surface stained with antibodies specific to CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend) for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone), with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, cells were analyzed using a BD FACSCelesta™ flow cytometer (Plotted Data-Mean Fluorescence Intensity; Figure. 18A and Figure 18B).

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 10^6/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either a cytokine mix of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL) (R&D), and hIL-15 (50 ng/mL) (NCI), or 0.01 nM to 10000 nM of the 18t15-12s complex at 37°C, 5% CO2 for 14-18 hrs. The cells were then treated with 10 µg/mL of Brefeldin A (Sigma) and 1X of Monensin (eBioscience) for 4 hrs before harvesting and staining with antibodies specific to CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes in room temperature) in FACS buffer (1X PBS (Hyclone), with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) and fixed for 10 minutes at room temperature. After fixation, cells were washed (1500 RPM for 5 minutes in room temperature) in 1x permeabilized buffer (eBioscience) and stained with IFN-γ- PE Ab (Biolegend) for 30 minutes at room temperature. Cells were washed once again with 1x permeabilized buffer and then washed with FACS buffer. Cell pellets were resuspended in 300 µls of FACS buffer and analyzed using a BD FACSCelesta™ flow cytometer (Plotted % of IFN-γ Positive Cells; Figure 19).

Example 12: In vitro cytotoxicity of NK cells against human tumor cells

Human myelogenous leukemia cells, K562 (CellTrace violet labelled), were incubated with purified human NK cells in the presence of increasing concentrations of the 18t15-12s complex or a mixture of cytokines as a control. After 20 hours, the cultures were harvested, stained with propidium iodide (PI), and assessed by flow cytometry. As shown in Figure 20, the 18t15-12s complex induced human NK cytotoxicity against K562, at levels similar or greater than the cytokine mixture, wherein both the 18t15-12s complex and the cytokine mixture induced greater cytotoxicity than the medium control.

Example 13: Creation of IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 DNA constructs

In a non-limiting example, IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 DNA constructs were created (Figure 21 and Figure 22). The human IL-12 subunit sequences, human IL-15RαSu sequence, human IL-15 sequence, human tissue factor 219 sequence, and human IL-18 sequence were synthesized by Genewiz. A DNA construct was made linking the IL-12 subunit beta (p40) to IL-12 subunit alpha (p35) with a GS (3) linker to generate a single chain version of IL-12, directly linking the IL-12 sequence to the IL-15RαSu sequence, and directly linking the IL-12/ IL-15RαSu construct to the N-terminus coding region of αCD16scFv.

The nucleic acid sequence of the IL-12/IL-15RαSu/αCD16scFv construct is as follows (SEQ ID NO: 123):

(Signal peptide)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTAT
CCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAA
GACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGC
CACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGG
AAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGA
ATAAGACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGT
TGGTGGCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCG
GGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCT
GAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAA
GAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGG
TGGACGCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATC
CGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCA
AAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCAC
ACCCCACAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCA

AGCGGGAGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCA
TCTGTCGGAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCC
AGCAGCTGGTCCGAGTGGGCCAGCGTGCCTTGTTCC

(*Linker)*

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(*Human IL-12 subunit alpha (p35)*)

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTAC
ACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAG
GCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT
ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGA
CAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGG
CTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTC
CATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCC
AAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGG
CTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCT
CAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGT
GCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATG
AGCTATTTAAACGCCAGC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(anti-Human CD16 light chain variable domain)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCAT

(Linker)

GGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAGGATCC

(anti-Human CD16 heavy chain variable domain)

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGG
CTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCA
TGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCAT
CAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTC
ACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCC
TGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCT
GTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

Constructs were also made linking the IL-18 sequence to the N-terminus coding region of tissue factor 219, and linking the IL-18/TF construct with the N-terminus coding region of IL-15 (Figure 22). The nucleic acid sequence of the IL-18/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 73):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCT
ACAGC

(Human IL-18)

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAAC
GACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGAC
CGACTCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGT
ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGA
GAAAATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATG
AACCCCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGC
GGTCCGTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGA
GGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCA
AGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGA
GGAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT

TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 14: Secretion of IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 fusion proteins

The IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 constructs were cloned into a pMSGV-1 modified retrovirus expression vector (Hughes, *Hum Gene Ther* 16:457–72, 2005, herein incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells resulted in secretion of a soluble IL-18/TF/IL-15:IL-12/IL-15RαSu/αCD16scFv protein complex (referred to as 18t15-12s/αCD16; Figure 23 and 24). Co-expression of the two constructs in CHO-K1 cells resulted in secretion of the soluble IL-18/TF/IL-15:IL-12/IL-15RαSu/αCD16scFv protein complex (referred to as 18t15-12s/αCD16; Figure 23 and Figure 24), which can be purified by anti-TF Ab affinity and other chromatography

methods. In some cases, the signal peptide is cleaved from the intact polypeptide to generate the mature form.

The amino acid sequence of the IL-12/IL-15RαSu/αCD16scFv fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 122):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-12 subunit beta (p40))

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

(*Linker*)

GGGGSGGGGSGGGGS

(*Human IL-12 subunit alpha (p35))*

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHE DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

(anti-Human CD16 light chain variable domain)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL TVGH

(*Linker*)

GGGGSGGGGSGGGGS

(anti-Human CD16 heavy chain variable domain)

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV
SGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRS
LLFDYWGQGTLVTVSR

The amino acid sequence of the IL-18/TF/IL-15 fusion protein (including leader sequence) is as follows (SEQ ID NO: 72):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISM
YKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPG
HDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Example 15: Creation of IL-18/IL-15RαSu and IL-12/TF/IL-15 DNA constructs

In a non-limiting example, IL-18/IL-15RαSu and IL-12/TF/IL-15 DNA constructs were created. The human IL-18 subunit sequences, human IL-15RαSu sequence, human IL-12 sequence, human tissue factor 219 sequence, and human IL-15 sequence were synthesized by Genewiz. A DNA construct was made linking IL-18 directly to IL-15RαSu. An additional construct was also made linking IL-12 sequence to the N-terminus coding region of human tissue factor 219 form, and further linking the IL-12/TF

construct to the N-terminus coding region of IL-15. As described above, a single-chain version of IL-12 (p40-linker-p35) was used.

The nucleic acid sequence of the IL-18/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 217):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCT
ACAGC

(Human IL-18)

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAAC
GACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGAC
CGACTCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGT
ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGA
GAAAATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATG
AACCCCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGC
GGTCCGTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGA
GGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCA
AGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGA
GGAT

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The nucleic acid sequence of the IL-12/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 218):

(Signal peptide)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCT
ACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTAT
CCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAA
GACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGC
CACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGG
AAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGA
ATAAGACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGT
TGGTGGCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCG
GGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCT
GAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAA
GAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGG
TGGACGCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATC
CGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCA
AAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCAC
ACCCCACAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCA
AGCGGGAGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCA
TCTGTCGGAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCC
AGCAGCTGGTCCGAGTGGGCCAGCGTGCCTTGTTCC

(*Linker*)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(*Human IL-12 subunit alpha (p35)*)

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTAC
ACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAG
GCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT
ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGA
CAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGG
CTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTC
CATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCC
AAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGG
CTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCT
CAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGT

GCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATG
AGCTATTTAAACGCCAGC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 16: Secretion of IL-18/IL-15RαSu and IL-12/TF/IL-15 fusion proteins

The IL-18/IL-15RαSu and IL-12/TF/IL-15 constructs were cloned into a pMSGV-1 modified retrovirus expression vector (Hughes, *Hum Gene Ther* 16:457–72, 2005 herein incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells resulted in secretion of a

soluble IL-12/TF/IL-15:IL-18/IL-15RαSu protein complex (referred to as 12t15/s18), which can be purified by anti-TF Ab affinity and other chromatography methods.

The amino acid sequence of the IL-18/IL-15RαSu fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 219):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISM YKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPG HDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

The amino acid sequence of the IL-12/TF/IL-15 fusion protein (including leader sequence) is as follows (SEQ ID NO: 220):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-12 subunit beta (p40))

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

(Linker)

GGGGSGGGGSGGGGS

(Human IL-12 subunit alpha (p35))

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHE DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE

DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

Example 17: Recombinant protein quantitation of the 18t15-12s16 complex

The 18t15-12s16 complex (comprising IL-12/IL-15RαSu/αCD16scFv;IL-18/TF/IL-15) was detected and quantified using standard sandwich ELISA methods (Figure 25). Anti-human tissue factor antibody /IL-2 or anti-TF Ab/IL-18 served as the capture antibody and biotinylated anti-human IL-12 or IL-18 antibody (BAF 219, D045-6, both R&D Systems) served as the detection antibody. Tissue factor was also detected using an anti-human tissue factor antibody (I43), and anti-human tissue factor antibody detection antibody.

Example 18: Creation of TGFβRII/IL-15RαSu and IL-21/TF/IL-15 DNA constructs

In a non-limiting example, a TGFβRII/IL-15RαSu DNA construct was created (Figure 26). The human TGFβRII dimer and human IL-21 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the TGFβRII to another TGFβRII with a linker to generate a single chain version of TGFβRII and then directly linking the TGFβRII single chain dimer sequence to the N-terminal coding region of IL-15RαSu.

The nucleic acid sequences of the TGFβRII/IL-15RαSu construct (including signal sequence) is as follows (SEQ ID NO: 93):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβRII-1st fragment)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT

(*Linker)*

GGAGGTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGT

(Human TGFβRII-2nd fragment)

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACC
GATAACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGA
GGTTTTCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCACAATCAC
CTCCATCTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAAT
GACGAGAATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACC
ACGATTTCATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAA
AAAGAAGCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGC
AACGACAATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATCACGTGTCCTCCTCCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAA
GCGTAAAGCCGGCACGTCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACG
AATGTCGCCCACTGGACAACCCCCAGTCTCAAATGTATTAGA

Additionally, an IL-21/TF/IL-15 construct was made linking the IL-21 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-21/TF construct to the N-terminus coding region of IL-15 (Figure 27). The nucleic acid sequence of the IL-21/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 89):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human Tissue Factor 219)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCAAGACAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC
CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAAC
ACATTTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTA
CTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAAC
GAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAG
CCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGA
GTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 19: Secretion of TGFβRII/IL-15RαSu and IL-21/TF/IL-15 fusion proteins

The TGFβRII/IL-15RαSu and IL-21/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described in Hughes et al., *Hum Gene Ther* 16:457–72, 2005, herein incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells resulted in secretion of the soluble IL-21/TF/IL-15:TGFβRII/IL-15RαSu protein complex (referred to as 21t15-TGFRs; Figure 28 and Figure 29). The 21t15-TGFRs complex was purified from CHO-K1 cell culture supernatant using anti-TF antibody affinity chromatography and other chromatography methods.

The amino acid sequence of the TGFβRII/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 92):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human TGFβRII-1st fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Linker)

GGGGSGGGGSGGGGS

(Human TGFβRII-2nd fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

The amino acid sequence of the mature IL-21/TF/IL-15 fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 88):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate a mature form that may be soluble or secreted.

Example 20: Purification of 21t15-TGFRs by immunoaffinity chromatography

An anti-TF antibody affinity column was connected to a GE Healthcare AKTA™ Avant protein purification system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Cell culture harvest of 21t15-TGFRs was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After loading the sample, the column was washed with 5 column volumes PBS, followed by elution with 6 column volumes 0.1M acetic acid, pH 2.9. Absorbance at 280 nm was collected and then the sample was then neutralized to pH 7.5-8.0 by adding 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon® centrifugal filters with a 30 KDa molecular weight cutoff. Figure 30 shows that the 21t15-TGFRs complex binds anti-TF antibody affinity column, wherein TF is a 21t15-TGFRs binding partner. The buffer-exchanged protein sample is stored at 2-8°C for further biochemical analysis and biological activity testing.

After each elution, the anti-TF antibody affinity column was then stripped using 6 column volumes 0.1M glycine, pH 2.5. The column was then neutralized using 10 column volumes PBS, 0.05% sodium azide, and stored at 2-8°C.

Example 21: Size exclusion chromatography of 21t15-TGFRs

A GE Healthcare Superdex® 200 Increase 10/300 GL gel filtration column was connected to a GE Healthcare AKTA™ Avant protein purification system. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.8 mL/min. A capillary loop was used to inject 200µL of 1 mg/mL of 21t15-TGFRs complex onto the column. The injection was then chased with 1.25 column volumes of PBS. The SEC chromatograph was shown in Figure 31. There were two protein peaks, likely representing a monomer and dimer forms of 21t15-TGFRs.

Example 22: SDS-PAGE of 21t15-TGFRs

To determine the purity and protein molecular weight, the purified 21t15-TGFRs complex protein sample was analyzed using 4-12% NuPage Bis-Tris protein gel SDS-PAGE under reduced conditions. The gel was stained with InstantBlue™ for about 30

min, followed by destaining overnight in purified water. Figure 32 shows an example SDS gel of anti-TF antibody affinity purified 21t15-TGFRs, with bands at 39.08 kDa and 53 kDa

Glycosylation of 21t15-TGFRs in CHO cells was confirmed using the Protein Deglycosylation Mix II kit (New England Biolabs) and the manufacturer's instructions. Deglycosylation reduces the molecular weight of 21t15-TGFRs, as seen in lane 4 of Figure 32.

Example 23: Recombinant protein quantitation of 21t15-TGFRs complexes

The 21t15-TGFRs complex was detected and quantified using standard sandwich ELISA methods (Figures. 33-37). Anti-human tissue factor antibody served as the capture antibody and biotinylated anti-human IL-21, IL-15, or TGFβRII served as the detection antibody. Tissue factor was also detected using an anti-human tissue factor capture antibody (I43), and anti-human tissue factor antibody detection antibody. The I43/anti-TF antibody ELISA was compared to purified tissue factor at similar concentrations.

Example 24: Immunostimulatory capacity of the 21t15-TGFRs complex

To assess the IL-15 immunostimulatory activity of the 21t15-TGFRs complexes, increasing concentrations of 21t15-TGFRs was added to 32Dβ cells (10^4 cell/well) in 200 µL IMDM:10% FBS media and cells were incubated for 3 days at 37°C. On the fourth day, WST-1 proliferation reagent (10 µL/well) then was added and after 4 hours, absorbance was measured at 450 nm to determine cell proliferation based on cleavage of WST-1 to a soluble formazan dye. Bioactivity of the human recombinant IL-15 was assessed as a positive control. As shown in Figure 37, 21t15-TGFRs demonstrated IL-15-dependent 32Dβ cell proliferation. The 21t15-TGFRs complex was reduced compared to that of human recombinant IL-15, possibly due to the linkage of IL-21 and tissue factor to the IL-15 domain.

Additionally, HEK-Blue TGFβ reporter cells (hkb-tgfb, InvivoGen) were used to measure the ability of 21t15-TGFRs to block TGFβ1 activity (Figure 38). Increasing concentrations of 21t15-TGFRs were mixed with 0.1 nM of TGFβ1

and added to HEK-Blue TGFβ reporter cells ($2.5x10^4$ cell/well) in 200 µL IMDM:10% heat-inactivated FBS media. Cells were incubated overnight at 37°C. The next day, 20 µl of induced HEK-Blue TGFβ reporter cell supernatant was added to 180 µl of QUANTI-Blue (InvivoGen) and incubated for 1-3 hours at 37°C. 21t15-TGFRs activity was assessed by measuring absorbance at 620 nm. Human recombinant TGFβRII/Fc activity was assessed as a positive control.

These results demonstrate that TGFβRII domain of the 21t15-TGFRs complex retains its ability to trap TGFβ1. The ability of 21t15-TGFRs to block TGFβ1 activity was reduced compared to that of human recombinant TGFβRII/Fc, possibly due to the linkage of TGFβRII to the IL-15Rα sushi domain.

Example 25: Induction of cytokine-induced memory-like NK cells by the 21t15-TGFRs complex

Cytokine-induced memory-like NK cells can be induced *ex vivo* following overnight stimulation of purified NK cells with saturating amounts of cytokines. These memory-like properties can be measured through expression of IL-2 receptor α (IL-2Rα, CD25), CD69 (and other activation markers), and increased IFN-γ production. To evaluate the ability of 21t15-TGFRs complexes to promote generation of cytokine-induced memory-like NK cells, purified human NK cells (>95% CD56+) were stimulated for 14-18 hours with 1 nM to 100 nM of the 21t15-TGFRs complex. Cell-surface CD25 and CD 69 expression and intracellular IFN-γ levels were assessed by antibody-staining and flow cytometry.

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 10^6/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-21 (50 ng/mL) (Biolegend) and hIL-15 (50 ng/mL) (NCI) or with 1 nM,

10 nM, or 100 nM 21t15-TGFRs complex overnight at 37°C, 5% CO_2 for 14-18 hrs. The cells were then harvested and surface stained with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, cells were analyzed using a BD FACSCelesta™ flow cytometer. (Plotted Data-Mean Fluorescence Intensity; Figure 39 and Figure 40).

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 106/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-21 (50 ng/mL) (Biolegend) and hIL-15 (50 ng/mL) (NCI) or with 1 nM, 10 nM, or 100 nM 21t15-TGFRs complex overnight at 37°C, 5% CO2 for 14-18 hrs. The cells were then treated with 10 µg/mL of Brefeldin A (Sigma) and 1X of Monensin (eBioscience) for 4 hrs. Cells were harvested and surface stained with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) and fixed for 10 minutes at room temperature. After fixation, cells were washed (1500 RPM for 5 minutes at room temperature) with 1x permeabilized buffer (eBioscience) and stained for intracellular IFN-γ- PE Ab (Biolegend) for 30 minutes at room temperature. Cells were washed once again with 1x permeabilized buffer and then washed with FACS buffer. Cell pellets were resuspended in 300 µls of FACS Buffer and analyzed using a BD FACSCelesta™ flow cytometer. (Plotted % of IFN-γ Positive Cells; Figure 41).

Example 26: In vitro cytotoxicity of NK cells against human tumor cells

K562 (CellTrace violet labelled), human myelogenous leukemia cells, were incubated with purified human NK cells (using StemCell human NK cell purification kit (E:T ratio; 2:1)) in the presence of increasing concentrations of the 21t15-TGFRs complex. After 20 hours, the cultures were harvested, stained with propidium iodide (PI), and assessed by flow cytometry. As shown in Figure 42, the 21t15-TGFRs complex induced human NK cytotoxicity against K562, as compared to control.

Example 27: Creation of an IL-21/TF mutant/IL-15 DNA construct and resulting fusion protein complex with TGFβRII /IL-15RαSu

In a non-limiting example, an IL-21/TF mutant/IL-15 DNA construct was made by linking IL-21 directly to the N-terminus coding region of a tissue factor 219 mutant, and further linking the IL-21/TF mutant to the N-terminus coding region of IL-15.

The nucleic acid sequence of the IL-21/TF mutant/IL-15 construct (including signal peptide sequence) is as follows (SEQ ID NO: 221, shaded nucleotides are mutant and the mutant codons are underlined):

(Signal sequence)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human Tissue Factor 219 mutants)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCGCGACAGCTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGCTTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC
CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGGCGCGGAATAA
CACAGCTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGT
ACTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAA
CGAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAA
GCCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTG
AGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of the IL-21/TF mutant/IL-15 construct (including signal peptide sequence) is as follows (SEQ ID NO: 222, substituted residues are shaded):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTKLEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate a mature form that may be soluble or secreted.

In some embodiments, the IL-21/TF mutant/IL-15 DNA construct may be combined with an TGFβRII /IL-15RαSu DNA construct, transfected into cells using a retroviral vector as described above, and expressed as IL-21/TF mutant/IL-15 and TGFβRII/IL-15RαSu fusion proteins. The IL-15RαSu domain of the TGFβRII/IL-15RαSu fusion protein binds to the IL-15 domain of the IL-21/TF mutant/IL-15 fusion protein to create an IL-21/TF mutant/IL-15:TGFβRII /IL-15RαSu complex.

Example 28: Creation of IL-21/IL-15RαSu and TGFβRII/TF/IL-15 DNA constructs and the resulting fusion protein complex

In a non-limiting example, an IL-21/IL-15RαSu DNA construct was made by linking IL-21 directly to the IL-15RαSu subunit sequence. The nucleic acid sequence of the IL-21/IL-15RαSu construct (including signal sequence) is as follows (SEQ ID NO: 111):

(Signal sequence)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of the IL-21/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 110):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

In some cases, the leader peptide is cleaved from the intact polypeptide to generate a mature form that may be soluble or secreted.

In some embodiments, the IL-21/IL-15RαSu DNA construct may be combined with a TGFβRII/TF/IL-15 DNA construct, transfected into a retroviral vector as described above, and expressed as IL-21/IL-15RαSu and TGFβRII/TF/IL-15 fusion

proteins. The IL-15RαSu domain of the IL-21/IL-15RαSu fusion protein binds to the IL-15 domain of the TGFβRII/TF/IL-15 fusion protein to create a TGFβRII/TF/IL-15:IL-21/IL-15RαSu complex.

The TGFβRII/TF/IL-15RαSu DNA construct was created by linking the TGFβRII sequence to the N-terminus coding region of human tissue factor 219 form, and then linking the TGFβRII/TF construct to the N-terminus coding region of IL-15. As described above, a single-chain version of TGFβRII (TGFβRII-linker-TGFβRII) was used. The nucleic acid sequence of the TGFβRII/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 136):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβRII-1st fragment)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT

(*Linker)*

GGAGGTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGT

(Human TGFβRII-2nd fragment)

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACC
GATAACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGA
GGTTTTCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCACAATCAC
CTCCATCTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAAT
GACGAGAATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACC
ACGATTTCATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAA

AAAGAAGCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGC
AACGACAATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCAAGACAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC
CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAAC
ACATTTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTA
CTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAAC
GAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAG
CCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGA
GTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of the TGFβRII/TF/IL-15 fusion protein (including signal peptide) is as follows (SEQ ID NO: 135):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human TGFβRII-1st fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Linker)

GGGGSGGGGSGGGGS

(Human TGFβRII-2nd fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

Example 29: Creation of an IL-7/IL-15RαSu DNA construct

In a non-limiting example, an IL-7/IL-15RαSu DNA construct was created (see Figure 43). The human IL-7 sequence, human IL-15RαSu sequence, human IL-15 sequence, and human tissue factor 219 sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-7 sequence to the IL-15RαSu sequence. The final IL-7/IL-15RαSu DNA construct sequence was synthesized by Genewiz.

The nucleic acid sequence encoding the second chimeric polypeptide of IL-7/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 103):

(Signal peptide)

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGG
CC

(Human IL-7)

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTA
ATGGTCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATT
GCCTGAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAG
GAAGGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAAT
GAATAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAA
CAATACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCT
GGGTGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGA
ACAGAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATA
AAAACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC

(Human IL-15R α sushi domain)

ATCACGTGCCCTCCCCCCATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAA
GCGTAAAGCCGGCACGTCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACG
AATGTCGCCCACTGGACAACCCCCAGTCTCAAATGCATTAGA

The second chimeric polypeptide of IL-7/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 102):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL

ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Example 30: Creation of an IL-21/TF/IL-15 DNA construct

In a non-limiting example, an IL-21/TF/IL-15 construct was made (Figure 44) by linking the IL-21 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-21/TF construct with the N-terminus coding region of IL-15.

The nucleic acid sequence encoding the first chimeric polypeptide of IL-21/TF/IL-15 construct (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 89):

(Signal peptide)

ATGGGAGTGAAAGTTCTTTTGCCCTTATTTGTATTGCTGTGGCCGAGG
CC

(Human IL-21 fragment)

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATT
GTTGATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGC
TCCAGAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGA
AGGCCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATG
TATCAATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAA
GACAGAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACC
ACCCAAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCAT
CAGCATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC

(Human Tissue Factor 219)

TCAGGCACTACAAATACTGTGGCAGCATATAATTTAACTTGGAAATCA
ACTAATTTCAAGACAATTTTGGAGTGGGAACCCAAACCCGTCAATCAAGTCT

ACACTGTTCAAATAAGCACTAAGTCAGGAGATTGGAAAAGCAAATGCTTTTA
CACAACAGACACAGAGTGTGACCTCACCGACGAGATTGTGAAGGATGTGAA
GCAGACGTACTTGGCACGGGTCTTCTCCTACCCGGCAGGGAATGTGGAGAGC
ACCGGTTCTGCTGGGGAGCCTCTGTATGAGAACTCCCCAGAGTTCACACCTTA
CCTGGAGACAAACCTCGGACAGCCAACAATTCAGAGTTTTGAACAGGTGGGA
ACAAAAGTGAATGTGACCGTAGAAGATGAACGGACTTTAGTCAGAAGGAAC
AACACTTTCCTAAGCCTCCGGGATGTTTTTGGCAAGGACTTAATTTATACACT
TTATTATTGGAAATCTTCAAGTTCAGGAAAGAAAACAGCCAAAACAAACACT
AATGAGTTTTTGATTGATGTGGATAAAGGAGAAAACTACTGTTTCAGTGTTCA
AGCAGTGATTCCCTCCCGAACAGTTAACCGGAAGAGTACAGACAGCCCGGTA
GAGTGTATGGGCCAGGAGAAAGGGGAATTCAGAGAA

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The first chimeric polypeptide of IL-21/TF/IL-15 construct including leader sequence is SEQ ID NO: 88:

(Signal peptide)

MGVKVLFALICIAVAEA (SEQ ID NO: 223)

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS

Example 31: Secretion of IL-7/IL-15RαSu and IL-21/TF/IL-15 fusion proteins

The IL-7/IL-15RαSu and IL-21/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of a soluble IL-21/TF/IL-15:IL-7/IL-15RαSu protein complex (referred to as 21t15-7s; Figures 45 and Figure 46). The 21t15-7s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody affinity chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-7/IL-15RαSu and IL-21/TF/IL-15 fusion proteins.

In some cases, the leader (signal sequence) peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

Example 32: Purification of 21t15-7s by immunoaffinity chromatography

An anti-TF antibody affinity column was connected to a GE Healthcare™ AKTA Avant protein purification system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Cell culture harvest of 21t15-7s was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After loading the sample, the column was washed with 5 column volumes PBS, followed by elution with 6 column volumes 0.1M acetic acid, pH 2.9. Absorbance at 280

nm was collected and then the sample was neutralized to pH 7.5-8.0 by adding 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon® centrifugal filters with a 30 KDa molecular weight cutoff. The buffer-exchanged protein sample was stored at 2-8°C for further biochemical analysis and biological activity testing.

After each elution, the anti-TF antibody affinity column was then stripped using 6 column volumes 0.1M glycine, pH 2.5. The column was then neutralized using 10 column volumes PBS, 0.05% sodium azide and stored at 2-8 °C.

Example 33: Size exclusion chromatography

A GE Healthcare Superdex® 200 Increase 10/300 GL gel filtration column was connected to a GE Healthcare AKTA™ Avant protein purification system. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A capillary loop was used to inject 200µL of 1 mg/mL of 7t15-21scomplex onto the column. The injection was chased with 1.25 column volumes of PBS.

Example 34: SDS-PAGE of 21t15-7s and 21t15-TGFRs

To determine the purity and protein molecular weight, the purified 21t15-7s or 21t15-TGFRs protein sample were analyzed using 4-12% NuPage Bis-Tris protein gel SDS-PAGE. The gel will be stained with InstantBlue™ for about 30 min, followed by destaining overnight in purified water.

Example 35: Glycosylation of 21t15-7s and 21t15-TGFRs in CHO-K1 cells

Glycosylation of 21t15-7s in CHO-K1 cells or 21t15-TGFRs in CHO-K1 cells were confirmed using the Protein Deglycosylation Mix II kit (New England Biolabs), according to the manufacturer's instructions.

Example 36: Recombinant protein quantitation of 21t15-7s and 21t15-TGFRs complexes

The 21t15-7s complex or the 21t15-TGFRs complex were detected and quantified using standard sandwich ELISA methods. Anti-human tissue factor antibody (IgG1)

served as the capture antibody and biotinylated anti-human IL-21, IL-15, or IL-7 antibody (21t15-7s) or biotinylated anti-human IL-21, IL-15, or TGF-βRII antibody (21t15-TGFRs) served as the detection antibody. Tissue factor in purified 21t15-7s or 21t15-TGFRs protein complexes was detected using an anti-human tissue factor capture antibody, and anti-human tissue factor antibody (IgG1) detection antibody. The anti-TF antibody ELISA will be compared to purified tissue factor at similar concentrations.

Example 37: Expansion capacity of primary natural killer (NK) cells by 21t15-7s complex + anti-TF IgG1 antibody or 21t15-TGFRs complex + anti-TF IgG1 antibody

To assess the 21t15-7s complex's ability to expand primary natural killer (NK) cells, 21t15-7s complex and 21t15-7s complex + anti-TF IgG1 antibody was added to NK cells obtained from samples of fresh human leukocytes. Cells were stimulated with 50nM of 21t15-7s complex with or without 25 nM of anti-TF IgG1 or anti-TF IgG4 antibody at 37°C and 5% CO_2. Cells were maintained at concentration at 0.5 x 10^6/mL not exceeding 2.0 x 10^6/mL by counting every 48-72 hours and media was replenished with fresh stimulator. Cells stimulated with 21t15-7s complex or anti-TF IgG1 antibody or anti-TF IgG4 antibody, or anti-TF IgG4 + 21t15-7s complex were maintained up to day 5. Figure 47 shows expansion of primary NK cells upon incubation with 21t15-7s complex + anti-TF IgG1 antibody. Figure 54 also shows a schematic of the results.

Example 38: Activation of expanded NK cells by the 21t15-7s complex + anti-TF IgG1 antibody or the 21t15-TGFRs complex + anti-TF IgG1 antibody

Primary NK cells can be induced *ex vivo* following overnight stimulation of purified NK cells with 21t15-7s complex + anti-TF IgG1 antibody. Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >80% and confirmed by staining with CD56-BV421 and CD16-BV510 specific antibodies (BioLegend). Cells were counted and resuspended in 1 x 10^6/mL in a 24 well flat bottom plate in 1 mL of complete media (RPMI 1640 (Gibco), supplemented with 4 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies),

streptomycin (Thermo Life Technologies), non-essential amino acid (Thermo Life Technologies), sodium pyruvate (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with 50 nM of 21t15-7s with or without 25 nM of anti-TF IgG1 antibody at 37°C and 5% CO_2. Cells were counted every 48-72 hours and maintained at a concentration of 0.5 x 10^6/mL to 2.0 x 10^6/mL until day14. Media was periodically replenished with fresh stimulator. Cells were harvested and surface stained at day 3 with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (Biolegend and analyzed by Flow Cytometry-Celeste-BD Bioscience). Figure 48 shows the activation markers CD25 MFI and CD69 MFI. The activation marker CD25 MFI increased with 21t15-7s complex + anti-TF IgG1 antibody stimulation, but not 21t15-7s complex stimulation. The activation marker CD69 MFI increased with both 21t15-7s complex + anti-TF IgG1 antibody and with 21t15-7s complex, alone.

Example 39: Cytotoxicity of NK cells against human tumor cells

Fresh human blood buffy coat was obtained from a blood bank. NK cells were isolated via negative selection using the RosetteSep/human NK cell reagent (StemCell Technologies). The NK cells were cultured in complete RPMI-1640 medium with 21t15-7s 100 nM and 50 nM of anti-TF IgG1 antibody for up to 11 days at 37°C and 5% CO_2. The activated NK cells were mixed with Celltrace violet–labeled K562 cells at E:T ratio equal to 2:1 and incubated at 37°C for 4 hours. The mixture was harvested and the percentage of dead K562 cells were determined by propidium iodide staining and flow cytometry. Figure 49 shows increased specific lysis of K562 cells when incubated with expanded NK cells.

Example 40: Creation of an IL-21/IL-15RαSu DNA construct

In a non-limiting example, an IL-21/IL-15RαSu DNA construct was created. The human IL-21 sequence and human IL-15RαSu sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-21 sequence to the IL-15RαSu sequence. The final IL-21/IL-15RαSu DNA construct sequence was synthesized by Genewiz. See Figure 50.

Example 41: Creation of an IL-7/TF/IL-15 DNA construct

In a non-limiting example, an IL-7/TF/IL-15 construct was made by linking the IL-7 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-7/TF construct with the N-terminus coding region of IL-15. See Figure 51.

Example 42: Creation of an IL-21/IL-15Rα Sushi DNA construct

In a non-limiting example, a second chimeric polypeptide of IL-21/IL-15RαSu was generated. The human IL-21 and human IL-15Rα sushi sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-21 sequence to the IL-15Rα sushi sequence. The final IL-21/IL-15RαSu DNA construct sequence was synthesized by Genewiz.

The nucleic acid sequence encoding the second chimeric polypeptide of IL-21/IL-15RαSu domain (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 111):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGA
GCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAG

GAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAAC
GTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The second chimeric polypeptide of IL-21/IL-15Rα sushi domain (including leader sequence) is as follows (SEQ ID NO: 110):

(Signal Sequence)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15Rα sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH
WTTPSLKCIR

Example 43: Creation of an IL-7/TF/IL-15 DNA construct

In a non-limiting example, an exemplary first chimeric polypeptide of IL-7/TF/IL-15 was made by linking the IL-7 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-7/TF construct with the N-terminus coding region of IL-15. The nucleic acid sequence encoding the first chimeric polypeptide of IL-7/TF/IL-15 (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 107):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL-7 fragment)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG

AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGT
CCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGT
AAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGA
GAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCC
AATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGT
GCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCA
CATTGTCCAGATGTTCATCAATACCTCC

The first chimeric polypeptide of IL-7/TF/IL-15 (including leader sequence), is as follows (SEQ ID NO: 106):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS

Example 44: Secretion of IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins

The IL-21/IL-15RαSu and IL-7/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of a soluble IL-7/TF/IL-15:IL-21/IL-15RαSu protein complex (referred to as 7t15-21s). The 7t15-21s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody (IgG1) affinity chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins. See Figure 52 and Figure 53.

Example 45: Analytical size exclusion chromatography (SEC) analysis of IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins

To determine if anti-tissue factor monoclonal antibody and 7t15-21s can form an antibody-fusion-molecule complex, analytical size exclusion chromatography (SEC) was performed. A Superdex 200 Increase 10/300 GL gel filtration column (from GE Healthcare) was connected to an AKTA Avant system (from GE Healthcare). The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. Samples of the anti-TF mAb (1 mg/mL), 7t15-21s (1mg/mL), and a mixture of combined at a 1:1 ratio, so the final concentration of each protein is 0.5mg/mL) were in PBS. Each sample was injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. The SEC chromatograph of each sample was shown in Figure 55. The SEC results indicated that there are two protein peaks for 7t15-21s, likely representing a dimer (with an apparent molecular weight of 199.2kDa) and a higher oligomer of 7t15-21s, and there is one peak (with an apparent molecular weight of 206.8kDa) for the anti-TF mAb. However, as expected, a new protein peak with a higher molecular weight (with an apparent molecular weight of 576.9 kDa) was formed in the mixture sample containing the anti-TF mAb and 7t15-21s, indicating that the anti-TF mAb and 7t15-21s form an antibody-antigen complex through the binding of anti-TF mAb to TF in the fusion protein complex.

Example 46: Expansion capacity of primary natural killer (NK) cells by 7t15-21s complex + anti-TF IgG1 antibody

To assess the 7t15-21s complex's ability to expand primary natural killer (NK) cells, 7t15-21s complex and 7t15-21s complex + anti-TF IgG1 antibody are added to NK cells obtained from samples of fresh human leukocytes. Cells are stimulated with 50nM of 7t15-21s complex with or without 25 nM of anti-TF IgG1 or anti-TF IgG4 antibody at 37°C and 5% CO_2. Cells are maintained at concentration at 0.5 x 10^6/mL not exceeding 2.0 x 10^6/mL by counting every 48-72 hours and media is replenished with fresh stimulator. Cells stimulated with 7t15-21s complex or anti-TF IgG1 antibody or anti-TF IgG4 antibody or anti-TF IgG4 + 7t15-21s complex are maintained up to day 5.

Expansion of primary NK cells upon incubation with 21t15-7s complex + anti-TF IgG1 antibody is observed.

Example 47: Activation of expanded NK cells by the 7t15-21s complex + anti-TF IgG1 antibody

Primary NK cells are induced *ex vivo* following overnight stimulation of purified NK cells with 7t15-21s complex + anti-TF IgG1 antibody. Fresh human leukocytes are obtained from a blood bank and CD56+ NK cells are isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells is >80% and is confirmed by staining with CD56-BV421 and CD16-BV510 specific antibodies (BioLegend). Cells are counted and resuspended in 1 x 10^6/mL in a 24 well flat bottom plate in 1 mL of complete media (RPMI 1640 (Gibco), supplemented with 4 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), non-essential amino acid (Thermo Life Technologies), sodium pyruvate (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells are stimulated with 50 nM of 7t15-21s with or without 25 nM of anti-TF IgG1 antibody at 37°C and 5% CO_2. Cells are counted every 48-72 hours and maintained at a concentration of 0.5 x 10^6/mL to 2.0 x 10^6/mL until day 14. Media is periodically replenished with fresh stimulator. Cells are harvested and surface stained at day 3 with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (Biolegend) and analyzed by Flow Cytometry-Celeste-BD Bioscience). The activation marker CD25 MFI are observed to increase with 7t15-21s complex + anti-TF IgG1 antibody stimulation, but not 7t15-21s complex stimulation. The activation marker CD69 MFI is observed to increase with both 7t15-21s complex + anti-TF IgG1 antibody and with 7t15-21s complex, alone.

Example 48: Increase in Glucose Metabolism in NK Cells Using 18t15-12s

A set of experiments was performed to determine the effect of the construct of 18t15-12s (Figure 6) on oxygen consumption rate and extracellular acidification rate (ECAR) on NK cells purified from human blood.

In these experiments, fresh human leukocytes were obtained from the blood bank from two different human donors and NK cells were isolated via negative selection using the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >80% and confirmed by staining with CD56-BV421 and CD16-BV510 specific Abs (BioLegend). The cells were counted and resuspended in 2 x 10^6/mL in 24-well, flat-bottom plates in 1 mL of complete media (RPMI 1640 (Gibco) supplemented with 4 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), non-essential amino acid (Thermo Life Technologies), sodium pyruvate (Thermo Life Technologies) and 10% FBS (Hyclone)). The cells were stimulated with either (1) media alone, (2) 100 nM 18t15-12s, or (3) mixture of single cytokines recombinant human IL-12 (0.25 μg), recombinant human IL-15 (1.25 μg), and recombinant human IL-18 (1.25 μg) overnight at 37 °C and 5% CO_2. On the next day, the cells were harvested and extracellular flux assays on expanded NK cells were performed using a XFp Analyzer (Seahorse Bioscience). The harvested cells washed and plated 2.0 x 10^5 cells/well in at least duplicate for extracellular flux analysis of OCR (Oxygen Consumption Rate) and ECAR (Extracellular Acidification Rate). The glycolysis stress tests were performed in Seahorse Media contain 2 mM of glutamine. The following were used during the assay: 10 mM glucose; 100 nM oligomycin; and 100 mM 2-deoxy-D-glycose (2DG).

The data show that the 18t15-12s results in significantly increased oxygen consumption rate (Figure 56) and extracellular acidification rate (ECAR) as compared to the same cells activated with a combination of recombinant human IL-12, recombinant human IL-15, and recombinant human IL-18 (Figure 57).

Example 49: 7t15-16s21 fusion protein generation and characterization

A fusion protein complex was generated comprising of anti-CD16scFv/IL-15RαSu/IL-21 and IL-7/TF/IL-15 fusion proteins. The human IL-7 and IL-21 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the IL-7/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC

AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of IL-7/TF/IL-15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by linking the anti-CD16scFv sequence to the N-terminus coding region of IL-15RαSu chain followed by the N-terminus coding region of

IL-21 which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the anti-CD16scFv linked to the N-terminus of IL-15RαSu chain followed by the N-terminus coding region of IL-21 are shown below.

The nucleic acid sequence of the anti-CD16SscFv/IL-15 RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

((Anti-human CD16scFv)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGA
GGAGGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCT
GGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTA
CGGCATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCC
GGCATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCA
GGTTCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAA
CTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCC
CTGCTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT

GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

The amino acid sequence of the anti-CD16scFv/IL-15RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Anti-human CD16scFv)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGM
SWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLR
AEDTAVYYCARGRSLLFDYWGQGTLVTVSR

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The anti-CD16scFv/IL-15RαSu/IL-21 and IL-7/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected

into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15:anti-CD16scFv/IL-15RαSu/IL-21 protein complex (referred to as 7t15-16s21; Figure 58 and Figure 59), which can be purified by anti-TF IgG1-based affinity and other chromatography methods.

Binding of 7t15-16s21 to CHO cells expressing human CD16b

CHO cells were transfected with human CD16b in a pMC plasmid and selected with 10 μg/mL of blasticidin for 10 days. The CHO cells stably expressing CD16b were stained with 1.2 μg/mL of 7t15-16s21, containing anti-human CD16 scFv or 18t15-12s, which does not contain anti-human CD16 scFv, as a negative control, and then stained with biotinylated anti-human tissue factor Ab and PE conjugated streptavidin. Only anti-human CD16scFv containing 7t15-16s21 stained the cells as shown in Figure 60A. 18t15-12s did not stain the CHO cells expressing human CD16b as showed in Figure 60B.

Detection of IL-15, IL-21, and IL-7 in 7t15-16s21 using ELISA

A 96-well plate was coated with 100 μL (8 μg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed 3 times and blocked with 100 μL of 1% BSA in PBS. Serial dilution of 7t15-16s21 (at a 1:3 ratio) were added to the wells, and incubated at RT for 60 min. Following 3 washes, 50 ng/mL of biotinylated-anti-IL15 antibody (BAM247, R&D Systems), 500 ng/mL of biotinylated-anti-IL-21 antibody (13-7218-81, R&D Systems), or 500 ng/mL of biotinylated-anti-IL-7 antibody (506602, R&D Systems) was added to the wells and incubated at RT for 60 min. The plate was washed 3 times, and incubated with 0.25 μg/mL of HRP-SA (Jackson ImmunoResearch) at 100 μL per well for 30 min at RT, followed by 4 washes and incubation with 100 μl of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figures 61A-61C, the IL-15, IL-21, and IL-7 domains in 7t15-16s21 were detected by the individual antibodies.

The IL-15 in 7t15-16s21 promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To analyze the activity of IL-15 in 7t15-16s21, the IL-15 activity of 7t15-16s21 was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted 7t15-16s21 or IL-15 were added to the cells (Figure 62). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µl of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The absorbance at 450 nm was measured by analyzing the amount of formazan dye produced. As shown in Figure 62, 7t15-16s21 and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of 7t15-16s21 and IL-15 being 172.2 pM and 16.63 pM, respectively.

Purification elution chromatograph of 7t15-16s21 from anti-TF antibody affinity column

7t15-16s21 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. The column was then washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. Figure 63 is a line graph showing the chromatographic profile of 7t15-16s21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin. As shown in Figure 63, the anti-TF antibody affinity column bound 7t15-16s21 which contains TF. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Analytical size exclusion chromatography (SEC) analysis of 7t15-16s21

To perform size exclusion chromatography (SEC) analysis for 7t15-16s21, a Superdex 200 Increase 10/300 GL gel filtration column (GE Healthcare) connected to an AKTA Avant system (GE Healthcare) was used. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A sample containing 7t15-16s21 in PBS was injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. As shown in Figure 64, the SEC results showed two protein peaks for 7t15-16s21.

Example 50: TGFRt15-16s21 fusion protein generation and characterization

A fusion protein complex was generated comprising anti-human CD16scFv/IL-15RαSu/IL21 and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 65 and 66). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the two TGFβ Receptor II/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Two Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT

GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT

TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFβ Receptor II/TF/IL-15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching anti-human CD16scFv directly linking to the N-terminus coding region of IL-15RαSu chain followed by the N-terminus coding region of IL-21 which was synthesized by Genewiz. The nucleic acid and protein

sequences of a construct comprising the anti-human CD16scFv linked to the N-terminus of IL-15RαSu followed by the N-terminus coding region of IL-21 are shown below.

The nucleic acid sequence of the anti-CD16scFv/IL-15 RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Anti-human CD16scFv)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGA
GGAGGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCT
GGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTA
CGGCATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCC
GGCATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCA
GGTTCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAA
CTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCC
CTGCTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC

AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

The amino acid sequence of the anti-CD16scFv/IL-15RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Anti-human CD16scFv)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGM
SWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLR
AEDTAVYYCARGRSLLFDYWGQGTLVTVSR

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The anti-CD16scFv/IL-15RαSu/IL-21 and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were

transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:CD16scFv/IL-15RαSu/IL-21 protein complex (referred to as TGFRt15-16s21), which can be purified by anti-TF IgG1-based affinity and other chromatography methods.

Interaction between TGFRt15-16s21 and CHO cells expressing human CD16b

CHO cells were transfected with human CD16b in a pMC plasmid and selected with 10 μg/mL of blasticidin for10 days. Cells stably expressing CD16b were stained with 1.2 μg/mL of TGFRt15-16s21, containing anti-human CD16 scFv, or 7t15-21s, not containing anti-human CD16 scFv, as a negative control, and with biotinylated anti-human tissue factor antibody and PE conjugated streptavidin. As shown in Figures 67A and 67B, TGFRt15-16s21, which contains anti-human CD16scFv, showed positive binding, while 7t15-21s did not show binding.

Effect of TGFRt15-16s21 on TGFβ1 activity in HEK-Blue TGFβ cells

To evaluate the activity of TGFβRII in TGFRt15-16s21, the effect of TGFRt15-16s21 on the activity of TGFβ1 in HEK-Blue TGFβ cells was analyzed. HEK-Blue TGFβ cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x glutamine) at 5 x 10^5 cells/mL. In a flat-bottom 96-well plate, 50 μl cells were added to each well (2.5 x 10^4 cells/well) and followed with 50 μL 0.1nM TGFβ1 (R&D systems). TGFRt15-16s21 or TGFR-Fc (R&D Systems) prepared at a 1:3 serial dilution was then added to the plate to reach a total volume of 200 μL. After 24 hrs of incubation at 37°C, 40 μL of induced HEK-Blue TGFβ cell supernatant was added to 160 μL pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM. The IC_{50} of each protein sample was calculated with GraphPad Prism 7.04. The IC_{50} of TGFRt15-16s21 and TGFR-Fc were 9127 pM and 460.6 pM respectively. These results showed that the TGFβRII domain in TGFRt15-16s21 was able to block the activity of TGFβ-1 in HEK-Blue TGFβ cells.

The IL-15 in TGFRt15-16s21 promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To analyze the activity of IL-15 in TGFRt15-16s21, the IL-15 activity of TGFRt15-16s21 was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted TGFRt15-16s21 or IL-15 were added to the cells (Figure 68). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The absorbance at 450 nm was measured by analyzing the amount of formazan dye produced. As shown in Figure 68, TGFRt15-16s21 and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of TGFRt15-16s21 and IL-15 being 51298 pM and 10.63 pM, respectively.

Detection of IL-15, IL-21, and TGFβRII in TGFRt15-16s21 using ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed 3 times and blocked with 100 µL of 1% BSA in PBS. TGFRt15-16s21 serially diluted at a 1:3 ratio was added and incubated at RT for 60 min. Following three washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), 500 ng/mL of biotinylated-anti-IL-21 antibody (13-7218-81, R&D Systems), or 200 ng/mL of biotinylated-anti-TGFβRII antibody (BAF241, R&D Systems) was applied per well, and incubated at RT for 60 min. Following three washes, incubation with 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch at 100 µL per well for 30 min at RT was carried out, followed by 4 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. The data are shown in Figure 69. As shown in Figures 70A-70C, the IL-15, IL-21, and TGFβRII domains in TGFRt15-16s21 were detected by the respective antibodies.

Purification elution chromatograph of TGFRt15-16s21 using anti-TF antibody affinity column

TGFRt15-16s21 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 71, the anti-TF antibody affinity column bound to TGFRt15-16s21, which contains tissue factor as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE of TGFRt15-16s21

To determine the purity and molecular weight of the TGFRt15-16s21 protein, protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGFRt15-16s21 protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs according to the manufacturer's instructions. Figure 72 shows results from the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results showed that the TGFRt15-16s21 protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample showed expected

molecular weights (69 kDa and 48 kDa) in the reduced SDS gel. Lane M was loaded with 10μL of SeeBlue Plus2 Prestained Standard.

Example 51: 7t15-7s fusion protein generation and characterization

A fusion protein complex was generated comprising IL-7/TF/IL-15 and IL-7/IL-15RαSu fusion proteins (Figure 73 and Figure 74). The human IL-7, tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT

TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL

ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by linking the IL-7 sequence to the N-terminus coding region of IL-15RαSu chain which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the IL-7 linked to the N-terminus of IL-15RαSu chain are shown below.

The nucleic acid sequence of 7s construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGG
CC

(Human IL7)

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTA
ATGGTCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATT
GCCTGAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAG
GAAGGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAAT
GAATAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAA
CAATACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCT
GGGTGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGA
ACAGAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATA
AAAACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC

(Human IL-15R α sushi domain)

ATCACGTGCCCTCCCCCCATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAA

GCGTAAAGCCGGCACGTCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACG
AATGTCGCCCACTGGACAACCCCCAGTCTCAAATGCATTAGA

The amino acid sequence of 7s fusion protein (including the leader sequence) is as follows:

(Signal peptide)

MGVKVLFALICIAVAEA

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

The IL-7/TF/IL-15 and IL-7/IL-15RαSu constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15:IL-7/IL-15RαSu protein complex referred to as 7t15-7s, which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Purification elution chromatograph of 7t15-7s using anti-TF antibody affinity column

7t15-7s harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 75, the anti-TF antibody affinity column bound to 7t15-7s which contains tissue

factor (TF) as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Immunostimulation of 7t15-7s in C57BL/6 mice

7t15-7s is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes the first polypeptide that is a soluble fusion of human IL-7, human tissue factor 219 fragment and human IL-15 (7t15), and the second polypeptide that is a soluble fusion of human IL-7 and sushi domain of human IL-15 receptor alpha chain (7s).

CHO cells were co-transfected with the IL7-TF-IL15 (7t15) and IL7-IL15Ra sushi domain (7s) vectors. The 7t15-7s complex was purified from the transfected CHO cell culture supernatant. The IL-7, IL-15 and tissue factor (TF) components were demonstrated in the complex by ELISA as shown in Figure 76. A humanized anti-TF monoclonal antibody (anti-TF IgG1) was used as the capture antibody to determine TF in 7t15-7s, and biotinylated anti-human IL-15 antibody (R&D systems) and biotinylated anti-human IL-7 antibody (R&D Systems) were used as the detection antibodies to respectively detect IL-15 and IL-7 in 7t15-7s, followed by peroxidase conjugated streptavidin (Jackson ImmunoResearch Lab) and ABTS substrate (Surmodics IVD, Inc.).

Example 52: TGFRt15-TGFRs fusion protein generation and characterization

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 77 and Figure 78). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then

directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the two TGFβ Receptor II/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Two Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT

TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFβ Receptor II/TF/IL-15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu are shown below.

The nucleic acid sequence of the TGFβ Receptor II/IL-15 RαSu construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Two human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC

GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of the two TGFβ Receptor II/IL-15RαSu construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Two human TGFβ Receptor II extra-cellular domains)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFβR/IL-15RαSu and TGFβR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFβR/TF/IL-15:TGFβR/IL-15RαSu protein complex (referred to as TGFRt15-TGFRs), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Effect of TGFRt15-TGFRs on TGFβ1 activity in HEK-Blue TGFβ cells

To evaluate the activity of TGFβRII in TGFRt15-TGFRs, the effect of TGFRt15-TGFRs on the activity of TGFβ1 in HEK-Blue TGFβ cells was analyzed. HEK-Blue TGFβ cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x glutamine) at 5 x 10^5 cells/mL. In a flat-bottom 96-well plate, 50 µL cells were added to each well (2.5 x 10^4 cells/well) and followed with 50 µL 0.1nM TGFβ1 (R&D systems). TGFRt15-TGFRsor TGFR-Fc (R&D Systems) prepared at a 1:3 serial dilution was then added to the plate to reach a total volume of 200 µL. After 24hrs of incubation at 37°C, 40 µL of induced HEK-Blue TGFβ cell supernatant was added to 160 µL pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM. The IC_{50} of each protein sample was calculated with GraphPad Prism 7.04. The IC_{50} of TGFRt15-TGFRs and TGFR-Fc were 216.9 pM and 460.6 pM respectively. These results showed that the TGFβRII domain in TGFRt15-TGFRs was able to block the activity of TGFβ1 in HEK-Blue TGFβ cells.

The IL-15 in TGFRt15-TGFRs promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To evaluate the activity of IL-15 in TGFRt15-TGFRs, the IL-15 activity of TGFRt15-TGFRs was compared to recombinant IL-15 using 32Dβ cells that express

IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted TGFRt15-TGFRs or IL-15 were added to the cells (Figure 79). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The absorbance at 450 nm was measured by analyzing the amount of formazan dye produced. As shown in Figure 79, TGFRt15-TGFRs and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of TGFRt15-TGFRsand IL-15 being 1901 pM and 10.63 pM, respectively.

Detection of IL-15 and TGFβRII domains in TGFRt15-TGFRs with corresponding antibodies using ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed 3 times and blocked with 100 µL of 1% BSA in PBS. TGFRt15-TGFRs was added at a 1:3 serial dilution, and incubated at RT for 60 min. After 3 washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), or 200 ng/mL of biotinylated-anti-TGFβRII antibody (BAF241, R&D Systems) was added to the wells and incubated at RT for 60 min. Next the plates were washed 3 times, and 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch) at 100 µL per well was added and incubated for 30 min at RT, followed by 4 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance at 405 nm was read. As shown in Figure 81A and 81B, the IL-15 and TGFβRII domains in TGFRt15-TGFRs were detected by the individual antibodies.

Purification elution chromatograph of TGFRt15-TGFRs from anti-TF antibody affinity column

TGFRt15-TGFRs harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then

buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 82, the anti-TF antibody affinity column bound to TGFRt15-TGFRs which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Analytical size exclusion chromatography (SEC) analysis of TGFRt15-TGFRs

A Superdex 200 Increase 10/300 GL gel filtration column (from GE Healthcare) was connected to an AKTA Avant system (from GE Healthcare). The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A sample containing TGFRt15-TGFRs in PBS was injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. The SEC chromatograph of the sample is shown in Figure 83. The SEC results showed four protein peaks for TGFRt15-TGFRs.

Reduced SDS-PAGE analysis of TGFRt15-TGFRs

To determine the purity and molecular weight of the TGFRt15-TGFRs protein, protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGFRt15-TGFRs protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 84 shows the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results showed that the TGFRt15-TGFRs protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular

weights (69 kDa and 39 kDa) in the reduced SDS gel. Lane M was loaded with 10 ul of SeeBlue Plus2 Prestained Standard.

Immunostimulatory activity of TGFRt15-TGFRs in C57BL/6 mice

TGFRt15-TGFRs is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes a first polypeptide that is a soluble fusion of two TGFβRII domains, human tissue factor 219 fragment and human IL-15, and the second polypeptide that is a soluble fusion of two TGFβRII domains and sushi domain of human IL-15 receptor alpha chain.

Wild type C57BL/6 mice were treated subcutaneously with either control solution or with TGFRt15-TGFRs at a dosage of 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg. Four days after treatment, spleen weight and the percentages of various immune cell types present in the spleen were evaluated. As shown in Figure 85A, the spleen weight in mice treated with TGFRt15-TGFRs increased with increasing dosage of TGFRt15-TGFRs. Moreover, the spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs were higher as compared to mice treated with the control solution, respectively. In addition, the percentages of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and $CD19^+$ B cells present in the spleen of control-treated and TGFRt15-TGFRs-treated mice were evaluated. As shown in Figure 85B, in the spleens of mice treated with TGFRt15-TGFRs, the percentages of $CD8^+$ T cells and NK cells both increased with increasing dosage of TGFRt15-TGFRs. Specifically, the percentages of $CD8^+$ T cells were higher in mice treated with 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs compared to control-treated mice, and the percentages of NK cells were higher in mice treated with 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs compared to control-treated mice. These results demonstrate that TGFRt15-TGFRs is able to stimulate immune cells in the spleen, in particular $CD8^+$ T cells and NK cells.

The pharmacokinetics of TGFRt15-TGFRs molecules were evaluated in wild type C57BL/6 mice. The mice were treated subcutaneously with TGFRt15-TGFRs at a dosage of 3 mg/kg. The mouse blood was drained from tail vein at various time points and the serum was prepared. The TGFRt15-TGFRs concentrations in mouse serum was determined with ELISA (capture: anti-human tissue factor antibody; detection:

biotinylated anti-human TGFβ receptor antibody and followed by peroxidase conjugated streptavidin and ABTS substrate). The results showed that the half-life of TGFRt15-TGFRs was 12.66 hours in C57BL/6 mice.

The mouse splenocytes were prepared in order to evaluate the immunostimulatory activity of TGFRt15-TGFRs over time in mice. As shown in Figure 86A, the spleen weight in mice treated with TGFRt15-TGFRs increased 48 hours posttreatment and continued to increase over time. In addition, the percentages of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and $CD19^+$ B cells present in the spleen of control-treated and TGFRt15-TGFRs-treated mice were evaluated. As shown in Figure 86B, in the spleens of mice treated with TGFRt15-TGFRs, the percentages of $CD8^+$ T cells and NK cells both increased at 48 hours after treatment and were higher and higher overtime after the single dose treatment. These results further demonstrate that TGFRt15-TGFRs is able to stimulate immune cells in the spleen, in particular $CD8^+$ T cells and NK cells.

Furthermore, the dynamic proliferation of immune cells based on Ki67 expression of splenocytes and cytotoxicity potential based on granzyme B expression were evaluated in splenocytes isolated from mice following a single dose (3 mg/kg) of TGFRt15-TGFRs. As shown in Figure 87A and 87B, in the spleens of mice treated with TGFRt15-TGFRs, the expression of Ki67 and granzyme B by NK cells increased at 24 hours after treatment and its expression of $CD8^+$ T cells and NK cells both increased at 48 hours and later time points after the single dose treatment. These results demonstrate that TGFRt15-TGFRs not only increases the numbers of $CD8^+$ T cells and NK cells but also enhance the cytotoxicity of these cells. The single dose treatment of TGFRt15-TGFRs led $CD8^+$ T cells and NK cells to proliferate for at least 4 days.

The cytotoxicity of the splenocytes from TGFRt15-TGFRs-treated mice against tumor cells was also evaluated. Mouse Moloney leukemia cells (Yac-1) were labeled with CellTrace Violet and were used as tumor target cells. Splenocytes were prepared from TGFRt15-TGFRs (3 mg/kg)-treated mouse spleens at various time points post treatment and were used as effector cells. The target cells were mixed with effector cells at an E:T ratio = 10:1 and incubated at 37°C for 20 hours. Target cell viability was assessed by analysis of propidium iodide positive, violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 tumor inhibition was calculated using the formula, (1-

[viable Yac-1 cell number in experimental sample]/[viable Yac-1 cell number in the sample without splenocytes]) x 100. As shown in Figure 88, splenocytes from TGFRt15-TGFRs-treated mice had stronger cytotoxicity against Yac-1 cells than the control mouse splenocytes.

Tumor size analysis in response to chemotherapy and/or TGFRt15-TGFRs

Pancreatic cancer cells (SW1990, ATCC® CRL-2172) were subcutaneously (s.c.) injected into C57BL/6 scid mice (The Jackson Laboratory, 001913, $2x10^6$ cells/mouse, in 100μL HBSS) to establish the pancreatic cancer mouse model. Two weeks after tumor cell injection, chemotherapy was initiated in these mice intraperitoneally with a combination of Abraxane (Celgene, 68817-134, 5 mg/kg, i.p.) and Gemcitabine (Sigma Aldrich, G6423, 40 mg/kg, i.p.), followed by immunotherapy with TGFRt15-TGFRs (3 mg/kg, s.c.) in 2 days. The procedure above was considered one treatment cycle and was repeated for another 3 cycles (1 cycle/week). Control groups were set up as the SW1990-injected mice that received PBS, chemotherapy (Gemcitabine and Abraxane), or TGFRt15-TGFRs alone. Along with the treatment cycles, tumor size of each animal was measured and recorded every other day, until the termination of the experiment 2 months after the SW1990 cells were injected. Measurement of the tumor volumes were analyzed by group and the results indicated that the animals receiving a combination of chemotherapy and TGFRt15-TGFRs had significantly smaller tumors comparing to the PBS group, whereas neither chemotherapy nor TGFRt15-TGFRs therapy alone work as sufficiently as the combination (Figure 89).

In vitro senescent B16F10 melanoma model

Next, in vitro killing of senescent B16F10 melanoma cells by activated mouse NK cells was evaluated. B16F10 senescence cells (B16F10-SNC) cells were labelled with CellTrace violet and incubated for 16 hrs with different E:T ratio of in vitro 2t2-activated mouse NK cells (isolated from spleen of C57BL/6 mice injected with TGFRt15-TGFRs10 mg/kg for 4 days). The cells were trypsinized, washed and resuspended in complete media containing propidium iodide (PI) solution. The cytotoxicity was assessed by flow cytometry (Figure 90).

Example 53: 7t15-21s137L (long version) fusion protein creation and characterization

A fusion protein complex was generated comprising of IL-21/IL-15RαSu/CD137L and IL-7/TF/IL-15 fusion proteins (Figure 91 and Figure 92). Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15. The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC

ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

The nucleic acid and protein sequences of the 21s137L are shown below. The nucleic acid sequence of the 21s137L construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCG
ATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGAC
GGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGC

TGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCG
AGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGA
GGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTG
GCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAA
ATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of 21s137L fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVS
LTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA
AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW
QLTQGATVLGLFRVTPEIPAGLPSPRSE

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The IL-21/IL-15RαSu/CD137L and IL-7/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY,

Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15: IL-21/IL-15RαSu/CD137L protein complex (referred to as 7t15-21s137L), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Purification elution chromatograph of 7t15-21s137L using anti-TF antibody affinity column

7t15-21s137L harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 93, the anti-TF antibody affinity column bound to 7t15-21s137L which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min. Figure 94 shows the analytical SEC profile of 7t15-21s137L.

Example 54: 7t15-21s137L (short version) fusion protein generation and characterization

A fusion protein complex was generated comprising of IL-21/IL-15RαSu/CD137L and IL-7/TF/IL-15 fusion proteins. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed

by the N-terminus coding region of IL-15. The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC

AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

The nucleic acid and protein sequences of the 21s137L (short version) are shown below. The nucleic acid sequence of 21s137L (short version) construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137 Ligand short version)

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTG
GTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACC
CAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACA
CGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTA
GAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGC
TGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACC
GTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCA
GGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCAC

ACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCT TGGGACTCTTCCGGGTGACCCCCGAAATC

The amino acid sequence of the 21s137L (short version) construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137 Ligand short version)

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKE DTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALAL TVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATV LGLFRVTPEI

The IL-21/IL-15RαSu/CD137L (short version) and IL-7/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15: IL-21/IL-15RαSu/CD137L protein complex (referred to as 7t15-21s137L (short version)), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Binding of 7t15-21s137L (short version) to CD137 (4.1BB)

On day 1, a 96-well plate was coated with 100 µL (2.5 µg/mL) of GAH IgG Fc (G-102-C, R&D Systems) in R5 (coating buffer) or R5 only and incubated at 4°C, overnight. On day 2, the plates were washed three times and blocked with 300 µL of 1% BSA in PBS at 37°C for 2 hrs. 10 ng/mL of 4.1BB/Fc (838-4B, R&D Systems) was added at 100 µL/well and incubated for 2 hrs at RT. After three washes, the 7t15-21s137L or 7t15-21s serially diluted at a 1/3 ratio (starting at 10 nM), and incubated at 4°C overnight. On day 3, following 3 washes, 300 ng/mL of biotinylated-anti-hTF antibody (BAF2339, R&D Systems) was added at 100 µL per well and incubated at RT for 2 hrs. The plate was then washed three times and incubated with 0.25 µg/mL of HRP-SA (Jackson ImmuneResearch) at 100 µL per well for 30 min, followed by 3 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figure 95, 7t15-21s137L (short version) showed significant interaction with 4.1BB/Fc (blue line) as compared to 7t15-21s.

Detection of IL-15, IL-21, and IL-7 in 7t15-21s137L (short version) with ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at RT for 2 hrs. The plates were washed 3 times and blocked with 100 µL of 1% BSA in PBS. 7t15-21s137L (short version), serially diluted at a 1:3 ratio was added, and incubated at RT for 60 min. After three washes, 50 ng/mL of biotinylated-anti-IL15 antibody (BAM247, R&D Systems), 500 ng/mL of biotinylated-anti-IL21 antibody (13-7218-81, R&D Systems), or 500 ng/mL of biotinylated-anti-IL7 antibody (506602, R&D Systems) was added to the wells and incubated at RT for 60 min. After three washes and incubation with 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch) at 100 µL per well was carried out for 30 min at RT, followed by four washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figures 96A-96C, the IL-15, IL-21, and IL-7 domains in 7t15-21s137L (short version) were detected by the respective antibodies.

The IL-15 in 7t15-1s137L (short version) promotes IL2Rαβγ containing CTLL2 cell proliferation

To evaluate the IL-15 activity of 7t15-21s137L (short version), 7t15-21s137L (short version) was compared with recombinant IL15 in promoting proliferation of IL2R$\alpha\beta\gamma$ expressing CTLL2 cells. IL-15-dependent CTLL2 cells were washed 5 times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serially diluted 7t15-21s137L (short version) or IL-15 were added to the cells (Figure 97). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubated for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 97, 7t15-21s137L (short version) and IL-15 promoted CTLL2 cell proliferation. The EC_{50} of 7t15-21s137L (short version) and IL-15 was 55.91 pM and 6.22 pM, respectively.

The IL-21 in 7t15-1s137L (short version) promotes IL21R containing B9 cell proliferation

To evaluate the IL-21 activity of 7t15-21s137L (short version), 7t15-21s137L (short version) was compared with recombinant IL-21 in promoting proliferation of IL-21R expressing B9 cells. IL-21R containing B9 cells were washed 5 times with RPMI-10% FBS and seeded to the wells at 1 x 10^4 cells/well. Serially diluted 7t15-21s137L (short version) or IL-21 were added to the cells (Figure 98). Cells were incubated in a CO_2 incubator at 37°C for 5 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 5 and incubated for an additional 4 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 98, 7t15-21s137L (short version) and IL-21 promoted B9 cell proliferation. The EC_{50} of 7t15-21s137L (short version) and IL-21 was 104.1 nM and 72.55 nM, respectively.

Example 55: 7t15-TGFRs fusion protein generation and characterization

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu and IL-7/TF/IL-15 fusion proteins (Figure 99 and Figure 100). The human TGFβ

Receptor II (Ile24-Asp159), tissue factor 219, IL-15, and IL-7 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15. The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT

GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu are shown below.

The nucleic acid sequence of the TGFRs construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA

AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of TGFRs fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

Effect of 7t15-TGFRs on TGFβ1 activity in HEK-Blue TGFβ cells

To evaluate the activity of TGFβR in 7t15-TGFRs, the effect of 7t15-TGFRs on the activity of TGFβ1 in HEK-Blue TGFβ cells was analyzed. HEK-Blue TGFβ cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x glutamine) at 5 x 10^5 cells/mL. In a flat-bottom 96-well plate, 50 µL cells were added to each well (2.5 x 10^4 cells/well) and followed with 50 µL 0.1nM TGFβ1 (R&D systems). 7t15-TGFRs or TGFR-Fc (R&D Systems) prepared at a1:3 serial dilution was then added to the plate to reach a total volume of 200 µL. After 24hrs of incubation at 37°C, 40 µL of induced HEK-Blue TGFβ cell supernatant was added to 160 µL pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM. The IC_{50} of each protein sample was calculated with GraphPad Prism 7.04. The

IC_{50} of 7t15-TGFRs and TGFR-Fc were 1142 pM and 558.6 pM respectively. These results showed that the TGFβR in 7t15-TGFRs was able to block the activity of TGFβ1 in HEK-Blue TGFβ cells.

Detection of IL-15, TGFβRII, and IL-7 in 7t15-TGFRs with ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed three times and blocked with 100 µL of 1% BSA in PBS. Serial dilution of 7t15-TGFRs (1:3 ratio) was added, and incubated at RT for 60 mins. After 3 washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), 200 ng/mL of biotinylated-anti-TGFbRII antibody (BAF241, R&D Systems), or 500 ng/mL of biotinylated-anti-IL-7 antibody (506602, R&D Systems) was added and incubated at RT for 60 min. Following three washes, incubation with 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch) at 100 µL per well was carried out for 30 min at RT, followed by 4 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. The data are shown in Figure 101. As shown in Figures 102A-102C, the IL-15, TGFR, and IL-7 in 7t15-TGFRs were detected by the respective antibodies.

The IL-15 in 7t15-TGFRs promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To evaluate the activity of IL-15 in 7t15-TGFRs, 7t15-TGFRs was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2×10^4 cells/well. Serially-diluted 7t15-TGFRs or IL-15 were added to the cells (Figure 103). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 103, 7t15-TGFRs and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of 7t15-TGFRs and IL-15 being 126 nM and 16.63 pM, respectively.

Purification elution chromatograph of 7t15-TGFRs using anti-TF antibody affinity column

7t15-TGFRs harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 104, the anti-TF antibody affinity column can bind 7t15-TGFRs which contains TF as a fusion partner of 7t15-TGFRs. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE analysis of 7t15-TGFRs

To determine the purity and molecular weight of the protein, 7t15-TGFRs protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the 7t15-TGFRs protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 105 shows reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. These results showed that the protein is glycosylated when it is expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (55 kDa

and 39 kDa) in reduced SDS gel. Lane M was loaded with 10 ul of SeeBlue Plus2 Prestained Standard.

Characterization of 7t15-TGFRs

7t15-TGFRs is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes the first polypeptide that is a soluble fusion of human IL-7, human tissue factor 219 fragment and human IL-15 (7t15), and the second polypeptide that is a soluble fusion of single chain two TGFβRII domains and sushi domain of human IL-15 receptor alpha chain (TGFRs).

CHO cells were co-transfected with 7t15 and TGFRs vectors. The 7t15-TGFRs complex was purified from the transfected CHO cell culture supernatant. The IL-7, IL-15, TGFβ receptor and tissue factor (TF) components were demonstrated in the complex by ELISA as shown in Figure 106. A humanized anti-TF monoclonal antibody (anti-TF IgG1) was used as the capture antibody to determine TF in 7t15-TGFRs, and biotinylated antibodies against human IL-15 antibody (R&D systems), human IL-7 (Biolegend), anti-TGFβ receptor (R&D Systems) were used as the detection antibodies to respectively determine IL-7, IL-15 and TGFβ receptor in 7t15-TGFRs. Peroxidase conjugated streptavidin (Jackson ImmunoResearch Lab) and ABTS substrate (Surmodics IVD, Inc.) were then used to detect the bound biotinylated antibodies. The results were analyzed by ELISA (Figure 106).

In vivo characterization of 7t15-TGFRs in C57BL/6 mice

To determine the immunostimulatory activity of 7t15-TGFRs in vivo, C57BL/6 mice were subcutaneously treated with control solution (PBS) or 7t15-TGFRs at 0.3, 1, 3 and 10 mg/kg. The treated mice were euthanized. The mouse spleens were collected and weighed day 4 post treatment. Single splenocyte suspensions were prepared and stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 antibodies and the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells was analyzed by flow cytometry. The results showed that 7t15-TGFRs was effective at expanding splenocytes based on spleen weight (Figure 107A), especially at 1-10 mg/kg. The percentages of $CD8^+$ T cells

and NK cells were higher compared to control-treated mice (Figure 107B) at all doses tested.

CD44 Expression of $CD4^+$ and $CD8^+$ T cells

It has been known that IL-15 induces CD44 expression on T cells and development of memory T cells. CD44 expression of $CD4^+$ and $CD8^+$ T cells in the 7t15-TGFRs treated mice were assessed. C57BL/6 mice were subcutaneously treated with 7t15-TGFRs. The splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8 and anti-CD44 monoclonal antibodies for immunocyte subsets. The percentages of $CD4^+CD44^{high}$ T cells of total CD4+ T cells and $CD8^+CD44^{high}$ T cells of total $CD8^+$ T cells were analyzed by flow cytometry. As shown in Figures 108A and 108B, 7t15-TGFRs significantly activated $CD4^+$ and $CD8^+$ T cells to differentiate into memory T cells.

Furthermore, the dynamic proliferation of immune cells based on Ki67 expression of splenocytes and cytotoxicity potential based on granzyme B expression of the splenocytes induced by 7t15-TGFRs after the single dose treatment of mouse were also evaluated. C57BL/6 mice were subcutaneously treated with 7t15-TGFRs at 3 mg/kg. The treated mice were euthanized and the splenocytes were prepared. The prepared splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 (NK) antibodies for immunocyte subsets and then intracellularly stained with anti-Ki67 antibody for cell proliferation and anti-granzyme B antibody for cytotoxic marker. The mean fluorescent intensity (MFI) of Ki67 and granzyme B of corresponding immunocyte subsets was analyzed by flow cytometry. As shown in Figures 109A and 109B, in the spleens of mice treated with 7t15-TGFRs, the expression of Ki67 and granzyme B by $CD8^+$ T cells and NK cells increased compared with PBS control treatment. These results demonstrate that 7t15-TGFRs is not only to increase numbers of $CD8^+$ T cells and NK cells but also enhance potential cytotoxicity of these cells.

Additionally, cytotoxicity of the mouse splenocytes against tumor cells was also evaluated. Mouse Yac-1 cells were labeled with CellTrace Violet and used as tumor target cells. The splenocytes were prepared from 7t15-TGFRs-treated mice and used as effector cells. The target cells were mixed with effector cells at E:T ratio = 10:1 in

RPMI-10 medium with or without 7t15-TGFRs at 100 nM and incubated at 37°C for 20 hours. Target Yac-1 cell inhibition was assessed by analysis of viable violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 inhibition was calculated using a formula, (1-viable Yac-1 cell number in experimental sample/viable Yac-1 cell number in the sample without splenocytes) x 100. As shown in Figure 110, 7t15-TGFRs-treated mouse splenocytes had stronger cytotoxicity against Yac-1 cells than the control mouse splenocytes and addition of 7t15-TGFRs during cytotoxic assay further enhanced cytotoxicity of splenocytes against Yac-1 target cells.

Example 56: TGFRt15-21s137L fusion protein generation and characterization

A fusion protein complex was generated comprising IL-21/IL-15RαSu/CD137L and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 111 and Figure 112). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT

AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG

GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

The nucleic acid and protein sequences of the 21s137L are shown below. The nucleic acid sequence of the 21s137L construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCG
ATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGAC
GGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGC
TGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCG
AGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGA
GGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTG
GCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAA
ATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of 21s137L fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVS
LTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA
AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW
QLTQGATVLGLFRVTPEIPAGLPSPRSE

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The IL-21/IL-15RαSu/CD137L and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15: IL-21/IL-15RαSu/CD137L protein complex (referred to as TGFRt15-21s137L), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Purification elution chromatograph of TGFRt15-21s137L using anti-TF antibody affinity column

TGFRt15-21s137L harvest from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 113, the anti-TF antibody affinity column bound to TGFRt15-21s137L which contains TF as a fusion partner of TGFRt15-21s137L. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Example 57: TGFRt15-TGFRs21 fusion protein generation and characterization

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu/IL-21 and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 114 and Figure 115). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, IL-21, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC

ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW

KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain, followed by the N-terminus coding region of IL-21, which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu following with the N-terminus of IL-21 are shown below.

The nucleic acid sequence of the TGFRs21 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC

TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Human IL-21)

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATT
GTTGATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGC
TCCAGAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGA
AGGCCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATG
TATCAATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAA
GACAGAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACC
ACCCAAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCAT
CAGCATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC

The amino acid sequence of TGFRs21 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK EFLERFKSLLQKMIHQHLSSRTHGSEDS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFR/IL-15RαSu/IL-21 and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:TGFR/IL-15RαSu/IL-21 protein complex (referred to as TGFRt15-TGFRs21), which can be purified by anti-TF antibody IgG1 affinity and other chromatography methods.

Purification elution chromatograph of TGFRt15-TGFRs21 using anti-TF antibody affinity column

TGFRt15-TGFRs21 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 116, the anti-TF antibody affinity column bound to TGFRt15-TGFRs21 which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column

volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE analysis of TGFRt15-TGFRs21

To determine the purity and molecular weight of the protein, TGFRt15-TGFRs21 protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGFRt15-TGFRs21 protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 117 shows the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. It is clear that the protein is glycosylated when it is expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (69 kDa and 55 kDa) in reduced SDS gel. Lane M was loaded with 10 ul of SeeBlue Plus2 Prestained Standard.

Immunostimulation of TGFRt15-TGFRs21 in C57BL/6 mice

TGFRt15-TGFRs21 is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes the first polypeptide that is a soluble fusion of single chain two TGFβRII domains, human tissue factor 219 fragment and human IL-15 (TGFRt15), and the second polypeptide that is a soluble fusion of single chain two TGFβRII domains, sushi domain of human IL-15 receptor alpha chain and human IL-21 (TGFRs21).

CHO cells were co-transfected with TGFRt15 and TGFRs21 vectors. The TGFRt15-TGFRs21 complex was purified from the transfected CHO cell culture supernatant. The TGFβ receptor, IL-15, IL-21 and tissue factor (TF) components were

demonstrated in the complex by ELISA as shown in Figure 118A-B. A humanized anti-TF monoclonal antibody (anti-TF IgG1) was used as the capture antibody to determine TF in TGFRt15-TGFRs21, biotinylated anti-human IL-15 antibody (R&D systems), biotinylated anti-human TGFβ receptor antibody (R&D systems, and biotinylated anti-human IL-21 antibody (R&D Systems) were used as the detection antibodies to respectively determine IL-15, TGFβ receptor, and IL-21 in TGFRt15-TGFRs21. For detection, peroxidase conjugated streptavidin (Jackson ImmunoResearch Lab) and ABTS were used.

Wild type C57BL/6 mice were treated subcutaneously with either control solution (PBS) or with TGFRt15-TGFRs21 at 3 mg/kg. Four days after treatment, spleen weight and the percentages of various immune cell types present in the spleen were evaluated. As shown in Figure 119A, the percentages of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells present in the spleen of control-treated and TGFRt15-TGFRs21-treated mice were evaluated. The dynamic proliferation of immune cells based on Ki67 expression after TGFRt15-TGFRs21 treatment was also evaluated. The splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 (NK) antibodies and then intracellularly stained with anti-Ki67 antibody. The percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells and the mean fluorescent intensity (MFI) of Ki67 of corresponding immunocyte subsets were analyzed by flow cytometry (Figures 119A and 119B). Furthermore, cytotoxicity potential based on granzyme B expression of the splenocytes induced by TGFRt15-TGFRs21 after the single dose treatment of mouse was also evaluated. As shown in Figure 120, in the spleens of mice treated with TGFRt15-TGFRs21, the expression of granzyme B by NK cells increased after treatment. The splenocytes from TGFRt15-TGFRs21-treated mice were stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 (NK) antibodies and then intracellularly stained with anti-granzyme B antibody. The mean fluorescent intensity (MFI) of granzyme B of corresponding immunocyte subsets was analyzed by flow cytometry (Figure 120).

As shown in Figure 119A, in the spleens of mice treated with TGFRt15-TGFRs21, the percentages of $CD8^+$ T cells and NK cells both increased on day 4 after a single TGFRt15-TGFRs21 treatment. These results demonstrate that TGFRt15-

TGFRs21 is able to induce immune cells to proliferate in mouse spleen, in particular $CD8^+$ T cells and NK cells.

Additionally, cytotoxicity of the mouse splenocytes against tumor cells was also evaluated. Mouse Yac-1 cells were labeled with CellTrace Violet and used as tumor target cells. The splenocytes were prepared from TGFRt15-TGFRs21-treated mice and used as effector cells. The target cells were mixed with effector cells at E:T ratio = 10:1 in RPMI-10 medium with or without TGFRt15-TGFRs21 at 100 nM and incubated at 37°C for 24 hours. Target Yac-1 cell inhibition was assessed by analysis of viable violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 inhibition was calculated using a formula, (1-[viable Yac-1 cell number in experimental sample]/[viable Yac-1 cell number in the sample without splenocytes]) x 100. As shown in Figure 121, TGFRt15-TGFRs21-treated mouse splenocytes had stronger cytotoxicity against Yac-1 cells than the control mouse cells in the presence of TGFRt15-TGFRs21 during cytotoxic assay (Figure 121).

Example 58: TGFRt15-TGFRs16 fusion protein generation

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu/ anti-CD16scFv and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 122 and Figure 123). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA

AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain, followed by the anti-CD16scFv sequence, which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu following with the anti-CD16scFv sequence are shown below.

The nucleic acid sequence of the TGFRs16 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA

GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Anti-human CD16scFv)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGA
GGAGGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCT
GGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTA
CGGCATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCC
GGCATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCA
GGTTCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAA
CTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCC
CTGCTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

The amino acid sequence of TGFRs16 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN

NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Anti-human CD16scFv)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGM
SWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLR
AEDTAVYYCARGRSLLFDYWGQGTLVTVSR

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFR/IL-15RαSu/anti-CD16scFv and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:TGFR/IL-15RαSu/anti-CD16scFv protein complex (referred to as TGFRt15-TGFRs16), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Example 59: The TGFRt15-TGFRs137L fusion protein generation

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu/ CD137L and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 124 and Figure 125). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, CD137L, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two

TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV

WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain, followed by a (G4S)3 linker and the CD137L sequence, which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu following with a (G4S)3 linker and the CD137L sequence are shown below.

The nucleic acid sequence of the TGFRs137L construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT

AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCG
ATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGAC
GGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGC
TGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCG
AGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGA
GGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTG
GCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAA
ATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of TGFRs137L fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVS LTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW QLTQGATVLGLFRVTPEIPAGLPSPRSE

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFR/IL-15RαSu/CD137L and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. Hum Gene Ther 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:TGFR/IL-15RαSu/CD137L

protein complex (referred to as TGFRt15-TGFRs137L), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Example 60: Stimulation of NK cells *in vitro* by multi-chain chimeric polypeptide constructs

A set of experiments was performed to assess changes in the surface phenotype of lymphocyte populations after stimulation with 18t15-12s, 18t15-12s16, and 7t15-21s. In these experiments, fresh human leukocytes were obtained from the blood bank. Peripheral blood lymphocytes were isolated with the Ficoll-PAQUE Plus (GE Healthcare) density gradient media. The cells were counted and resuspended at 0.2 x 10^6/mL in a 96-well flat-bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with: 18t15-12s (100 nM); 18t15-12s16 (100 nM), a mixture of single cytokines rhIL15 (50 ng/mL) (Miltenyi), rhIL18 (50 ng/mL) (Invivogen), and rhIL-12 (10 ng/mL) (Peprotech); 7t15-21s (100 nM) + anti-TF antibody (50 nM); 7t15-21s (100 nM); or anti-TF antibody (50 nM) at 37 °C and 5% CO_2 for 16 hours. The next day, the cells were harvested and surface stained for 30 minutes with antibodies specific for CD4 or CD8, CD62L, and CD69. After surface staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 126 shows that overnight incubation of purified lymphocyte populations (CD4 and CD8 T cells) with 18t15-12s, 18t15-12s16, or 7t15-21s + anti-TF antibody resulted in an increase in the percentage of CD8 and CD4 T cells expressing CD69. Additionally, incubation with 7t15-21s + anti-TF antibody resulted in an increase in the percentage of CD8 and CD4 T cells expressing CD62L (Figure 126).

A set of experiments was performed to determine the increase in phospho-STAT4 and phospho-STAT5 levels in NK cells after stimulation with 18t15-12s. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies).

The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 specific antibodies (BioLegend). The cells were counted and resuspended in 0.05 x 10^6/mL in a 96-well flat-bottom plate in 0.1 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with hIL-12 (10 ng/mL) (Biolegend) or hIL-15 (50 ng/mL) (NCI) (Single cytokines), or 18t15-12s (100 nM) at 37 °C and 5% CO_2 for 90 minutes. Unstimulated NK cells (US) were used as a control. The cells were harvested and fixed in paraformaldehyde (Sigma) to a final concentration of 1.6%. Plates were incubated in the dark at room temperature for 10 minutes. FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) (100 µL) was added and cells were transferred to 96-well "V" bottom plate. The cells were washed for 1500 RPM for 5 minutes at room temperature. The cell pellet was mixed with 100 µL chilled methanol by gently pipetting up and down, and cells were incubated for 30 minutes at 4 °C. The cells were mixed with 100 mL of FACS buffer and washed for 1500 RPM for 5 minutes at room temperature. The cell pellets were mixed with 50 mL of FACS buffer containing 4 mL of pSTAT4 (BD Bioscience) and pSTAT5 antibodies (BD Bioscience) followed by incubation for 30 minutes at room temperature in the dark. The cells were mixed with 100 mL of FACS buffer and washed for 1500 RPM for 5 minutes at room temperature. The cell pellets were mixed with 50 mL of FACS buffer and cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 127 shows that incubation of NK cells with 18t15-12s induced an increase in pSTAT4 and pSTAT5 (plotted data, normalized fold-change).

Example 61: Stimulation of NK cells *in vivo* by TGFRt15-TGFRs

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct on immune stimulation in $ApoE^{-/-}$ mice fed with a Western diet. In these experiments, 6-week old female B6.129P2-$ApoE^{tm1Unc}$/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-weeks of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs at 3 mg/kg.

Three days post treatment, mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. The blood was mixed with 10 μL 0.5 M EDTA, and 20 μL blood was taken for lymphocyte subsets analysis. The red blood cells were lysed with ACK (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4) and the lymphocytes were stained with anti-mouse CD8a and anti-mouse NK1.1 antibodies for 30 minutes at 4 °C in FACS staining buffer (1% BSA in PBS). The cells were washed once and analyzed with a BD FACS Celesta. For Treg staining, ACK treated blood lymphocytes were stained with anti-mouse CD4 and anti-mouse CD25 antibodies for 30 minutes at 4 °C in FACS staining buffer. The cells were washed once and resuspended in fixation/permeabilization working solution and incubated at room temperature for 60 minutes. The cells were washed once and resuspended in permeabilization buffer. The samples were centrifuged at 300-400 x g for 5 minutes at room temperature and the supernatant was then discarded. The cell pellet was resuspended in residual volume and the volume adjusted to about 100 μL with 1 x permeabilization buffer. Anti-Foxp3 antibody was added to the cells, and the cells were incubated for 30 minutes at room temperature. Permeabilization buffer (200 μL) was added to the cells, and the cells were centrifuged at 300-400 x g for 5 minutes at room temperature. The cells were resuspended in flow cytometry staining buffer and analyzed on a flow cytometer. Figures 128A-128C show that treatment with TGFRt15-TGFRs increased the percentage of NK cells and $CD8^+$ T cells in $ApoE^{-/-}$ mice fed with Western diet.

Example 62: Induction of proliferation of immune cells *in vivo*

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct on immune stimulation in C57BL/6 mice. In these experiments, C57BL/6 mice were subcutaneously treated with control solution (PBS) or TGFRt15-TGFRs at 0.1, 0.3, 1, 3, and 10 mg/kg. The treated mice were euthanized 4 days post-treatment. Spleen weight was measured and splenocyte suspensions were prepared. The splenocyte suspensions were stained with conjugated anti-CD4, anti-CD8, and anti-NK1.1 (NK) antibodies. The cells were additionally stained for proliferation marker Ki67. Figure 129A shows that spleen weight in mice treated with TGFRt15-TGFRs

increased with increasing dosage of TGFRt15-TGFRs. Additionally, spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs was higher as compared to mice treated with just the control solution. The percentages of $CD8^+$ T cells and NK cells both increased with increasing dosage of TGFRt15-TGFRs (Figure 129B). Finally, TGFRt15-TGFRs significantly upregulated expression of cell proliferation marker Ki67 in both $CD8^+$ T cells and NK cells at all doses of TGFRt15-TGFRs tested (Figure 129C). These results demonstrate that TGFRt15-TGFRs treatment induced proliferation of both $CD8^+$ T cells and NK cells in C57BL/6 mice.

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct on immune stimulation in $ApoE^{-/-}$ mice fed with a Western diet. In these experiments, 6-week old female B6.129P2-$ApoE^{tm1Unc}$/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-week of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs at 3 mg/kg. Three days post-treatment, the mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. The blood was mixed with 10 μL 0.5 M EDTA and 20 μL blood was taken for lymphocyte subsets analysis. The red blood cells were lysed with ACK (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4) and the lymphocytes were stained with anti-mouse CD8a and anti-mouse NK1.1 antibodies for 30 minutes at 4 °C in FACS staining buffer (1% BSA in PBS). The cells were washed once and resuspended in Fixation Buffer (BioLegend Cat# 420801) for 20 minutes at room temperature. The cells were centrifuged at 350 x g for 5 minutes, the fixed cells were resuspended in Intracellular Staining Permeabilization Wash Buffer (BioLegend Cat# 421002) and then centrifuged at 350 x g for 5 minutes. The cells were then stained with anti-Ki67 antibody for 20 minutes at RT. The cells were washed twice with Intracellular Staining Permeabilization Wash Buffer and centrifuged at 350 x g for 5 minutes. The cells were then resuspended in FACS staining buffer. Lymphocyte subsets were analyzed with a BD FACS Celesta. As described in Figure 130A and 130B, treatment of $ApoE^{-/-}$ mice with TGFRt15-TGFRs induced proliferation (Ki67-positive staining) in NK and $CD8^+$ T cells.

Example 63: NK-mediated cytotoxicity following treatment with multi-chain construct

A set of experiments was performed to determine if treatment of NK cells with TGFRt15-TGFRs enhanced cytotoxicity of NK cells. In these experiments, Human Daudi B lymphoma cells were labeled with CellTrace Violet (CTV) and used as tumor target cells. Mouse NK effector cells were isolated with NK1.1-positive selection using a magnetic cell sorting method (Miltenyi Biotec) of C57BL/6 female mouse spleens 4 days post TGFRt15-TGFRs subcutaneous treatment at 3 mg/kg. Human NK effector cells were isolated from peripheral blood mononuclear cells derived from human blood buffy coats with the RosetteSep/human NK cell reagent (Stemcell Technologies). The target cells (Human Daudi B lymphoma cells) were mixed with effector cells (either mouse NK effector cells or human NK effector cells) in the presence of 50 nM TGFRt15-TGFRs or in the absence of TGFRt15-TGFRs (control) and incubated at 37 °C for 44 hours for mouse NK cells and for 20 hours for human NK cells. Target cell (Daudi) viability was assessed by analysis of propidium iodide-positive, CTV-labeled cells using flow cytometry. The percentage of Daudi inhibition was calculated using the formula (1-viable tumor cell number in experimental sample/viable tumor cell number in the sample without NK cells) x 100. Figure 131 shows that mouse (Figure 131A) and human (Figure 131B) NK cells had significantly stronger cytotoxicity against Daudi B cells following NK cell activation with TGFRt15-TGFRs than in the absence of TGFRt15-TGFRs activation.

A set of experiments was performed to determine antibody-dependent cellular cytotoxicity (ADCC) of mouse and human NK cells following treatment with TGFRt15-TGFRs. In these experiments, human Daudi B lymphoma cells were labeled with CellTrace Violet (CTV) and used as tumor target cells. Mouse NK effector cells were isolated with NK1.1-positive selection using a magnetic cell sorting method (Miltenyi Biotec) of C57BL/6 female mouse spleens 4 days post-TGFRt15-TGFRs subcutaneous treatment at 3 mg/kg. Human NK effector cells were isolated from peripheral blood mononuclear cells derived from human blood buffy coats with the RosetteSep/human NK cell reagent (Stemcell Technologies). The target cells (Daudi B cells) were mixed with effector cells (either mouse NK effector cells or human NK effector cells) in the presence

of anti-CD20 antibody (10 nM Rituximab, Genentech) and in the presence of 50 nM TGFRt15-TGFRs, or in the absence of TGFRt15-TGFRs (control) and incubated at 37 °C for 44 hours for mouse NK cells and for 20 hours for human NK cells. The Daudi B cells express the CD20 targets for the anti-CD20 antibody. Target cell viability was assessed after incubation by analysis of propidium iodide-positive, CTV-labeled target cells using flow cytometry. The percentage of Daudi inhibition was calculated using the formula (1-viable tumor cell number in experimental sample/viable tumor cell number in the sample without NK cells) x 100. Figure 132 shows that mouse NK cells (Figure 132A) and human NK cells (Figure 132B) had stronger ADCC activity against Daudi B cells following NK cell activation with TGFRt15-TGFRs than in the absence of TGFRt15-TGFRs activation.

Example 64: Treatment of Cancer

A set of experiments was performed to assess antitumor activity of TGFRt15-TGFRs plus anti-TRP1 antibody (TA99) in combination with chemotherapy in a melanoma mouse model. In these experiments, C57BL/6 mice were subcutaneously injected with 0.5 x 10^6 B16F10 melanoma cells. The mice were treated with three doses of chemotherapy docetaxel (10 mg/kg) (DTX) on day 1, day 4, and day 7, followed by treatment with single dose of combination immunotherapy TGFRt15-TGFRs (3 mg/kg) + anti-TRP1 antibody TA99 (200 μg) on day 9. Figure 133A shows a schematic of the treatement regimen. Tumor growth was monitored by caliper measurement, and tumor volume was calculated using the formula $V = (L \times W^2)/2$, where L is the largest tumor diameter and W is the perpendicular tumor diameter. Figure 133B shows that treatment with DTX + TGFRt15-TGFRs + TA99 significantly reduced tumor growth compared to saline control and DTX treatment groups (N=10, ****p <0.001, Multiple t test analyses).

To assess immune cell subsets in the B16F10 tumor model, peripheral blood analysis was performed. In these experiments, C57BL/6 mice were injected with B16F10 cells and treated with DTX, DTX + TGFRt15-TGFRs + TA99, or saline. Blood was drawn from the submandibular vein of B16F10 tumor-bearing mice on days 2, 5, and 8 post-immunotherapy for the DTX + TGFRt15-TGFRs + TA99 group and day 11 post-tumor injection for the DTX and saline groups. RBCs were lysed in ACK lysis buffer

and the lymphocytes were washed and stained with anti-NK1.1, anti-CD8, and anti-CD4 antibodies. The cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figures 133C-133E show that DTX + TGFRt15-TGFRs + TA99 treatment induced an increase in the percentage of NK cells and $CD8^+$ T cells in the tumors compared to the saline and DTX treatment groups.

On day 17, total RNA was extracted from tumors of mice treated with saline, DTX or DTX + TGFRt15-TGFRs + TA99 using Trizol. Total RNA (1 μg) was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was carried out with CFX96 Detection System (Bio-Rad) using FAM-labeled predesigned primers for senescence cell markers, (F) p21 (G) DPP4 and (H) IL6. The housekeeping gene 18S ribosomal RNA was used as an internal control to normalize the variability in expression levels. The expression of each target mRNA relative to *18S* rRNA was calculated based on Ct as $2^{-\Delta(\Delta Ct)}$, in which $\Delta Ct = Ct_{target} - Ct_{18S}$. The data is presented as fold-change as compared to saline control. Figure 133F-133H show that DTX treatment induced an increase in senescent tumor cells that were subsequently reduced following treatment with TGFRt15-TGFRs + TA99 immunotherapy.

A set of experiments was performed to investigate amelioration of Western diet-induced hyperglycemia in $ApoE^{-/-}$ mice by TGFRt15-TGFRs. In these experiments, 6-week old female B6.129P2-$ApoE^{tm1Unc}$/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-weeks of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs at 3 mg/kg. Three days post-treatment, the mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. Blood glucose was detected with a glucose meter (OneTouch UltraMini) and GenUltimated test strips using a drop of fresh blood. As shown in Figure 134A, TGFRt15-TGFRs treatment reduced hyperglycemia induced by the Western diet. The plasma insulin and resistin levels were analyzed with Mouse Rat Metabolic Array by Eve Technologies. HOMA-IR was calculated using the following formula: homeostatic model assessment-insulin resistance = Glucose (mg/dL) * Insulin (mU/mL)/405. As shown in Figure 134B, TGFRt15-TGFRs treatment reduced insulin resistance compared to the untreated group. TGFRt15-TGFRs ($p<0.05$) reduced

resistin levels significantly compared to the untreated group as shown in Figure 142C, which may relate to the reduced insulin resistance induced by TGFRt15-TGFRs (Figure 134B).

Example 65: Induction of differentiation of NK cells into cytokine-induced memory like NK cells

A set of experiments was performed to assess the differentiation of NK cells into cytokine-induced memory like NK Cells (CIMK-NK Cells) after stimulation with 18t15-12s. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >90% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 antibodies (BioLegend). The cells were counted and resuspended in 2 x 10^6/mL in a 24-well flat-bottom plate in 2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were unstimulated ("No Spike") or stimulated with 18t15-12s (100 nM) or a mixture of single cytokines including rhIL15 (50 ng/mL) (Miltenyi), rhIL18 (50 ng/mL) (Invivogen), and rhIL-12 (10 ng/mL) (Peprotech) ("single cytokines") at 37 °C and 5% CO_2 for 16 hrs. The next day, the cells were harvested, and washed two times with warm complete media at 1000 RPM for 10 minutes at room temperature. The cells were resuspended at 2 x 10^6/mL in a 24-well flat-bottom plate in 2 mL of complete media with rhIL15 (1 ng/mL). After every 2 days, half of the medium was replaced with fresh complete media containing rhIL15.

To assess the change in memory phenotype of NK cells at day 7, the cells were stained with antibodies to cell-surface CD56, CD16, CD27, CD62L, NKp30, and NKp44 (BioLegend). After surface staining, the cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 135 shows that incubation of NK cells with 18t15-12s resulted in an increase in the percentage of $CD16^+CD56^+$ NK cells expressing

CD27, CD62L, and NKp44, and an increase in the levels (MFI) of NKp30 in $CD16^{+}CD56^{+}$ NK cells.

Example 66: Upregulation of CD44 memory T cells

A set of experiments was performed to assess upregulation of CD44 memory T cells upon treatment with TGFRt15-TGFRs. In these experiments, C57BL/6 mice were subcutaneously treated with TGFRt15-TGFRs. The treated mice were euthanized and the single splenocyte suspensions were prepared 4 days (TGFRt15-TGFRs) following the treatment. The prepared splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8 and anti-CD44 antibodies and the percentages of $CD44^{high}$ T cells in $CD4^{+}$ T cells or $CD8^{+}$ T cells were analyzed by flow cytometry. The results show that TGFRt15-TGFRs upregulated expression of the memory marker CD44 on $CD4^{+}$ and $CD8^{+}$ T cells (Figures 136). These findings indicate that TGFRt15-TGFRs was able to induce mouse T cells to differentiate into memory T cells.

Example 67: Tissue factor coagulation assays following treatment with single-chain or multi-chain chimeric polypeptides

A set of experiments was performed to assess blood coagulation following treatment with single-chain or multi-chain chimeric polypeptides. To initiate the blood coagulation cascade pathway, tissue factor (TF) binds to Factor VIIa (FVIIa) to form a TF/FVIIa complex. The TF/FVIIa complex then binds Factor X (FX) and converts FX to FXa.

Factor VIIa (FVIIa) activity Assay

One assay to measure blood coagulation involves measuring Factor VIIa (FVIIa) activity. This type of assay requires the presence of tissue factor and calcium. The TF/FVIIa complex activity can be measured by a small substrate or by a natural protein substrate, for example, Factor X (FX). When FX is used as a substrate, phospholipids are also required for TF/FVIIa activity. In this assay, FVIIa activity is determined with FVIIa-specific chromogenic substrate S-2288 (Diapharma, West Chester, OH). The

color change of the S-2288 substrate can be measured spectrophotometrically and is proportional to the proteolytic activity of FVIIa (e.g., the TF/FVIIa complex).

In these experiments, the FVIIa activity of the following groups were compared: the 219-amino acid extracellular domain of tissue factor domain (TF_{219}), a multi-chain chimeric polypeptide with a wild-type tissue factor domain, and a multi-chain chimeric polypeptide with a mutant tissue factor domain. The chimeric polypeptides containing mutant tissue factor molecules were constructed with mutations to the TF domain at amino acid sites: Lys20, Ile22, Asp58, Arg135, and Phe140.

In order to assess activity of FVIIa, FVIIa, and TF_{219} or a TF_{219} -containing multi-chain chimeric polypeptide were mixed at an equal molar concentration (10 nM) in all wells of a 96-well ELISA plate in a total volume of 70 µL. After incubation for 10 minutes at 37 °C, 10 µL of 8 mM S-2288 substrate was added to start the reaction. The incubation was then kept at 37 °C for 20 minutes. Finally, color change was monitored by reading absorbance at 405 nm. The OD values of different TF/VIIa complexes are shown in Table 1 and Table 2. Table 1 shows a comparison of TF_{219}, 21t15-21s wild-type (WT) and 21t15-21s mutant (Mut). Table 2 shows a comparison of TF_{219}, 21t15-TGFRs wild-type (WT), and 21t15-TGFRs mutant (Mut). These data show that TF_{219}-containing multi-chain chimeric polypeptides (e.g., 21t15-21s-WT, 21t15-21s-Mut, 21t15-TGFRS-WT, and 21t15-TGFRS-Mut) have lower FVIIa activity than TF_{219} when the chromogenic S-2288 was used as a substrate. Notably, the multi-chain chimeric polypeptides containing TF_{219} mutations showed much lower FVIIa activity when compared to multi-chain chimeric polypeptides containing wild type TF_{219}.

Table 1. FVIIa activity

Molecule	**OD value at 405 nm**
TF_{219}	0.307
21t15/21S-WT	0.136
21t15/21S-Mut	0.095

WT: wild type of TF_{219}, Mut: TF_{219} containing mutations.

Table 2. FVIIa activity

Molecule	OD value at 405 nm
TF_{219}	0.345
21t15/TGFRS-WT	0.227
21t15/TGFRS-Mut	0.100

WT: wild type of TF_{219}, Mut: TF_{219} containing mutations.

Factor X (FX) Activation Assay

An additional assay to measure blood coagulation involves measuring activation of Factor X (FX). Briefly, TF/VIIa activates blood coagulation Factor X (FX) to Factor Xa (FXa) in the presence of calcium and phospholipids. TF_{243}, which contains the transmembrane domain of TF, has much higher activity in activating FX to FXa than TF_{219}, which does not contain the transmembrane domain. TF/VIIa dependent activation of FX is determined by measuring FXa activity using an FXa-specific chromogenic substrate S-2765 (Diapharma, West Chester, OH). The color change of S-2765 can be monitored spectrophotometrically and is proportional to the proteolytic activity of FXa.

In these experiments, FX activation with a multi-chain chimeric polypeptide (18t15-12s, mouse (m)21t15, 21t15-TGFRs, and 21t15-7s) was compared with a positive control (Innovin) or TF_{219}. TF_{219} (or TF_{219}-containing multi-chain chimeric polypeptides)/FVIIa complexes were mixed at an equal molar concentration (0.1 nM each) in a volume of 50 µL in round bottom wells of a 96-well ELISA plate, after which 10 µL of 180 nM FX was added. After 15 minutes of incubation at 37 °C, during which time FX was converted to FXa, 8 µL of 0.5 M EDTA (which chelates calcium and thus terminates FX activation by TF/VIIa) was added to each well to stop FX activation. Next, 10 µL of 3.2 mM S-2765 substrate was added to the reaction mixture. Immediately, the plate absorbance was measured at 405 nm and was recorded as the absorbance at time 0. The plate was then incubated for 10-20 minutes at 37 °C. The color change was monitored by reading absorbance at 405 nm following the incubation. Results of FX activation as measured by FXa activity using chromogenic substrate S-2765 are shown in Figure 137. In this experiment, Innovin, which is a commercial prothrombin reagent containing lipidated recombinant human TF_{243}, was used as a

positive control for FX activation. Innovin was reconstituted with purified water to about 10 nM of TF_{243}. Next, 0.1 nM TF/VIIa complex was made by mixing an equal volume of 0.2nM of FVIIa with 0.2 nM of Innovin. Innovin demonstrated very potent FX activation activity, while TF_{219} and TF_{219}-containing multi-chain chimeric polypeptides had very low FX activation activity, confirming that TF_{219} is not active in a TF/FVIIa complex for activating natural substrate FX *in vivo*.

Prothrombin Time Test

A third assay to measure blood coagulation is the prothrombin time (PT) test, which measures blood clotting activity. Here, the PT test was performed using commercially available normal human plasma (Ci-Trol Coagulation Control, Level I). For a standard PT test, clot reactions were initiated by addition of Innovin, a lipidated recombinant human TF_{243}, in the presence of calcium. Clotting time was monitored and reported by STart PT analyzer (Diagnostica Stago, Parsippany, N.J.). PT assays were started by injecting 0.2 mL of various dilutions of Innovin diluted in PT assay buffer (50 mM Tris-HCl, pH 7.5, 14.6 mM $CaCl_2$, 0.1% BSA) into cuvettes containing 0.1 mL of normal human plasma prewarmed at 37 °C. In the PT assay, shorter PT time (clotting time) indicates a higher TF-dependent clotting activity while longer PT (clotting time) means lower TF-dependent clotting activity.

As seen in Figure 138, addition of different amounts of Innovin (e.g., Innovin reconstituted with purified water equivalent to 10 nM of lipidated recombinant human TF_{243} was considered to be 100% Innovin) to the PT assay demonstrated a dose-response relationship, where lower concentrations of TF_{243} resulted in a longer PT time (lower clotting activity). For example, 0.001% Innovin had a PT time greater than 110 seconds, which was almost the same as buffer alone.

In another experiment, the PT test was conducted on TF_{219} and multi-chain chimeric polypeptides including: 18t15-12s, 7t15-21s, 21t15-TGFRs-WT, and 21t15-TGFRs-Mut. Figure 139 show that TF_{219} and TF_{219}-containing multi-chain chimeric polypeptides (at a concentration of 100 nM) had prolonged PT times indicating extremely low or no clotting activity.

Studies were also conducted to evaluate whether incubating the multi-chain chimeric polypeptides in the presence of other cells carrying receptors for the cytokine components of the multi-chain chimeric polypeptide (32Dβ or human PBMCs) would affect the clotting time in the PT assay. To examine whether cells that express IL-15 receptor (32Dβ cells) or IL-15 and IL-21 receptors (PBMCs) would bind IL-15 -containing multi-chain chimeric polypeptides to mimic natural TF as a cellular FVIIa receptor, TF_{219}-containing multi-chain chimeric polypeptides (at a concentration of 100 nM for each molecule) were diluted in the PT assay buffer and preincubated with 32Dβ cells (at 2 x 10^5 cells/mL) or PBMC (at 1 x 10^5 cells/mL) for 20-30 minutes at room temperature. The PT assay was then conducted as described above. Figures 140 and 141 shows that TF_{219} and TF_{219}-containing multi-chain chimeric polypeptides mixed with 32Dβ cells (Figure 140) or PBMC (Figure 141) at a final concentration of 100 nM had prolonged PT times similar to 0.001-0.01% Innovin (equivalent to 0.1 pM to 1.0 pM of TF_{243}). Expressed in percentage of relative TF_{243} activity, TF_{219}-containing multi-chain chimeric polypeptides had 100,000 to 1,000,000 times lower TF dependent clotting activity when compared to Innovin. This demonstrated that TF_{219}-containing multi-chain chimeric polypeptides had extremely low or no TF-dependent clotting activity, even while the molecules were bound to an intact cell membrane surface, such as 32Dβ or PBMCs.

Example 68: Characterization of 7t15-21s137L (long version)

The nucleic acid sequence of the 7t15 construct (including signal peptide sequence) is as follows (SEQ ID NO: 107):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA

GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGT
CCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGT
AAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGA
GAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCC
AATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGT
GCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCA
CATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows (SEQ ID NO: 106):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

The nucleic acid sequence of the 21s137L construct (including signal peptide sequence) is as follows (SEQ ID NO: 225):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC

CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGA
GCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAG
GAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAAC
GTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG
GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of 21s137L fusion protein (including the leader sequence) is as follows (SEQ ID NO: 226):

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH
WTTPS
LKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE

The following experiment was conducted to evaluate whether the CD137L portion in 7t15-21s137L was intact to bind to CD137 (4.1BB). On day 1, a 96-well plate was coated with 100 µL (2.5 µg/mL) of GAH IgG Fc (G-102-C, R&D Systems) in R5 (coating buffer), overnight. On day 2, the plates were washed three times and blocked with 300 µL of 1% BSA in PBS at 37°C for 2 hrs. 10 ng/ml of 4.1BB/Fc (838-4B, R&D Systems) was added at 100 µl/well for 2 hrs at room temperature. Following three washes, 7t15-21s137L (long version) or 7t15-21s137Ls (short version) was added starting at 10 nM, or recombinant human 4.1BBL starting at 180ng/mL, with 1/3 dilution, followed by incubation at 4°C overnight. On day 3, the plates were washed three times, and 500 ng/mL of biotinylate-goat anti-human 4.1BBL (BAF2295, R&D Systems) was applied at 100 µL per well, followed by incubation at RT for 2 hrs. The plates were washed three times, and incubated with 0.25 µg/mL of HRP-SA (Jackson ImmuneResearch) at 100 µL per well for 30 min. The plates were then washed three times, and incubated with 100 µL of ABTS for 2 mins at RT. The results were read at 405 nm. As shown in Figure 142, both 7t15-21s137L (long version) and 7t15-21s137L (short version) could interact with 4.1BB/Fc (dark diamond and gray square) compared to the recombinant human 4.1BB ligand (rhCD137L, light gray star). 7t15-21s137L (long version) (dark diamond) interacted better with 4.1BB/Fc as compared to 7t15-21s137L (short version) (gray square).

The following experiments were conducted to evaluate whether the components IL7, IL21, IL15, and 4.1BBL in 7t15-21s137L (long version) were intact to be detected by the individual antibody using ELISA. A 96-well plate was coated with 100 μL (4 μg/mL) of anti-TF (human IgG1) in R5 (coating buffer) and incubated at RT for 2 hrs. The plates were washed three times, and blocked with 100 μL of 1% BSA in PBS. Purified 7t15-21s137L (long version) was added starting at 10 nM, and at 1/3 dilution, followed by incubation at RT for 60 min. The plates were washed three times, and 500 ng/mL of biotinylate-anti-IL7 (506602, R&D Systems), 500 ng/mL of biotinylate-anti-IL21 (13-7218-81, R&D Systems), 50 ng/mL of biotinylate-anti-IL15 (BAM247, R&D Systems), or 500 ng/ml of biotinylate-goat anti-human 4.1BBL (BAF2295, R&D Systems) was added per well and incubated at room temperature for 60 min. The plates were washed three times and incubated with 0.25 μg/mL of HRP-SA (Jackson ImmunoResearch) at 100 μL per well for 30 min at RT. The plates were washed four times, and incubated with 100 μL of ABTS for 2 mins at room temperature. The absorbance results were read at 405 nm. As shown in Figure 143A-143D, the components including IL7, IL21, IL15, and 4.1BBL in 7t15-21s137L (long version) were detected by the individual antibodies.

The following experiment was conducted to evaluate the activity of IL15 in 7t15-21s137L (long version) and 7t15-21s137L (short version). The ability of 7t15-21s137L (long version) and 7t15-21s137L (short version) to promote proliferation of IL2Rαβγ-expressing CTLL2 cells was compared with that of recombinant IL15. IL15 dependent CTLL2 cells were washed five times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serially diluted 7t15-21s137L (long version), 7t15-21s137L (short version), or IL15 were added to the cells. Cells were incubated in a CO_2 incubator at 37 °C for 3 days. Cell proliferation was detected by adding 20 μL of PrestoBlue (A13261, ThermoFisher) to each well on day 3 and incubated for an additional 4 hours in a CO_2 incubator at 37 °C. Raw absorbance at 570-610 nm was read in a micro-titer plate reader. As shown in Figure 144, 7t15-21s137L (long version), 7t15-21s137L (short version), and IL15 all promoted CTLL2 cell proliferation. The EC_{50} of 7t15-21s137L (long version), 7t15-21s137L (short version), and IL15 is 51.19 pM, 55.75 pM, and 4.947 pM, respectively.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Exemplary Embodiments

Embodiment A1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

Embodiment A2. The multi-chain chimeric polypeptide of embodiment A1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment A3. The multi-chain chimeric polypeptide of embodiment A1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment A4. The multi-chain chimeric polypeptide of any one of embodiments A1-A3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment A5. The multi-chain chimeric polypeptide of any one of embodiments A1-A3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A6. The multi-chain chimeric polypeptide of any one of embodiments A1-A5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment A7. The multi-chain chimeric polypeptide of any one of embodiments A1-A5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment A8. The multi-chain chimeric polypeptide of any one of embodiments A1-A7, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment A9. The multi-chain chimeric polypeptide of embodiment A8, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment A10. The multi-chain chimeric polypeptide of embodiment A9, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment A11. The multi-chain chimeric polypeptide of any one of embodiments A1-A7, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment A12. The multi-chain chimeric polypeptide of any one of embodiments A1-A11, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment A13. The multi-chain chimeric polypeptide of embodiment A12, wherein the first target-binding domain and the second target-binding domain are each antigen-binding domains.

Embodiment A14. The multi-chain chimeric polypeptide of embodiment A12 or A13, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment A15. The multi-chain chimeric polypeptide of any one of embodiments A1-A14, wherein one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF- β receptor II (TGF- βRII), a ligand of TGF- βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

Embodiment A16. The multi-chain chimeric polypeptide of any one of embodiments A1-A14, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment A17. The multi-chain chimeric polypeptide of embodiment A16, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group

consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

Embodiment A18. The multi-chain chimeric polypeptide of any one of embodiments A1-A14, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment A19. The multi-chain chimeric polypeptide of embodiment A18, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28.

Embodiment A20. The multi-chain chimeric polypeptide of any one of embodiments A1-A19, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment A21. The multi-chain chimeric polypeptide of embodiment A20, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

Embodiment A22. The multi-chain chimeric polypeptide of any one of embodiments A1-A19, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment A23. The multi-chain chimeric polypeptide of embodiment A22, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A24. The multi-chain chimeric polypeptide of embodiment A22, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment A25. The multi-chain chimeric polypeptide of embodiment A22, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment A26. The multi-chain chimeric polypeptide of embodiment A22, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment A27. The multi-chain chimeric polypeptide of embodiment A22, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A28. The multi-chain chimeric polypeptide of embodiment A27, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A29. The multi-chain chimeric polypeptide of embodiment A27, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding

domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A30. The multi-chain chimeric polypeptide of embodiment A27, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A31. The multi-chain chimeric polypeptide of embodiment A27, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment A32. The multi-chain chimeric polypeptide of embodiment A27, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment A33. The multi-chain chimeric polypeptide of embodiment A27, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment A34. The multi-chain chimeric polypeptide of any one of embodiments A1-A33, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment A35. The multi-chain chimeric polypeptide of embodiment A34, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment A36. The multi-chain chimeric polypeptide of embodiment A34, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment A37. The multi-chain chimeric polypeptide of embodiment A34, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment A38. The multi-chain chimeric polypeptide of embodiment A34, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment A39. The multi-chain chimeric polypeptide of any one of embodiments A20-A38, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment A40. The multi-chain chimeric polypeptide of embodiment A39, wherein two or more of the first target-binding domain, the second target-binding

domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment A41. The multi-chain chimeric polypeptide of embodiment A40, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment A42. The multi-chain chimeric polypeptide of embodiment A39, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen.

Embodiment A43. The multi-chain chimeric polypeptide of embodiment A42, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope.

Embodiment A44. The multi-chain chimeric polypeptide of embodiment A43, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence.

Embodiment A45. The multi-chain chimeric polypeptide of any one of embodiments A20-A38, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment A46. The multi-chain chimeric polypeptide of any one of embodiments A20-A45, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain.

Embodiment A47. The multi-chain chimeric polypeptide of embodiment A46, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain.

Embodiment A48. The multi-chain chimeric polypeptide of embodiment A47, wherein antigen-binding domain comprises a scFv.

Embodiment A49. The multi-chain chimeric polypeptide of any one of embodiments A20-A48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD3, and a receptor for CD28.

Embodiment A50. The multi-chain chimeric polypeptide of any one of embodiments A20-A48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment A51. The multi-chain chimeric polypeptide of embodiment A50, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

Embodiment A52. The multi-chain chimeric polypeptide of any one of embodiments A20-A48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment A53. The multi-chain chimeric polypeptide of embodiment A52, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

Embodiment A54. The multi-chain chimeric polypeptide of any one of embodiments A1-A53, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment A55. The multi-chain chimeric polypeptide of any one of embodiments A1-A53, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment A56. The multi-chain chimeric polypeptide of any one of embodiments A1-A55, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment A57. The multi-chain chimeric polypeptide of embodiment A56, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

Embodiment A58. The multi-chain chimeric polypeptide of embodiment A57, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

Embodiment A59. The multi-chain chimeric polypeptide of embodiment A58, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

Embodiment A60. The multi-chain chimeric polypeptide of any one of embodiments A56-A59, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment A61. The multi-chain chimeric polypeptide of embodiment A60, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment A62. The multi-chain chimeric polypeptide of any one of embodiments A1-A61, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment A63. The multi-chain chimeric polypeptide of any one of embodiments A1-A62, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment A64. The multi-chain chimeric polypeptide of any one of embodiments A1-A63, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment A65. The multi-chain chimeric polypeptide of any one of embodiments A1-A64, wherein the pair of affinity domains is a sushi domain from an

alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15.

Embodiment A66. The multi-chain chimeric polypeptide of embodiment A65, wherein the soluble IL15 has a D8N or D8A amino acid substitution.

Embodiment A67. The multi-chain chimeric polypeptide of embodiment A65 or A66, wherein the human IL15Rα is a mature full-length IL15Rα.

Embodiment A68. The multi-chain chimeric polypeptide of any one of embodiments A1-A64, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment A69. The multi-chain chimeric polypeptide of any one of embodiments A1-A68, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment A70. A composition comprising any of the multi-chain chimeric polypeptides of embodiments A1-A69.

Embodiment A71. The composition of embodiment A70, wherein the composition is a pharmaceutical composition.

Embodiment A72. A kit comprising at least one dose of the composition of embodiment A70 or A71.

Embodiment A73. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments A1-A69 or the composition of embodiment A70 or A71.

Embodiment A74. The method of embodiment A73, wherein the immune cell is contacted in vitro.

Embodiment A75. The method of embodiment A74, wherein the immune cell was previously obtained from a subject.

Embodiment A76. The method of embodiment A75, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment A77. The method of embodiment A73, wherein the immune cell is contacted in vivo.

Embodiment A78. The method of any one of embodiments A73-A77, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment A79. The method of any one of embodiments A73-A78, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment A80. The method of any one of embodiments A73-A78, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment A81. The method of any one of embodiments A73-A80, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment A82. The method of embodiment A81, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment A83. The method of embodiment A82, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment A84. The method of embodiment A81, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment A85. The method of embodiment A84, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and

neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment A86. The method of embodiment A81, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment A87. The method of embodiment A86, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment A88. A method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments A1-A69 or the composition of embodiment A70 or A71.

Embodiment A89. The method of embodiment A88, wherein the immune cell is contacted in vitro.

Embodiment A90. The method of embodiment A89, wherein the immune cell was previously obtained from a subject.

Embodiment A91. The method of embodiment A90, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment A92. The method of embodiment A88, wherein the immune cell is contacted in vivo.

Embodiment A93. The method of any one of embodiments A88-A92, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a

peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment A94. The method of any one of embodiments A88-A93, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment A95. The method of any one of embodiments A88-A93, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment A96. The method of any one of embodiments A88-A95, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment A97. The method of embodiment A96, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment A98. The method of embodiment A97, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment A99. The method of embodiment A96, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment A100. The method of embodiment A99, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment A101. The method of embodiment A96, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment A102. The method of embodiment A96, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment A103. A method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments A1-A69 or the composition of embodiment A70 or A71.

Embodiment A104. The method of embodiment A103, wherein the immune cell is contacted in vitro.

Embodiment A105. The method of embodiment A104, wherein the immune cell was previously obtained from a subject.

Embodiment A106. The method of embodiment A105, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment A107. The method of embodiment A103, wherein the immune cell is contacted in vivo.

Embodiment A108. The method of any one of embodiments A103-A107, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment A109. The method of any one of embodiments A103-A108, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment A110. The method of any one of embodiments A103-A108, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment A111. The method of any one of embodiments A103-A110, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment A112. The method of embodiment A111, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment A113. The method of embodiment A112, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment A114. The method of embodiment A111, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment A115. The method of embodiment A114, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment A116. The method of embodiment A111, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment A117. The method of embodiment A116, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment A118. A method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments A1-A69 or the composition of embodiment A70 or A71.

Embodiment A119. The method of embodiment A118, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment A120. The method of embodiment A119, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment A121. The method of embodiment A118, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment A122. The method of embodiment A121, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment A123. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments A1-A69 or the composition of embodiment A70 or A71.

Embodiment A124. The method of embodiment A123, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment A125. The method of embodiment A124, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and

neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment A126. The method of embodiment A123, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment A127. The method of embodiment A126, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment A128. The method of embodiment A123, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment A129. The method of embodiment A128, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment A130. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments A1-A69.

Embodiment A131. A vector comprising the nucleic acid of embodiment A130.

Embodiment A132. The vector of embodiment A131, wherein the vector is an expression vector.

Embodiment A133. A cell comprising the nucleic acid of embodiment A130 or the vector of embodiment A131 or A132.

Embodiment A134. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment A133 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment A135. A multi-chain chimeric polypeptide produced by the method of embodiment A134.

Embodiment A136. The multi-chain chimeric polypeptide of embodiment A56, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 3.

Embodiment A137. The multi-chain chimeric polypeptide of embodiment A136, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 3.

Embodiment A138. The multi-chain chimeric polypeptide of embodiment A137, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

Embodiment A139. The multi-chain chimeric polypeptide of embodiment A138, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 3.

Embodiment A140. The multi-chain chimeric polypeptide of embodiment A56, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

Embodiment A141. The multi-chain chimeric polypeptide of embodiment A140, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

Embodiment A142. The multi-chain chimeric polypeptide of embodiment A141, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 4.

Embodiment A143. The multi-chain chimeric polypeptide of embodiment A142, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 4.

Embodiment A144. The multi-chain chimeric polypeptide of embodiment 56, wherein the human soluble tissue factor domain does not initiate coagulation.

Embodiment A145. The multi-chain chimeric polypeptide of any one of claims 1-59, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

B. Exemplary Embodiments

Embodiment B1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-18 or a receptor of IL-12.

Embodiment B2. The multi-chain chimeric polypeptide of embodiment B1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment B3. The multi-chain chimeric polypeptide of embodiment B1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment B4. The multi-chain chimeric polypeptide of any one of embodiments B1-B3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment B5. The multi-chain chimeric polypeptide of any one of embodiments B1-B3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B6. The multi-chain chimeric polypeptide of any one of embodiments B1-B5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment B7. The multi-chain chimeric polypeptide of any one of embodiments B1-B5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment B8. The multi-chain chimeric polypeptide of any one of embodiments B1-B7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment B9. The multi-chain chimeric polypeptide of embodiment B8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

Embodiment B10. The multi-chain chimeric polypeptide of embodiment B9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

Embodiment B11. The multi-chain chimeric polypeptide of embodiment B10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

Embodiment B12. The multi-chain chimeric polypeptide of any one of embodiments B8-B11, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment B13. The multi-chain chimeric polypeptide of embodiment B12, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment B14. The multi-chain chimeric polypeptide of any one of embodiments B1-B13, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment B15. The multi-chain chimeric polypeptide of any one of embodiments B1-B14, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment B16. The multi-chain chimeric polypeptide of any one of embodiments B1-B15, wherein the multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

Embodiment B17. The multi-chain chimeric polypeptide of any one of embodiments B1-B16, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment B18. The multi-chain chimeric polypeptide of any one of embodiments B1-B17, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment B19. The multi-chain chimeric polypeptide of any one of embodiments B 1-18, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment B20. The multi-chain chimeric polypeptide of embodiment B19, wherein the signal sequence comprises SEQ ID NO: 31.

Embodiment B21. The multi-chain chimeric polypeptide of embodiment B20, wherein the signal sequence is SEQ ID NO: 31.

Embodiment B22. The multi-chain chimeric polypeptide of any one of embodiments B1-B21, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15.

Embodiment B23. The multi-chain chimeric polypeptide of embodiment B22, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment B24. The multi-chain chimeric polypeptide of embodiment B22, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 14.

Embodiment B25. The multi-chain chimeric polypeptide of embodiment B24, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 14.

Embodiment B26. The multi-chain chimeric polypeptide of embodiment B25, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 14.

Embodiment B27. The multi-chain chimeric polypeptide of embodiment B26, wherein the soluble IL-15 comprises SEQ ID NO: 14.

Embodiment B28. The multi-chain chimeric polypeptide of any one of embodiments B22-B27, wherein the sushi domain of IL15Rα comprises a sushi domain from human IL15Rα.

Embodiment B29. The multi-chain chimeric polypeptide of embodiment B28, wherein the sushi domain from human IL15Rα comprises a sequence that is 80% identical to SEQ ID NO: 28.

Embodiment B30. The multi-chain chimeric polypeptide of embodiment B29, wherein the sushi domain from human IL15Rα comprises a sequence that is 90% identical to SEQ ID NO: 28.

Embodiment B31. The multi-chain chimeric polypeptide of embodiment B30, wherein the sushi domain from human IL15Rα comprises a sequence that is 95% identical to SEQ ID NO: 28.

Embodiment B32. The multi-chain chimeric polypeptide of embodiment B31, wherein the sushi domain from human IL15Rα comprises SEQ ID NO: 28.

Embodiment B33. The multi-chain chimeric polypeptide of embodiment B28, wherein the sushi domain from human IL15Rα is a mature full-length IL15Rα.

Embodiment B34. The multi-chain chimeric polypeptide of any one of embodiments B1-B21, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment B35. The multi-chain chimeric polypeptide of any one of embodiments B1-B34, wherein one or both of the first target-binding domain and the second target-binding domain is an agonistic antigen-binding domain.

Embodiment B36. The multi-chain chimeric polypeptide of embodiment B35, wherein the first target-binding domain and the second target-binding domain are each agonistic antigen-binding domains.

Embodiment B37. The multi-chain chimeric polypeptide of embodiment B35 or B36, wherein antigen-binding domain comprises a scFv or single-domain antibody.

Embodiment B38. The multi-chain chimeric polypeptide of any one of embodiments B1-B34, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble IL-15 or a soluble IL-18.

Embodiment B39. The multi-chain chimeric polypeptide of embodiment B38, wherein the first target-binding domain and the second target-binding domain are each independently a soluble IL-15 or a soluble IL-18.

Embodiment B40. The multi-chain chimeric polypeptide of any one of embodiments B1-B39, wherein the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-18 or a receptor of IL-12.

Embodiment B41. The multi-chain chimeric polypeptide of embodiment B40, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment B42. The multi-chain chimeric polypeptide of embodiment B41, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment B43. The multi-chain chimeric polypeptide of any one of embodiments B1-B39, wherein the first target-binding domain binds specifically to a receptor for IL-12, and the second target-binding domain binds specifically to a receptor for IL-18.

Embodiment B44. The multi-chain chimeric polypeptide of any one of embodiments B1-B39, wherein the first target-binding domain binds specifically to a

receptor for IL-18, and the second target-binding domain bind specifically to a receptor for IL-12.

Embodiment B45. The multi-chain chimeric polypeptide of embodiment B44, wherein the first target-binding domain comprises a soluble IL-18.

Embodiment B46. The multi-chain chimeric polypeptide of embodiment B45, wherein the soluble IL-18 is a soluble human IL-18.

Embodiment B47. The multi-chain chimeric polypeptide of embodiment B46, wherein the soluble human IL-18 comprises a sequence at least 80% identical to SEQ ID NO: 16.

Embodiment B48. The multi-chain chimeric polypeptide of embodiment B47, wherein the soluble human IL-18 comprises a sequence at least 90% identical to SEQ ID NO: 16.

Embodiment B49. The multi-chain chimeric polypeptide of embodiment B48, wherein the soluble human IL-18 comprises a sequence at least 95% identical to SEQ ID NO: 16.

Embodiment B50. The multi-chain chimeric polypeptide of embodiment B49, wherein the soluble human IL-18 comprises a sequence of SEQ ID NO: 16.

Embodiment B51. The multi-chain chimeric polypeptide of any one of embodiments B44-B50, wherein the second target-binding domain comprises a soluble IL-12.

Embodiment B52. The multi-chain chimeric polypeptide of embodiment B51, wherein the soluble IL-18 is a soluble human IL-12.

Embodiment B53. The multi-chain chimeric polypeptide of embodiment B52, wherein the soluble human IL-15 comprises a sequence of soluble human IL-12β (p40) and a sequence of soluble human IL-12α (p35).

Embodiment B54. The multi-chain chimeric polypeptide of embodiment B53, wherein the soluble human IL-15 further comprises a linker sequence between the sequence of soluble IL-12β (p40) and the sequence of soluble human IL-12α (p35).

Embodiment B55. The multi-chain chimeric polypeptide of embodiment B54, wherein the linker sequence comprises SEQ ID NO: 7.

Embodiment B56. The multi-chain chimeric polypeptide of any one of embodiments B53-B55, wherein the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 80% identical to SEQ ID NO: 66.

Embodiment B57. The multi-chain chimeric polypeptide of embodiment B56, wherein the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 90% identical to SEQ ID NO: 66.

Embodiment B58. The multi-chain chimeric polypeptide of embodiment B57, wherein the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 95% identical to SEQ ID NO: 66.

Embodiment B59. The multi-chain chimeric polypeptide of embodiment B58, wherein the sequence of soluble human IL-12β (p40) comprises SEQ ID NO: 66.

Embodiment B60. The multi-chain chimeric polypeptide of any one of embodiments B53-B59, wherein the sequence of soluble human IL-12α (p35) comprises a sequence that is at least 80% identical to SEQ ID NO: 68.

Embodiment B61. The multi-chain chimeric polypeptide of embodiment B60, wherein the sequence of soluble human IL-12α (p35) comprises a sequence that is at least 90% identical to SEQ ID NO: 68.

Embodiment B62. The mule-chain chimeric polypeptide of embodiment B61, wherein the sequence of soluble human IL-12α (p35) comprises a sequence that is at least 95% identical to SEQ ID NO: 68.

Embodiment B63. The multi-chain chimeric polypeptide of embodiment B62, wherein the sequence of soluble human IL-12α (p35) comprises SEQ ID NO: 68.

Embodiment B64. The multi-chain chimeric polypeptide of embodiment B1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 70.

Embodiment B65. The multi-chain chimeric polypeptide of embodiment B64, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 70.

Embodiment B66. The multi-chain chimeric polypeptide of embodiment B65, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 70.

Embodiment B67. The multi-chain chimeric polypeptide of embodiment B66, wherein the first chimeric polypeptide comprises SEQ ID NO: 70.

Embodiment B68. The multi-chain chimeric polypeptide of embodiment B67, wherein the first chimeric polypeptide comprises SEQ ID NO: 72.

Embodiment B69. The multi-chain chimeric polypeptide of any one of embodiments B1 and B64-B68, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 74.

Embodiment B70. The multi-chain chimeric polypeptide of embodiment B69, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 74.

Embodiment B71. The multi-chain chimeric polypeptide of embodiment B70, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 74.

Embodiment B72. The multi-chain chimeric polypeptide of embodiment B71, wherein the second chimeric polypeptide comprises SEQ ID NO: 74.

Embodiment B73. The multi-chain chimeric polypeptide of embodiment B72, wherein the second chimeric polypeptide comprises SEQ ID NO: 74.

Embodiment B74. The multi-chain chimeric polypeptide of any one of embodiments B1-B63, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment B75. The multi-chain chimeric polypeptide of embodiment B74, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s)and the first domain of the pair of affinity domains.

Embodiment B76. The multi-chain chimeric polypeptide of any one of embodiments B1-B63, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment B77. The multi-chain chimeric polypeptide of embodiment B76, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B78. The multi-chain chimeric polypeptide of embodiment B76, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment B79. The multi-chain chimeric polypeptide of embodiment B76, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment B80. The multi-chain chimeric polypeptide of embodiment B76, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment B81. The multi-chain chimeric polypeptide of embodiment B76, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B82. The multi-chain chimeric polypeptide of embodiment B81, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B83. The multi-chain chimeric polypeptide of embodiment B81, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B84. The multi-chain chimeric polypeptide of embodiment B81, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B85. The multi-chain chimeric polypeptide of embodiment B81, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment B86. The multi-chain chimeric polypeptide of embodiment B81, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment B87. The multi-chain chimeric polypeptide of embodiment B81, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment B88. The multi-chain chimeric polypeptide of any one of embodiments B1-B63 and B74-B87, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment B89. The multi-chain chimeric polypeptide of embodiment B88, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment B90. The multi-chain chimeric polypeptide of embodiment B88, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment B91. The multi-chain chimeric polypeptide of embodiment B88, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment B92. The multi-chain chimeric polypeptide of embodiment B88, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment B93. The multi-chain chimeric polypeptide of any one of embodiments B74-B92, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment B94. The multi-chain chimeric polypeptide of embodiment B93, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment B95. The multi-chain chimeric polypeptide of embodiment B94, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment B96. The multi-chain chimeric polypeptide of any one of embodiments B74-B92, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment B97. The multi-chain chimeric polypeptide of any one of embodiments B74-B96, wherein the one or more additional antigen-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for

IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD28.

Embodiment B98. The multi-chain chimeric polypeptide of any one of embodiments B74-B96, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment B99. The multi-chain chimeric polypeptide of embodiment B98, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

Embodiment B100. The multi-chain chimeric polypeptide of any one of embodiments B74-B96, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment B101. The multi-chain chimeric polypeptide of embodiment B100, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, or a soluble CD28.

Embodiment B102. A composition comprising any of the multi-chain chimeric polypeptides of embodiments B1-B101.

Embodiment B103. The composition of embodiment B102, wherein the composition is a pharmaceutical composition.

Embodiment B104. A kit comprising at least one dose of the composition of embodiment B102 or B103.

Embodiment B105. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments B1-B101 or the composition of embodiment B102 or B103.

Embodiment B106. The method of embodiment B105, wherein the immune cell is contacted in vitro.

Embodiment B107. The method of embodiment B106, wherein the immune cell was previously obtained from a subject.

Embodiment B108. The method of embodiment B107, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment B109. The method of embodiment B105, wherein the immune cell is contacted in vivo.

Embodiment B110. The method of any one of embodiments B105-B109, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment B111. The method of any one of embodiments B105-B110, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment B112. The method of any one of embodiments B105-B110, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment B113. The method of any one of embodiments B105-B112, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment B114. The method of embodiment B113, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment B115. The method of embodiment B114, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment B116. The method of embodiment B113, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment B117. The method of embodiment B116, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma,

osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment B118. The method of embodiment B113, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment B119. The method of embodiment B118, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment B120. A method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments B1-B101 or the composition of embodiment B102 or B103.

Embodiment B121. The method of embodiment B120, wherein the immune cell is contacted in vitro.

Embodiment B122. The method of embodiment B121, wherein the immune cell was previously obtained from a subject.

Embodiment B123. The method of embodiment B122, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment B124. The method of embodiment B120, wherein the immune cell is contacted in vivo.

Embodiment B125. The method of any one of embodiments B120-B124, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment B126. The method of any one of embodiments B120-B125, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment B127. The method of any one of embodiments B120-B125, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment B128. The method of any one of embodiments B120-B127, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment B129. The method of embodiment B128, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment B130. The method of embodiment B129, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic

pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment B131. The method of embodiment B128, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment B132. The method of embodiment B131, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment B133. The method of embodiment B128, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment B134. The method of embodiment B128, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus,

coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment B135. A method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments B1-B101 or the composition of embodiment B102 or B103.

Embodiment B136. The method of embodiment B135, wherein the immune cell is contacted in vitro.

Embodiment B137. The method of embodiment B136, wherein the immune cell was previously obtained from a subject.

Embodiment B138. The method of embodiment B137, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment B139. The method of embodiment B135, wherein the immune cell is contacted in vivo.

Embodiment B140. The method of any one of embodiments B135-B139, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment B141. The method of any one of embodiments B135-B140, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment B142. The method of any one of embodiments B135-B140, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment B143. The method of any one of embodiments B135-B142, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment B144. The method of embodiment B143, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment B145. The method of embodiment B144, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment B146. The method of embodiment B143, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment B147. The method of embodiment B146, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma,

osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment B148. The method of embodiment B143, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment B149. The method of embodiment B148, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment B150. A method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments B1-B101 or the composition of embodiment B102 or B103.

Embodiment B151. The method of embodiment B150, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment B152. The method of embodiment B151, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell

non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment B153. The method of embodiment B150, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment B154. The method of embodiment B153, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction

Embodiment B155. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments B1-B101 or the composition of embodiment B102 or B103.

Embodiment B156. The method of embodiment B155, wherein the subject has been identified or diagnosed as having a cancer or infectious disease.

Embodiment B157. The method of embodiment B156, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment B158. The method of embodiment B155, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment B159. The method of embodiment B158, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment B160. The method of embodiment B155, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment B161. The method of embodiment B160, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment B162. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments B1-B101.

Embodiment B163. A vector comprising the nucleic acid of embodiment B162.

Embodiment B164. The vector of embodiment B163, wherein the vector is an expression vector.

Embodiment B165. A cell comprising the nucleic acid of embodiment B162 or the vector of embodiment B163 or B164.

Embodiment B166. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment B165 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment B167. A multi-chain chimeric polypeptide produced by the method of embodiment B166.

Embodiment B168. The multi-chain chimeric polypeptide of embodiment B8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 3.

Embodiment B169. The multi-chain chimeric polypeptide of embodiment B168, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 3.

Embodiment B170. The multi-chain chimeric polypeptide of embodiment B169, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

Embodiment B171. The multi-chain chimeric polypeptide of embodiment B170, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 3.

Embodiment B172. The multi-chain chimeric polypeptide of embodiment B8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

Embodiment B173. The multi-chain chimeric polypeptide of embodiment B172, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

Embodiment B174. The multi-chain chimeric polypeptide of embodiment B173, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 4.

Embodiment B175. The multi-chain chimeric polypeptide of embodiment B174, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 4.

C. Exemplary Embodiments

Embodiment C1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-21 or a ligand of tumor growth factor receptor β II (TGFβRII).

Embodiment C2. The multi-chain chimeric polypeptide of embodiment C1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment C3. The multi-chain chimeric polypeptide of embodiments C1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment C4. The multi-chain chimeric polypeptide of any one of embodiments C1-C3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment C5. The multi-chain chimeric polypeptide of any one of embodiments C1-C3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C6. The multi-chain chimeric polypeptide of any one of embodiments C1-C5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment C7. The multi-chain chimeric polypeptide of any one of embodiments C1-C5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment C8. The multi-chain chimeric polypeptide of any one of embodiments C1-C7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment C9. The multi-chain chimeric polypeptide of embodiment C8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

Embodiment C10. The multi-chain chimeric polypeptide of embodiment C9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

Embodiment C11. The multi-chain chimeric polypeptide of embodiment C10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

Embodiment C12. The multi-chain chimeric polypeptide of any one of embodiments C8-C11, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment C13. The multi-chain chimeric polypeptide of embodiment C12, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment C14. The multi-chain chimeric polypeptide of any one of embodiments C1-C13, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment C15. The multi-chain chimeric polypeptide of any one of embodiments C1-C14, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment C16. The multi-chain chimeric polypeptide of any one of embodiments C1-C15, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment C17. The multi-chain chimeric polypeptide of any one of embodiments C1-C16, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment C18. The multi-chain chimeric polypeptide of any one of embodiments C1-C17, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment C19. The multi-chain chimeric polypeptide of any one of embodiments C1-C18, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment C20. The multi-chain chimeric polypeptide of embodiment C19, wherein the signal sequence comprises SEQ ID NO: 31.

Embodiment C21. The multi-chain chimeric polypeptide of embodiment C20, wherein the signal sequence is SEQ ID NO: 31.

Embodiment C22. The multi-chain chimeric polypeptide of any one of embodiments C1-C21, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα and a soluble IL-15.

Embodiment C23. The multi-chain chimeric polypeptide of embodiment C22, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment C24. The multi-chain chimeric polypeptide of embodiment C22, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 14.

Embodiment C25. The multi-chain chimeric polypeptide of embodiment C24, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 14.

Embodiment C26. The multi-chain chimeric polypeptide of embodiment C25, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 14.

Embodiment C27. The multi-chain chimeric polypeptide of embodiment C26, wherein the soluble IL-15 comprises SEQ ID NO: 14.

Embodiment C28. The multi-chain chimeric polypeptide of any one of embodiments C22-C27, wherein the sushi domain of IL15Rα comprises a sushi domain from human IL15Rα.

Embodiment C29. The multi-chain chimeric polypeptide of embodiment C28, wherein the sushi domain from human IL15Rα comprises a sequence that is 80% identical to SEQ ID NO: 28.

Embodiment C30. The multi-chain chimeric polypeptide of embodiment C29, wherein the sushi domain from human IL15Rα comprises a sequence that is 90% identical to SEQ ID NO: 28.

Embodiment C31. The multi-chain chimeric polypeptide of embodiment C30, wherein the sushi domain from human IL15Rα comprises a sequence that is 95% identical to SEQ ID NO: 28.

Embodiment C32. The multi-chain chimeric polypeptide of embodiment C31, wherein the sushi domain from human IL15Rα comprises SEQ ID NO: 28.

Embodiment C33. The multi-chain chimeric polypeptide of embodiment C28, wherein the sushi domain from human IL15Rα is a mature full-length IL15Rα.

Embodiment C34. The multi-chain chimeric polypeptide of any one of embodiments C1-C21, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment C35. The multi-chain chimeric polypeptide of any one of embodiments C1-C34, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment C36. The multi-chain chimeric polypeptide of embodiment C35, wherein the first target-binding domain and the second target-binding domain are antigen-binding domains.

Embodiment C37. The multi-chain chimeric polypeptide of embodiment C35 or C36, wherein antigen-binding domain comprises a scFv or single-domain antibody.

Embodiment C38. The multi-chain chimeric polypeptide of any one of embodiments C1-C34, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 or a soluble TGFβRII.

Embodiment C39. The multi-chain chimeric polypeptide of any one of embodiments C1-C38, wherein the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a ligand of TGFβRII.

Embodiment C40. The multi-chain chimeric polypeptide of embodiment C39, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment C41. The multi-chain chimeric polypeptide of embodiment C40, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment C42. The multi-chain chimeric polypeptide of any one of embodiments C1-C38, wherein the first target-binding domain binds specifically to a ligand of TGFβRII, and the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment C43. The multi-chain chimeric polypeptide of any one of embodiments C1-C38, wherein the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain bind specifically to a ligand of TGFβRII.

Embodiment C44. The multi-chain chimeric polypeptide of embodiment C43, wherein the first target-binding domain comprises a soluble IL-21.

Embodiment C45. The multi-chain chimeric polypeptide of embodiment C44, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment C46. The multi-chain chimeric polypeptide of embodiment C45, wherein the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 78.

Embodiment C47. The multi-chain chimeric polypeptide of embodiment C46, wherein the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 78.

Embodiment C48. The multi-chain chimeric polypeptide of embodiment C47, wherein the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 78.

Embodiment C49. The multi-chain chimeric polypeptide of embodiment C48, wherein the soluble human IL-21 comprises a sequence of SEQ ID NO: 78.

Embodiment C50. The multi-chain chimeric polypeptide of any one of embodiments C43-C49, wherein the second target-binding domain comprises a soluble TGFβRII.

Embodiment C51. The multi-chain chimeric polypeptide of embodiment C50, wherein the soluble TGFβRII is a soluble human TGFβRII.

Embodiment C52. The multi-chain chimeric polypeptide of embodiment C51, wherein the soluble human TGFβRII comprises a first sequence of soluble human TGFβRII and a second sequence of soluble human TGFβRII.

Embodiment C53. The multi-chain chimeric polypeptide of embodiment C52, wherein the soluble human TGFβRII further comprises a linker sequence between the

first sequence of soluble human TGFβRII and the second sequence of soluble human TGFβRII.

Embodiment C54. The multi-chain chimeric polypeptide of embodiment C53, wherein the linker sequence comprises SEQ ID NO: 7.

Embodiment C55. The multi-chain chimeric polypeptide of any one of embodiments C52-C54, wherein the first sequence of soluble human TGFβRII comprises a sequence that is at least 80% identical to SEQ ID NO: 80.

Embodiment C56. The multi-chain chimeric polypeptide of embodiment C55, wherein the first sequence of soluble human TGFβRII comprises a sequence that is at least 90% identical to SEQ ID NO: 80.

Embodiment C57. The multi-chain chimeric polypeptide of embodiment C56, wherein the first sequence of soluble human TGFβRII comprises a sequence that is at least 95% identical to SEQ ID NO: 80.

Embodiment C58. The multi-chain chimeric polypeptide of embodiment C57, wherein the first sequence of soluble human TGFβRII comprises SEQ ID NO: 80.

Embodiment C59. The multi-chain chimeric polypeptide of any one of embodiments C52-C58, wherein the second sequence of soluble human TGFβRII comprises a sequence that is at least 80% identical to SEQ ID NO: 81.

Embodiment C60. The multi-chain chimeric polypeptide of embodiment C59, wherein the second sequence of soluble human TGFβRII comprises a sequence that is at least 90% identical to SEQ ID NO: 81.

Embodiment C61. The mule-chain chimeric polypeptide of embodiment C60, wherein the second sequence of soluble human TGFβRII comprises a sequence that is at least 95% identical to SEQ ID NO: 81.

Embodiment C62. The multi-chain chimeric polypeptide of embodiment C61, wherein the second sequence of soluble human TGFβRII comprises SEQ ID NO: 81.

Embodiment C63. The multi-chain chimeric polypeptide of embodiment C1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 86.

Embodiment C64. The multi-chain chimeric polypeptide of embodiment C63, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 86.

Embodiment C65. The multi-chain chimeric polypeptide of embodiment C64, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 86.

Embodiment C66. The multi-chain chimeric polypeptide of embodiment C65, wherein the first chimeric polypeptide comprises SEQ ID NO: 86.

Embodiment C67. The multi-chain chimeric polypeptide of embodiment C66, wherein the first chimeric polypeptide comprises SEQ ID NO: 88.

Embodiment C68. The multi-chain chimeric polypeptide of any one of embodiments C1 and C63-C67, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 90.

Embodiment C69. The multi-chain chimeric polypeptide of embodiment C68, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 90.

Embodiment C70. The multi-chain chimeric polypeptide of embodiment C69, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 90.

Embodiment C71. The multi-chain chimeric polypeptide of embodiment C70, wherein the second chimeric polypeptide comprises SEQ ID NO: 90.

Embodiment C72. The multi-chain chimeric polypeptide of embodiment C71, wherein the second chimeric polypeptide comprises SEQ ID NO: 92.

Embodiment C73. The multi-chain chimeric polypeptide of any one of embodiments C1-C62, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment C74. The multi-chain chimeric polypeptide of embodiment C73, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

Embodiment C75. The multi-chain chimeric polypeptide of any one of embodiments C1-C62, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment C76. The multi-chain chimeric polypeptide of embodiment C75, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C77. The multi-chain chimeric polypeptide of embodiment C75, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment C78. The multi-chain chimeric polypeptide of embodiment C75, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment C79. The multi-chain chimeric polypeptide of embodiment C75, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment C80. The multi-chain chimeric polypeptide of embodiment C75, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C81. The multi-chain chimeric polypeptide of embodiment C80, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C82. The multi-chain chimeric polypeptide of embodiment C80, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C83. The multi-chain chimeric polypeptide of embodiment C80, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C84. The multi-chain chimeric polypeptide of embodiment C80, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C85. The multi-chain chimeric polypeptide of embodiment C80, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment C86. The multi-chain chimeric polypeptide of embodiment C80, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-

binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment C87. The multi-chain chimeric polypeptide of any one of embodiments C1-C62 and C73-C86, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment C88. The multi-chain chimeric polypeptide of embodiment C87, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment C89. The multi-chain chimeric polypeptide of embodiment C87, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment C90. The multi-chain chimeric polypeptide of embodiment C87, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment C91. The multi-chain chimeric polypeptide of embodiment C87, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment C92. The multi-chain chimeric polypeptide of any one of embodiments C73-C91, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment C93. The multi-chain chimeric polypeptide of embodiment C92, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment C94. The multi-chain chimeric polypeptide of embodiment C93, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment C95. The multi-chain chimeric polypeptide of any one of embodiments C73-C91, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment C96. The multi-chain chimeric polypeptide of any one of embodiments C73-C95, wherein the one or more additional antigen-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-D, a ligand of TGF-β receptor II (TGF-RII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a

receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD28.

Embodiment C97. The multi-chain chimeric polypeptide of any one of embodiments C73-C95, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment C98. The multi-chain chimeric polypeptide of embodiment C97, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

Embodiment C99. The multi-chain chimeric polypeptide of any one of embodiments C73-C95, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment C100. The multi-chain chimeric polypeptide of embodiment C99, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, , or a soluble CD28.

Embodiment C101. A composition comprising any of the multi-chain chimeric polypeptides of embodiments C1-C100.

Embodiment C102. The composition of embodiment C101, wherein the composition is a pharmaceutical composition.

Embodiment C103. A kit comprising at least one dose of the composition of embodiment C101 or C102.

Embodiment C104. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments C1-C100 or the composition of embodiment C101 or C102.

Embodiment C105. The method of embodiment C104, wherein the immune cell is contacted in vitro.

Embodiment C106. The method of embodiment C105, wherein the immune cell was previously obtained from a subject.

Embodiment C107. The method of embodiment C106, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment C108. The method of embodiment C104, wherein the immune cell is contacted in vivo.

Embodiment C109. The method of any one of embodiments C104-C108, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment C110. The method of any one of embodiments C104-C109, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment C111. The method of any one of embodiments C104-C109, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment C112. The method of any one of embodiments C104-C111, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment C113. The method of embodiment C112, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment C114. The method of embodiment C113, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment C115. The method of embodiment C112, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment C116. The method of embodiment C115, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma,

retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment C117. The method of embodiment C112, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment C118. The method of embodiment C117, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment C119. A method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments C1-C100 or the composition of embodiment C101 or C102.

Embodiment C120. The method of embodiment C119, wherein the immune cell is contacted in vitro.

Embodiment C121. The method of embodiment C120, wherein the immune cell was previously obtained from a subject.

Embodiment C122. The method of embodiment C121, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment C123. The method of embodiment C119, wherein the immune cell is contacted in vivo.

Embodiment C124. The method of any one of embodiments C119-C123, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment C125. The method of any one of embodiments C119-C124, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment C126. The method of any one of embodiments C119-C124, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment C127. The method of any one of embodiments C119-C126, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment C128. The method of embodiment C127, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment C129. The method of embodiment C128, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia,

glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment C130. The method of embodiment C127, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment C131. The method of embodiment C130, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment C132. The method of embodiment C127, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment C133. The method of embodiment C127, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment C134. A method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments C1-C100 or the composition of embodiment C101 or C102.

Embodiment C135. The method of embodiment C134, wherein the immune cell is contacted in vitro.

Embodiment C136. The method of embodiment C135, wherein the immune cell was previously obtained from a subject.

Embodiment C137. The method of embodiment C136, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment C138. The method of embodiment C134, wherein the immune cell is contacted in vivo.

Embodiment C139. The method of any one of embodiments C134-C138, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment C140. The method of any one of embodiments C134-C139, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment C141. The method of any one of embodiments C134-C139, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment C142. The method of any one of embodiments C134-C141, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment C143. The method of embodiment C142, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment C144. The method of embodiment C143, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment C145. The method of embodiment C142, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment C146. The method of embodiment C145, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and

neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment C147. The method of embodiment C142, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment C148. The method of embodiment C147, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment C149. A method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments C1-C100 or the composition of embodiment C101 or C102.

Embodiment C150. The method of embodiment C149, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment C151. The method of embodiment C150, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment C152. The method of embodiment C149, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment C153. The method of embodiment C152, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in panceatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment C154. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments C1-C100 or the composition of embodiment C101 or C102.

Embodiment C155. The method of embodiment C154, wherein the subject has been identified or diagnosed as having a cancer or infectious disease.

Embodiment C156. The method of embodiment C157, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma,

gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment C157. The method of embodiment C154, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment C158. The method of embodiment C157, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment C159. The method of embodiment C154, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment C160. The method of embodiment C159, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment C161. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments C1-C100.

Embodiment C162. A vector comprising the nucleic acid of embodiment C161.

Embodiment C163. The vector of embodiment C162, wherein the vector is an expression vector.

Embodiment C164. A cell comprising the nucleic acid of embodiment C161 or the vector of embodiment C162 or C163.

Embodiment C165. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment C164 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment C166. A multi-chain chimeric polypeptide produced by the method of embodiment C165.

Embodiment C167. The multi-chain chimeric polypeptide of embodiment C12, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 3.

Embodiment C168. The multi-chain chimeric polypeptide of embodiment C167, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 3.

Embodiment C169. The multi-chain chimeric polypeptide of embodiment C168, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

Embodiment C170. The multi-chain chimeric polypeptide of embodiment C169, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 3.

Embodiment C171. The multi-chain chimeric polypeptide of embodiment C12, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

Embodiment C172. The multi-chain chimeric polypeptide of embodiment C171, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

Embodiment C173. The multi-chain chimeric polypeptide of embodiment C172, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 4.

Embodiment C174. The multi-chain chimeric polypeptide of embodiment C173, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 4.

D. Exemplary Embodiments

Embodiment D1. A multi-chain chimeric polypeptide comprising:

(c) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(d) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-21 or a receptor of IL-7.

Embodiment D2. The multi-chain chimeric polypeptide of embodiment D1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment D3. The multi-chain chimeric polypeptide of embodiment D1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment D4. The multi-chain chimeric polypeptide of any one of embodiments D1-D3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment D5. The multi-chain chimeric polypeptide of any one of embodiments D1-D3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D6. The multi-chain chimeric polypeptide of any one of embodiments D1-D5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment D7. The multi-chain chimeric polypeptide of any one of embodiments D1-D5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment D8. The multi-chain chimeric polypeptide of any one of embodiments D1-D7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment D9. The multi-chain chimeric polypeptide of embodiment D8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

Embodiment D10. The multi-chain chimeric polypeptide of embodiment D9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

Embodiment D11. The multi-chain chimeric polypeptide of embodiment D10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

Embodiment D12. The multi-chain chimeric polypeptide of embodiment D11, wherein the soluble human tissue factor domain comprises SEQ ID NO: 1.

Embodiment D13. The multi-chain chimeric polypeptide of embodiment D8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 3.

Embodiment D14. The multi-chain chimeric polypeptide of embodiment D13, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 3.

Embodiment D15. The multi-chain chimeric polypeptide of embodiment D14, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

Embodiment D16. The multi-chain chimeric polypeptide of embodiment D15, wherein the soluble human tissue factor domain comprises SEQ ID NO: 3.

Embodiment D17. The multi-chain chimeric polypeptide of embodiment D8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

Embodiment D18. The multi-chain chimeric polypeptide of embodiment D17, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

Embodiment D19. The multi-chain chimeric polypeptide of embodiment D18, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 4.

Embodiment D20. The multi-chain chimeric polypeptide of embodiment D19, wherein the soluble human tissue factor domain comprises SEQ ID NO: 4.

Embodiment D21. The multi-chain chimeric polypeptide of any one of embodiments D8-D11, D13-D15, and D17-D19, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment D22. The multi-chain chimeric polypeptide of embodiment D21, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment D23. The multi-chain chimeric polypeptide of any one of embodiments D1-D22, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment D24. The multi-chain chimeric polypeptide of any one of embodiments D1-D23, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment D25. The multi-chain chimeric polypeptide of any one of embodiments D1-D24, wherein the multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

Embodiment D26. The multi-chain chimeric polypeptide of any one of embodiments D1-D25, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment D27. The multi-chain chimeric polypeptide of any one of embodiments D1-D26, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment D28. The multi-chain chimeric polypeptide of any one of embodiments D1-D27, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment D29. The multi-chain chimeric polypeptide of embodiment D28, wherein the signal sequence comprises SEQ ID NO: 31.

Embodiment D30. The multi-chain chimeric polypeptide of embodiment D28, wherein the signal sequence is SEQ ID NO: 223.

Embodiment D31. The multi-chain chimeric polypeptide of any one of embodiments D1-D30, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15.

Embodiment D32. The multi-chain chimeric polypeptide of embodiment D31, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment D33. The multi-chain chimeric polypeptide of embodiment D31, wherein the soluble IL-15 comprises a sequence that is at least 80% identical to SEQ ID NO: 14.

Embodiment D34. The multi-chain chimeric polypeptide of embodiment D33, wherein the soluble IL-15 comprises a sequence that is at least 90% identical to SEQ ID NO: 14.

Embodiment D35. The multi-chain chimeric polypeptide of embodiment D34, wherein the soluble IL-15 comprises a sequence that is at least 95% identical to SEQ ID NO: 14.

Embodiment D36. The multi-chain chimeric polypeptide of embodiment D35, wherein the soluble IL-15 comprises SEQ ID NO: 14.

Embodiment D37. The multi-chain chimeric polypeptide of any one of embodiments D31-D36, wherein the sushi domain of IL15Rα comprises a sushi domain from human IL15Rα.

Embodiment D38. The multi-chain chimeric polypeptide of embodiment D37, wherein the sushi domain from human IL15Rα comprises a sequence that is at least 80% identical to SEQ ID NO: 28.

Embodiment D39. The multi-chain chimeric polypeptide of embodiment D38, wherein the sushi domain from human IL15Rα comprises a sequence that is at least 90% identical to SEQ ID NO: 28.

Embodiment D40. The multi-chain chimeric polypeptide of embodiment D39, wherein the sushi domain from human IL15Rα comprises a sequence that is at least 95% identical to SEQ ID NO: 28.

Embodiment D41. The multi-chain chimeric polypeptide of embodiment D40, wherein the sushi domain from human IL15Rα comprises SEQ ID NO: 28.

Embodiment D42. The multi-chain chimeric polypeptide of embodiment D37, wherein the sushi domain from human IL15Rα is a mature full-length IL15Rα.

Embodiment D43. The multi-chain chimeric polypeptide of any one of embodiments D1-D30, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment D44. The multi-chain chimeric polypeptide of any one of embodiments D1-D43, wherein one or both of the first target-binding domain and the second target-binding domain is an agonistic antigen-binding domain.

Embodiment D45. The multi-chain chimeric polypeptide of embodiment D44, wherein the first target-binding domain and the second target-binding domain are each agonistic antigen-binding domains.

Embodiment D46. The multi-chain chimeric polypeptide of embodiment D44 or D45, wherein antigen-binding domain comprises a scFv or single-domain antibody.

Embodiment D47. The multi-chain chimeric polypeptide of any one of embodiments D1-D43, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 or a soluble IL-7.

Embodiment D48. The multi-chain chimeric polypeptide of embodiment D47, wherein the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble IL-7.

Embodiment D49. The multi-chain chimeric polypeptide of any one of embodiments D1-D48, wherein the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a receptor of IL-7.

Embodiment D50. The multi-chain chimeric polypeptide of embodiment D49, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment D51. The multi-chain chimeric polypeptide of embodiment D50, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment D52. The multi-chain chimeric polypeptide of any one of embodiments D1-D48, wherein the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to a receptor for IL-7.

Embodiment D53. The multi-chain chimeric polypeptide of any one of embodiments D1-D48, wherein the first target-binding domain binds specifically to a

receptor for IL-7, and the second target-binding domain bind specifically to a receptor for IL-21.

Embodiment D54. The multi-chain chimeric polypeptide of embodiment D53, wherein the first target-binding domain comprises a soluble IL-21.

Embodiment D55. The multi-chain chimeric polypeptide of embodiment D54, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment D56. The multi-chain chimeric polypeptide of embodiment D55, wherein the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 78.

Embodiment D57. The multi-chain chimeric polypeptide of embodiment D56, wherein the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 78.

Embodiment D58. The multi-chain chimeric polypeptide of embodiment D57, wherein the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 78.

Embodiment D59. The multi-chain chimeric polypeptide of embodiment D58, wherein the soluble human IL-21 comprises a sequence of SEQ ID NO: 78.

Embodiment D60. The multi-chain chimeric polypeptide of any one of embodiments D53-D59, wherein the second target-binding domain comprises a soluble IL-7.

Embodiment D61. The multi-chain chimeric polypeptide of embodiment D60, wherein the soluble IL-7 is a soluble human IL-7.

Embodiment D62. The multi-chain chimeric polypeptide of embodiment D61, wherein the soluble human IL-7 comprises a sequence at least 80% identical to SEQ ID NO: 11.

Embodiment D63. The multi-chain chimeric polypeptide of embodiment D62, wherein the soluble human IL-7 comprises a sequence at least 90% identical to SEQ ID NO: 11.

Embodiment D64. The multi-chain chimeric polypeptide of embodiment D63, wherein the soluble human IL-7 comprises a sequence at least 95% identical to SEQ ID NO: 11.

Embodiment D65. The multi-chain chimeric polypeptide of embodiment D64, wherein the soluble human IL-7 comprises a sequence of SEQ ID NO: 11.

Embodiment D66. The multi-chain chimeric polypeptide of embodiment D1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 104.

Embodiment D67. The multi-chain chimeric polypeptide of embodiment D66, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104.

Embodiment D68. The multi-chain chimeric polypeptide of embodiment D67, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 104.

Embodiment D69. The multi-chain chimeric polypeptide of embodiment D68, wherein the first chimeric polypeptide comprises SEQ ID NO: 104.

Embodiment D70. The multi-chain chimeric polypeptide of embodiment D69, wherein the first chimeric polypeptide comprises SEQ ID NO: 106.

Embodiment D71. The multi-chain chimeric polypeptide of any one of embodiments D1 and D66-D70, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 108.

Embodiment D72. The multi-chain chimeric polypeptide of embodiment D71, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108.

Embodiment D73. The multi-chain chimeric polypeptide of embodiment D72, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 108.

Embodiment D74. The multi-chain chimeric polypeptide of embodiment D73, wherein the second chimeric polypeptide comprises SEQ ID NO: 108.

Embodiment D75. The multi-chain chimeric polypeptide of embodiment D74, wherein the second chimeric polypeptide comprises SEQ ID NO: 110.

Embodiment D76. The multi-chain chimeric polypeptide of embodiment D1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 96.

Embodiment D77. The multi-chain chimeric polypeptide of embodiment D76, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 96.

Embodiment D78. The multi-chain chimeric polypeptide of embodiment D77, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 96.

Embodiment D79. The multi-chain chimeric polypeptide of embodiment D68, wherein the first chimeric polypeptide comprises SEQ ID NO: 96.

Embodiment D80. The multi-chain chimeric polypeptide of embodiment D69, wherein the first chimeric polypeptide comprises SEQ ID NO: 98.

Embodiment D81. The multi-chain chimeric polypeptide of any one of embodiments D1 and D76-D80, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 100.

Embodiment D82. The multi-chain chimeric polypeptide of embodiment D81, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 100.

Embodiment D83. The multi-chain chimeric polypeptide of embodiment D82, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 100.

Embodiment D84. The multi-chain chimeric polypeptide of embodiment D83, wherein the second chimeric polypeptide comprises SEQ ID NO: 100.

Embodiment D85. The multi-chain chimeric polypeptide of embodiment D84, wherein the second chimeric polypeptide comprises SEQ ID NO: 106.

Embodiment D86. The multi-chain chimeric polypeptide of any one of embodiments D1-D65, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more

additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment D87. The multi-chain chimeric polypeptide of embodiment D86, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s)and the first domain of the pair of affinity domains.

Embodiment D88. The multi-chain chimeric polypeptide of any one of embodiments D1-D65, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment D89. The multi-chain chimeric polypeptide of embodiment D88, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D90. The multi-chain chimeric polypeptide of embodiment D88, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment D91. The multi-chain chimeric polypeptide of embodiment D88, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment D92. The multi-chain chimeric polypeptide of embodiment D88, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment D93. The multi-chain chimeric polypeptide of embodiment D88, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D94. The multi-chain chimeric polypeptide of embodiment D93, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D95. The multi-chain chimeric polypeptide of embodiment D93, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D96. The multi-chain chimeric polypeptide of embodiment D93, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D97. The multi-chain chimeric polypeptide of embodiment D93, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D98. The multi-chain chimeric polypeptide of embodiment D93, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment D99. The multi-chain chimeric polypeptide of embodiment D93, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment D100. The multi-chain chimeric polypeptide of any one of embodiments D1-D65 and D86-D99, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment D101. The multi-chain chimeric polypeptide of embodiment D100, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment D102. The multi-chain chimeric polypeptide of embodiment D100, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment D103. The multi-chain chimeric polypeptide of embodiment D100, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment D104. The multi-chain chimeric polypeptide of embodiment D100, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment D105. The multi-chain chimeric polypeptide of any one of embodiments D86-D104, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment D106. The multi-chain chimeric polypeptide of embodiment D105, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment D107. The multi-chain chimeric polypeptide of embodiment D106, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment D108. The multi-chain chimeric polypeptide of any one of embodiments D86-D104, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment D109. The multi-chain chimeric polypeptide of any one of embodiments D86-D108, wherein the one or more additional antigen-binding domains

bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD28.

Embodiment D110. The multi-chain chimeric polypeptide of any one of embodiments D86-D108, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment D111. The multi-chain chimeric polypeptide of embodiment D110, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

Embodiment D112. The multi-chain chimeric polypeptide of any one of embodiments D86-D108, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment D113. The multi-chain chimeric polypeptide of embodiment D112, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-

βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, or a soluble CD28.

Embodiment D114. A composition comprising any of the multi-chain chimeric polypeptides of embodiments D1-D113.

Embodiment D115. The composition of embodiment D114, wherein the composition is a pharmaceutical composition.

Embodiment D116. A kit comprising at least one dose of the composition of embodiment D114 or D115.

Embodiment D117. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments D1-D113 or the composition of embodiment D114 or D115.

Embodiment D118. The method of embodiment D117, wherein the immune cell is contacted *in vitro*.

Embodiment D119. The method of embodiment D118, wherein the immune cell was previously obtained from a subject.

Embodiment D120. The method of embodiment D119, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment D121. The method of embodiment D117, wherein the immune cell is contacted in vivo.

Embodiment D122. The method of any one of embodiments D117-D121, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment D123. The method of any one of embodiments D117-D122, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment D124. The method of any one of embodiments D117-D122, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment D125. The method of any one of embodiments D117-D124, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment D126. The method of embodiment D125, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment D127. The method of embodiment D126, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia,

glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment D128. The method of embodiment D125, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment D129. The method of embodiment D128, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment D130. The method of embodiment D125, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment D131. The method of embodiment D130, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Embodiment D132. A method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments D1-D113 or the composition of embodiment D114 or D115.

Embodiment D133. The method of embodiment D132, wherein the immune cell is contacted *in vitro*.

Embodiment D134. The method of embodiment D133, wherein the immune cell was previously obtained from a subject.

Embodiment D135. The method of embodiment D134, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment D136. The method of embodiment D132, wherein the immune cell is contacted in vivo.

Embodiment D137. The method of any one of embodiments D132-D136, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment D138. The method of any one of embodiments D132-D137, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment D139. The method of any one of embodiments D132-D137, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment D140. The method of any one of embodiments D132-D139, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment D141. The method of embodiment D140, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment D142. The method of embodiment D141, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment D143. The method of embodiment D140, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment D144. The method of embodiment D143, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and

neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment D145. The method of embodiment D140, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment D146. The method of embodiment D140, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Embodiment D147. A method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments D1-D113 or the composition of embodiment D114 or D115.

Embodiment D148. The method of embodiment D147, wherein the immune cell is contacted *in vitro*.

Embodiment D149. The method of embodiment D148, wherein the immune cell was previously obtained from a subject.

Embodiment D150. The method of embodiment D149, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment D151. The method of embodiment D147, wherein the immune cell is contacted in vivo.

Embodiment D152. The method of any one of embodiments D147-D151, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a

peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment D153. The method of any one of embodiments D147-D152, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment D154. The method of any one of embodiments D147-D152, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment D155: The method of any one of embodiments D147-D153, wherein the immune cell is contacted with anti-TF IgG antibody to create a memory or memory like immune cell.

Embodiment D156. The method of any one of embodiments D147-D155, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment D157. The method of embodiment D156, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment D158. The method of embodiment D156, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia,

wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment D159. The method of embodiment D156, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment D160. The method of embodiment D159, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment D161. The method of embodiment D156, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment D162. The method of embodiment D161, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Embodiment D163. A method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method comprising administering to the

subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments D1-D113 or the composition of embodiment D114 or D115.

Embodiment D164. The method of embodiment D163, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment D165. The method of embodiment D164, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment D166. The method of embodiment D163, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment D167. The method of embodiment D166, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease,

age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction

Embodiment D168. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments D1-D113 or the composition of embodiment D114 or D115.

Embodiment D169. The method of embodiment D168, wherein the subject has been identified or diagnosed as having a cancer or infectious disease.

Embodiment D170. The method of embodiment D169, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment D171. The method of embodiment D168, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment D172. The method of embodiment D171, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic

pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment D173. The method of embodiment D168, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment D174. The method of embodiment D173, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Embodiment D175. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments D1-D113.

Embodiment D176. A vector comprising the nucleic acid of embodiment D174.

Embodiment D177. The vector of embodiment D176, wherein the vector is an expression vector.

Embodiment D178. A cell comprising the nucleic acid of embodiment D175 or the vector of embodiment D175 or D176.

Embodiment D179. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment D177 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment D180. A multi-chain chimeric polypeptide produced by the method of embodiment D179.

E. Exemplary Embodiments

Embodiment E1. A multi-chain chimeric polypeptide comprising:

(e) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(f) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain and the second targeting-binding domain each independently bind specifically to: a receptor for IL-7, CD16, a receptor for IL-21, TGF-β, or a receptor for CD137L.

Embodiment E2. The multi-chain chimeric polypeptide of embodiment E1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment E3. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment E4. The multi-chain chimeric polypeptide of any one of embodiments E1-E3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment E5. The multi-chain chimeric polypeptide of any one of embodiments E1-E3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E6. The multi-chain chimeric polypeptide of any one of embodiments E1-E5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment E7. The multi-chain chimeric polypeptide of any one of embodiments E1-E5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment E8. The multi-chain chimeric polypeptide of any one of embodiments E1-E7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment E9. The multi-chain chimeric polypeptide of embodiment E8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

Embodiment E10. The multi-chain chimeric polypeptide of embodiment E9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

Embodiment E11. The multi-chain chimeric polypeptide of embodiment E10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

Embodiment E12. The multi-chain chimeric polypeptide of any one of embodiments E8-E11, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment E13. The multi-chain chimeric polypeptide of embodiment E12, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment E14. The multi-chain chimeric polypeptide of any one of embodiments E1-E13, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment E15. The multi-chain chimeric polypeptide of any one of embodiments E1-E14, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment E16. The multi-chain chimeric polypeptide of any one of embodiments E1-E15, wherein the multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

Embodiment E17. The multi-chain chimeric polypeptide of any one of embodiments E1-E16, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment E18. The multi-chain chimeric polypeptide of any one of embodiments E1-E17, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment E19. The multi-chain chimeric polypeptide of any one of embodiments E1-E18, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment E20. The multi-chain chimeric polypeptide of embodiment E19, wherein the signal sequence comprises SEQ ID NO: 31.

Embodiment E21. The multi-chain chimeric polypeptide of embodiment E20, wherein the signal sequence is SEQ ID NO: 31.

Embodiment E22. The multi-chain chimeric polypeptide of any one of embodiments E1-E21, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15.

Embodiment E23. The multi-chain chimeric polypeptide of embodiment E22, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment E24. The multi-chain chimeric polypeptide of embodiment E22, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 14.

Embodiment E25. The multi-chain chimeric polypeptide of embodiment E24, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 14.

Embodiment E26. The multi-chain chimeric polypeptide of embodiment E25, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 14.

Embodiment E27. The multi-chain chimeric polypeptide of embodiment E26, wherein the soluble IL-15 comprises SEQ ID NO: 14.

Embodiment E28. The multi-chain chimeric polypeptide of any one of embodiments E22-E27, wherein the sushi domain of IL15Rα comprises a sushi domain from human IL15Rα.

Embodiment E29. The multi-chain chimeric polypeptide of embodiment E28, wherein the sushi domain from human IL15Rα comprises a sequence that is 80% identical to SEQ ID NO: 28.

Embodiment E30. The multi-chain chimeric polypeptide of embodiment E29, wherein the sushi domain from human IL15Rα comprises a sequence that is 90% identical to SEQ ID NO: 28.

Embodiment E31. The multi-chain chimeric polypeptide of embodiment E30, wherein the sushi domain from human IL15Rα comprises a sequence that is 95% identical to SEQ ID NO: 28.

Embodiment E32. The multi-chain chimeric polypeptide of embodiment E31, wherein the sushi domain from human IL15Rα comprises SEQ ID NO: 28.

Embodiment E33. The multi-chain chimeric polypeptide of embodiment E28, wherein the sushi domain from human IL15Rα is a mature full-length IL15Rα.

Embodiment E34. The multi-chain chimeric polypeptide of any one of embodiments E1-E21, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment E35. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7, CD16, or a receptor for IL-21.

Embodiment E36. The multi-chain chimeric polypeptide of embodiment E35, wherein the first target-binding domain binds specifically to a receptor IL-7 and the second target-binding domain binds specifically to CD16 or a receptor for IL-21.

Embodiment E37. The multi-chain chimeric polypeptide of embodiment E36, wherein the first target-binding domain comprises a soluble IL-7 protein.

Embodiment E38. The multi-chain chimeric polypeptide of embodiment E37, wherein the soluble IL-7 protein is a soluble human IL-7.

Embodiment E39. The multi-chain chimeric polypeptide of any one of embodiments E36-E38, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to CD16.

Embodiment E40. The multi-chain chimeric polypeptide of embodiment E39, wherein the second antigen-binding domain comprises an scFv that binds specifically to CD16.

Embodiment E41. The multi-chain chimeric polypeptide of any one of embodiments E36-E38, wherein the second antigen-binding domain bind specifically to a receptor for IL-21.

Embodiment E42. The multi-chain chimeric polypeptide of embodiment E41, wherein the second antigen-binding domain comprises a soluble IL-21.

Embodiment E43. The multi-chain chimeric polypeptide of embodiment E42, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment E44. The multi-chain chimeric polypeptide of any one of embodiments E36-E40, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for IL-21.

Embodiment E45. The multi-chain chimeric polypeptide of embodiment E44, wherein the additional target-binding domain comprises a soluble IL-21.

Embodiment E46. The multi-chain chimeric polypeptide of embodiment E45, wherein the soluble IL-21 is a soluble human IL-12.

Embodiment E47. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, CD16, or a receptor for IL-21.

Embodiment E48. The multi-chain chimeric polypeptide of embodiment E47, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to CD16 or a receptor of IL-21.

Embodiment E49. The multi-specific chimeric polypeptide of embodiment E48, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E50. The multi-specific chimeric polypeptide of embodiment E49, wherein soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E51. The multi-specific chimeric polypeptide of any one of embodiments E48-E50, wherein the second target-binding domain binds specifically to CD16.

Embodiment E52. The multi-specific chimeric polypeptide of embodiment E51, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to CD16.

Embodiment E53. The multi-chain chimeric polypeptide of embodiment E52, wherein the second antigen-binding domain comprises an scFv that binds specifically to CD16.

Embodiment E54. The multi-chain chimeric polypeptide of any one of embodiments E48-E50, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment E55. The multi-chain chimeric polypeptide of embodiment E54, wherein the second target-binding domain comprises a soluble IL-21.

Embodiment E56. The multi-chain chimeric polypeptide of embodiment E55, wherein the second target-binding domain comprises a soluble human IL-21.

Embodiment E57. The multi-chain chimeric polypeptide of any one of embodiments E48-E53, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for IL-21.

Embodiment E58. The multi-chain chimeric polypeptide of embodiment E57, wherein the additional target-binding domain comprises a soluble IL-21.

Embodiment E59. The multi-chain chimeric polypeptide of embodiment E58, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment E60. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor for IL-7.

Embodiment E61. The multi-chain chimeric polypeptide of embodiment E60, wherein the first target-binding domain and the second target-binding domain include a soluble IL-7.

Embodiment E62. The multi-chain chimeric polypeptide of embodiment E61, wherein the soluble IL-7 is a soluble human IL-7.

Embodiment E63. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second target-binding domain each independently bind specifically to TGF-β.

Embodiment E64. The multi-specific chimeric polypeptide of embodiment E63, wherein the first target-binding domain and the second target-binding domain is a soluble TGF- β receptor.

Embodiment E65. The multi-specific chimeric polypeptide of embodiment E64, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E66. The multi-specific chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7, a receptor for IL-21, or a receptor for CD137L.

Embodiment E67. The multi-chain chimeric polypeptide of embodiment E66, wherein the first target-binding domain binds specifically to a receptor for IL-7 and the second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

Embodiment E68. The multi-specific chimeric polypeptide of embodiment E67, wherein the first target-binding domain is a soluble IL-7.

Embodiment E69. The multi-specific chimeric polypeptide of embodiment E68, wherein the soluble IL-7 is a soluble human IL-7.

Embodiment E70. The multi-chain chimeric polypeptide of any one of embodiments E67-E69, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment E71. The multi-chain chimeric polypeptide of embodiment E70, wherein the second target-binding domain is a soluble IL-21.

Embodiment E72. The multi-chain chimeric polypeptide of embodiment E71, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment E73. The multi-chain chimeric polypeptide of any one of embodiments E67-E69, wherein the second antigen-binding domain binds specifically to a receptor for CD137L.

Embodiment E74. The multi-chain chimeric polypeptide of embodiment E73, wherein the second antigen-binding domain is a soluble CD137L.

Embodiment E75. The multi-chain chimeric polypeptide of embodiment E74, wherein the soluble CD137L is a soluble human CD137L.

Embodiment E76. The multi-chain chimeric polypeptide of any one of embodiments E67-E72, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for CD137L.

Embodiment E77. The multi-chain chimeric polypeptide of embodiment E76, wherein the additional target-binding domain comprises a soluble CD137L.

Embodiment E78. The multi-chain chimeric polypeptide of embodiment E77, wherein the soluble CD137L is a soluble human CD137L.

Embodiment E79. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7 or TGF-β.

Embodiment E80. The multi-chain chimeric polypeptide of embodiment E79, wherein the first target-binding domain binds specifically to a receptor IL-7 and the second target-binding domain binds specifically to TGF-β.

Embodiment E81. The multi-chain chimeric polypeptide of embodiment E80, wherein the first target-binding domain comprises a soluble IL-7 protein.

Embodiment E82. The multi-chain chimeric polypeptide of embodiment E81, wherein the soluble IL-7 protein is a soluble human IL-7.

Embodiment E83. The multi-chain chimeric polypeptide of any one of embodiments E80-E82, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to TGF-β.

Embodiment E84. The multi-specific chimeric polypeptide of embodiment E83, wherein the second target-binding domain is a soluble TGF-β receptor.

Embodiment E85. The multi-specific chimeric polypeptide of embodiment E84, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E86. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, a receptor for IL-21, or a receptor for CD137L.

Embodiment E87. The multi-chain chimeric polypeptide of embodiment E86, wherein the first target-binding domain binds specifically to a TGF-β and the second

target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

Embodiment E88. The multi-specific chimeric polypeptide of embodiment E87, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E89. The multi-specific chimeric polypeptide of embodiment E88, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E90. The multi-specific chimeric polypeptide of any one of embodiments E87-E89, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment E91. The multi-chain chimeric polypeptide of embodiment E90, wherein the second target-binding domain comprises a soluble IL-21.

Embodiment E92. The multi-chain chimeric polypeptide of embodiment E91, wherein the second target-binding domain comprises a soluble human IL-21.

Embodiment E93. The multi-specific chimeric polypeptide of any one of embodiments E87-E89, wherein the second target-binding domain binds specifically to a receptor for CD137L.

Embodiment E94. The multi-chain chimeric polypeptide of embodiment E93, wherein the second target-binding domain comprises a soluble CD137L.

Embodiment E95. The multi-chain chimeric polypeptide of embodiment E94, wherein the second target-binding domain comprises a soluble human CD137L.

Embodiment E96. The multi-chain chimeric polypeptide of any one of embodiments E87-E92, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for CD137L.

Embodiment E97. The multi-chain chimeric polypeptide of embodiment E96, wherein the additional target-binding domain comprises a soluble CD137L.

Embodiment E98. The multi-chain chimeric polypeptide of embodiment E97, wherein the soluble CD137L is a soluble human CD137L.

Embodiment E99. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or a receptor for IL-21.

Embodiment E100. The multi-chain chimeric polypeptide of embodiment E99, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to TGF-β or a receptor for IL-21.

Embodiment E101. The multi-specific chimeric polypeptide of embodiment E100, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E102. The multi-specific chimeric polypeptide of embodiment E101, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E103. The multi-specific chimeric polypeptide of any one of embodiments E100-E102, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment E104. The multi-chain chimeric polypeptide of embodiment E103, wherein the second target-binding domain comprises a soluble IL-21.

Embodiment E105. The multi-chain chimeric polypeptide of embodiment E104, wherein the second target-binding domain comprises a soluble human IL-21.

Embodiment E106. The multi-specific chimeric polypeptide of any one of embodiments E100-E102, wherein the second target-binding domain binds specifically to TGF-β.

Embodiment E107. The multi-specific chimeric polypeptide of embodiment E106, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E108. The multi-specific chimeric polypeptide of embodiment E107, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E109. The multi-specific chimeric polypeptide of any one of embodiments E100-E105, wherein the second polypeptide further comprises an additional target-binding domain that binds specifically to TGF-β.

Embodiment E110. The multi-specific chimeric polypeptide of embodiment E109, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E111. The multi-specific chimeric polypeptide of embodiment E110, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E112. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or IL-16.

Embodiment E113. The multi-chain chimeric polypeptide of embodiment E112, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to TGF-β or IL-16.

Embodiment E114. The multi-specific chimeric polypeptide of embodiment E113, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E115. The multi-specific chimeric polypeptide of embodiment E114, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E116. The multi-specific chimeric polypeptide of any one of embodiments E113-E115, wherein the second target-binding domain binds specifically to IL-16.

Embodiment E117. The multi-specific chimeric polypeptide of embodiment E116, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to CD16.

Embodiment E118. The multi-chain chimeric polypeptide of embodiment E117, wherein the second antigen-binding domain comprises an scFv that binds specifically to CD16.

Embodiment E119. The multi-specific chimeric polypeptide of any one of embodiments E113-E115, wherein the second target-binding domain binds specifically to TGF-β.

Embodiment E120. The multi-specific chimeric polypeptide of embodiment E119, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E121. The multi-specific chimeric polypeptide of embodiment E120, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E122. The multi-specific chimeric polypeptide of any one of embodiments E113-E118, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to TGF-β.

Embodiment E123. The multi-specific chimeric polypeptide of embodiment E122, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E124. The multi-specific chimeric polypeptide of embodiment E123, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E125. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a TGF-β or a receptor for CD137L.

Embodiment E126. The multi-chain chimeric polypeptide of embodiment E125, wherein the first target-binding domain binds specifically to TGF-β and the second target-binding domain binds specifically to a receptor for CD137L.

Embodiment E127. The multi-specific chimeric polypeptide of embodiment E126, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment E128. The multi-specific chimeric polypeptide of embodiment E127, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E129. The multi-chain chimeric polypeptide of embodiment E128, wherein the second target-binding domain comprises a soluble CD137L protein.

Embodiment E130. The multi-chain chimeric polypeptide of embodiment E129, wherein the soluble CD137L protein is a soluble human CD137L.

Embodiment E131. The multi-chain chimeric polypeptide of any one of embodiments E126-E130, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to TGF-β.

Embodiment E132. The multi-specific chimeric polypeptide of embodiment E131, wherein the additional target-binding domain is a soluble TGF-β receptor.

Embodiment E133. The multi-specific chimeric polypeptide of embodiment E132, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment E134. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 104.

Embodiment E135. The multi-chain chimeric polypeptide of embodiment E134, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104.

Embodiment E136. The multi-chain chimeric polypeptide of embodiment E135, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 104.

Embodiment E137. The multi-chain chimeric polypeptide of embodiment E136, wherein the first chimeric polypeptide comprises SEQ ID NO: 104.

Embodiment E138. The multi-chain chimeric polypeptide of embodiment E137, wherein the first chimeric polypeptide comprises SEQ ID NO: 106.

Embodiment E139. The multi-chain chimeric polypeptide of any one of embodiments E1 and E134-E138, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 129.

Embodiment E140. The multi-chain chimeric polypeptide of embodiment E139, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 129.

Embodiment E141. The multi-chain chimeric polypeptide of embodiment E140, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 129.

Embodiment E142. The multi-chain chimeric polypeptide of embodiment E141, wherein the second chimeric polypeptide comprises SEQ ID NO: 129.

Embodiment E143. The multi-chain chimeric polypeptide of embodiment E142, wherein the second chimeric polypeptide comprises SEQ ID NO: 131.

Embodiment E144. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 133.

Embodiment E145. The multi-chain chimeric polypeptide of embodiment E144, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 133.

Embodiment E146. The multi-chain chimeric polypeptide of embodiment E145, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 133.

Embodiment E147. The multi-chain chimeric polypeptide of embodiment E146, wherein the first chimeric polypeptide comprises SEQ ID NO: 133.

Embodiment E148. The multi-chain chimeric polypeptide of embodiment E147, wherein the first chimeric polypeptide comprises SEQ ID NO: 135.

Embodiment E149. The multi-chain chimeric polypeptide of any one of embodiments E1 and E144-E148, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 137.

Embodiment E150. The multi-chain chimeric polypeptide of embodiment E149, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 137.

Embodiment E151. The multi-chain chimeric polypeptide of embodiment E150, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 137.

Embodiment E152. The multi-chain chimeric polypeptide of embodiment E151, wherein the second chimeric polypeptide comprises SEQ ID NO: 137.

Embodiment E153. The multi-chain chimeric polypeptide of embodiment E152, wherein the second chimeric polypeptide comprises SEQ ID NO: 139.

Embodiment E154. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 141.

Embodiment E155. The multi-chain chimeric polypeptide of embodiment E154, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 141.

Embodiment E156. The multi-chain chimeric polypeptide of embodiment E155, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 141.

Embodiment E157. The multi-chain chimeric polypeptide of embodiment E156, wherein the first chimeric polypeptide comprises SEQ ID NO: 141.

Embodiment E158. The multi-chain chimeric polypeptide of embodiment E157, wherein the first chimeric polypeptide comprises SEQ ID NO: 143.

Embodiment E159. The multi-chain chimeric polypeptide of any one of embodiments E1 and E154-E158, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 145.

Embodiment E160. The multi-chain chimeric polypeptide of embodiment E159, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 145.

Embodiment E161. The multi-chain chimeric polypeptide of embodiment E160, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 145.

Embodiment E162. The multi-chain chimeric polypeptide of embodiment E161, wherein the second chimeric polypeptide comprises SEQ ID NO: 145.

Embodiment E163. The multi-chain chimeric polypeptide of embodiment E162, wherein the second chimeric polypeptide comprises SEQ ID NO: 147.

Embodiment E164. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 149.

Embodiment E165. The multi-chain chimeric polypeptide of embodiment E164, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 149.

Embodiment E166. The multi-chain chimeric polypeptide of embodiment E165, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 149.

Embodiment E167. The multi-chain chimeric polypeptide of embodiment E166, wherein the first chimeric polypeptide comprises SEQ ID NO: 149.

Embodiment E168. The multi-chain chimeric polypeptide of embodiment E167, wherein the first chimeric polypeptide comprises SEQ ID NO: 151.

Embodiment E169. The multi-chain chimeric polypeptide of any one of embodiments E1 and E164-E168, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 153.

Embodiment E170. The multi-chain chimeric polypeptide of embodiment E169, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 153.

Embodiment E171. The multi-chain chimeric polypeptide of embodiment E170, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 153.

Embodiment E172. The multi-chain chimeric polypeptide of embodiment E171, wherein the second chimeric polypeptide comprises SEQ ID NO: 153.

Embodiment E173. The multi-chain chimeric polypeptide of embodiment E172, wherein the second chimeric polypeptide comprises SEQ ID NO: 155.

Embodiment E174. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 161.

Embodiment E175. The multi-chain chimeric polypeptide of embodiment E174, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 161.

Embodiment E176. The multi-chain chimeric polypeptide of embodiment E175, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 161.

Embodiment E177. The multi-chain chimeric polypeptide of embodiment E176, wherein the first chimeric polypeptide comprises SEQ ID NO: 161.

Embodiment E178. The multi-chain chimeric polypeptide of embodiment E177, wherein the first chimeric polypeptide comprises SEQ ID NO: 163.

Embodiment E179. The multi-chain chimeric polypeptide of any one of embodiments E1 and E174-E178, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 165.

Embodiment E180. The multi-chain chimeric polypeptide of embodiment E179, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 165.

Embodiment E181. The multi-chain chimeric polypeptide of embodiment E180, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 165.

Embodiment E182. The multi-chain chimeric polypeptide of embodiment E181, wherein the second chimeric polypeptide comprises SEQ ID NO: 165.

Embodiment E183. The multi-chain chimeric polypeptide of embodiment E182, wherein the second chimeric polypeptide comprises SEQ ID NO: 167.

Embodiment E184. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 173.

Embodiment E185. The multi-chain chimeric polypeptide of embodiment E184, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 173.

Embodiment E186. The multi-chain chimeric polypeptide of embodiment E185, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 173.

Embodiment E187. The multi-chain chimeric polypeptide of embodiment E186, wherein the first chimeric polypeptide comprises SEQ ID NO: 173.

Embodiment E188. The multi-chain chimeric polypeptide of embodiment E187, wherein the first chimeric polypeptide comprises SEQ ID NO: 175.

Embodiment E189. The multi-chain chimeric polypeptide of any one of embodiments E1 and E184-E188, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 189.

Embodiment E190. The multi-chain chimeric polypeptide of embodiment E189, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 189.

Embodiment E191. The multi-chain chimeric polypeptide of embodiment E190, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 189.

Embodiment E192. The multi-chain chimeric polypeptide of embodiment E191, wherein the second chimeric polypeptide comprises SEQ ID NO: 189.

Embodiment E193. The multi-chain chimeric polypeptide of embodiment E192, wherein the second chimeric polypeptide comprises SEQ ID NO: 191.

Embodiment E194. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 173.

Embodiment E195. The multi-chain chimeric polypeptide of embodiment E194, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 173.

Embodiment E196. The multi-chain chimeric polypeptide of embodiment E195, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 173.

Embodiment E197. The multi-chain chimeric polypeptide of embodiment E196, wherein the first chimeric polypeptide comprises SEQ ID NO: 173.

Embodiment E198. The multi-chain chimeric polypeptide of embodiment E197, wherein the first chimeric polypeptide comprises SEQ ID NO: 175.

Embodiment E199. The multi-chain chimeric polypeptide of any one of embodiments E1 and E194-E198, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 177.

Embodiment E200. The multi-chain chimeric polypeptide of embodiment E199, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 177.

Embodiment E201. The multi-chain chimeric polypeptide of embodiment E200, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 177.

Embodiment E202. The multi-chain chimeric polypeptide of embodiment E201, wherein the second chimeric polypeptide comprises SEQ ID NO: 177.

Embodiment E203. The multi-chain chimeric polypeptide of embodiment E202, wherein the second chimeric polypeptide comprises SEQ ID NO: 179.

Embodiment E204. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 181.

Embodiment E205. The multi-chain chimeric polypeptide of embodiment E204, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 181.

Embodiment E206. The multi-chain chimeric polypeptide of embodiment E205, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 181.

Embodiment E207. The multi-chain chimeric polypeptide of embodiment E206, wherein the first chimeric polypeptide comprises SEQ ID NO: 181.

Embodiment E208. The multi-chain chimeric polypeptide of embodiment E207, wherein the first chimeric polypeptide comprises SEQ ID NO: 183.

Embodiment E209. The multi-chain chimeric polypeptide of any one of embodiments E1 and E204-E208, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 185.

Embodiment E210. The multi-chain chimeric polypeptide of embodiment E209, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 185.

Embodiment E211. The multi-chain chimeric polypeptide of embodiment E210, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 185.

Embodiment E212. The multi-chain chimeric polypeptide of embodiment E211, wherein the second chimeric polypeptide comprises SEQ ID NO: 185.

Embodiment E213. The multi-chain chimeric polypeptide of embodiment E212, wherein the second chimeric polypeptide comprises SEQ ID NO: 187.

Embodiment E214. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 193.

Embodiment E215. The multi-chain chimeric polypeptide of embodiment E214, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 193.

Embodiment E216. The multi-chain chimeric polypeptide of embodiment E215, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 193.

Embodiment E217. The multi-chain chimeric polypeptide of embodiment E216, wherein the first chimeric polypeptide comprises SEQ ID NO: 193.

Embodiment E218. The multi-chain chimeric polypeptide of embodiment E217, wherein the first chimeric polypeptide comprises SEQ ID NO: 195.

Embodiment E219. The multi-chain chimeric polypeptide of any one of embodiments E1 and E214-E218, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 197.

Embodiment E220. The multi-chain chimeric polypeptide of embodiment E219, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 197.

Embodiment E221. The multi-chain chimeric polypeptide of embodiment E220, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 197.

Embodiment E222. The multi-chain chimeric polypeptide of embodiment E221, wherein the second chimeric polypeptide comprises SEQ ID NO: 197.

Embodiment E223. The multi-chain chimeric polypeptide of embodiment E222, wherein the second chimeric polypeptide comprises SEQ ID NO: 199.

Embodiment E224. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 201.

Embodiment E225. The multi-chain chimeric polypeptide of embodiment E224, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 201.

Embodiment E226. The multi-chain chimeric polypeptide of embodiment E225, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 201.

Embodiment E227. The multi-chain chimeric polypeptide of embodiment E226, wherein the first chimeric polypeptide comprises SEQ ID NO: 201.

Embodiment E228. The multi-chain chimeric polypeptide of embodiment E227, wherein the first chimeric polypeptide comprises SEQ ID NO: 203.

Embodiment E229. The multi-chain chimeric polypeptide of any one of embodiments E1 and E224-E228, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 205.

Embodiment E230. The multi-chain chimeric polypeptide of embodiment E229, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 205.

Embodiment E231. The multi-chain chimeric polypeptide of embodiment E230, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 205.

Embodiment E232. The multi-chain chimeric polypeptide of embodiment E231, wherein the second chimeric polypeptide comprises SEQ ID NO: 205.

Embodiment E233. The multi-chain chimeric polypeptide of embodiment E232, wherein the second chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment E234. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 209.

Embodiment E235. The multi-chain chimeric polypeptide of embodiment E234, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 209.

Embodiment E236. The multi-chain chimeric polypeptide of embodiment E235, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 209.

Embodiment E237. The multi-chain chimeric polypeptide of embodiment E236, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment E238. The multi-chain chimeric polypeptide of embodiment E237, wherein the first chimeric polypeptide comprises SEQ ID NO: 211.

Embodiment E239. The multi-chain chimeric polypeptide of any one of embodiments E1 and E234-E238, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 213.

Embodiment E240. The multi-chain chimeric polypeptide of embodiment E239, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 213.

Embodiment E241. The multi-chain chimeric polypeptide of embodiment E240, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 213.

Embodiment E242. The multi-chain chimeric polypeptide of embodiment E241, wherein the second chimeric polypeptide comprises SEQ ID NO: 213.

Embodiment E243. The multi-chain chimeric polypeptide of embodiment E242, wherein the second chimeric polypeptide comprises SEQ ID NO: 215.

Embodiment E244. The multi-chain chimeric polypeptide of any one of embodiments E1-E133, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment E245. The multi-chain chimeric polypeptide of embodiment E244, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s)and the first domain of the pair of affinity domains.

Embodiment E246. The multi-chain chimeric polypeptide of any one of embodiments E1-E133, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment E247. The multi-chain chimeric polypeptide of embodiment E246, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E248. The multi-chain chimeric polypeptide of embodiment E246, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment E249. The multi-chain chimeric polypeptide of embodiment E246, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment E250. The multi-chain chimeric polypeptide of embodiment E246, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment E251. The multi-chain chimeric polypeptide of embodiment E246, wherein at least one of the one or more additional target-binding domains is disposed at

the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E252. The multi-chain chimeric polypeptide of embodiment E251, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E253. The multi-chain chimeric polypeptide of embodiment E251, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E254. The multi-chain chimeric polypeptide of embodiment E251, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E255. The multi-chain chimeric polypeptide of embodiment E251, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E256. The multi-chain chimeric polypeptide of embodiment E251, wherein the at least one of the one or more additional target-binding domains positioned

between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment E257. The multi-chain chimeric polypeptide of embodiment E251, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment E258. The multi-chain chimeric polypeptide of any one of embodiments E44-E46, E57-E59, E76-E78, E96-E98, E109-E111, E122-E124, and E131-E133, wherein the second chimeric polypeptide further comprises the additional target-binding domain at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment E259. The multi-chain chimeric polypeptide of embodiment E258, wherein the additional target-binding domain directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment E260. The multi-chain chimeric polypeptide of embodiment E258, wherein the second chimeric polypeptide further comprises a linker sequence between the additional target-binding domain and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment E261. The multi-chain chimeric polypeptide of embodiment E258, wherein the additional target-binding domain directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment E262. The multi-chain chimeric polypeptide of embodiment E258, wherein the second chimeric polypeptide further comprises a linker sequence between the additional target-binding domain and the second target-binding domain in the second chimeric polypeptide.

Embodiment E263. A composition comprising any of the multi-chain chimeric polypeptides of embodiments E1-E262.

Embodiment E264. The composition of embodiment E263, wherein the composition is a pharmaceutical composition.

Embodiment E265. A kit comprising at least one dose of the composition of embodiment E263 or E264.

Embodiment E266. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments E1-E262 or the composition of embodiment E263 or E264.

Embodiment E267. The method of embodiment E266, wherein the immune cell is contacted in vitro.

Embodiment E268. The method of embodiment E267, wherein the immune cell was previously obtained from a subject.

Embodiment E269. The method of embodiment E268, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment E270. The method of embodiment E266, wherein the immune cell is contacted in vivo.

Embodiment E271. The method of any one of embodiments E266-E270, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a $CD8^+$ T cell, a $CD4^+$ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment E272. The method of any one of embodiments E266-E271, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment E273. The method of any one of embodiments E266-E271, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment E274. The method of any one of embodiments E266-E273, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment E275. The method of embodiment E274, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment E276. The method of embodiment E275, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia,

glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment E277. The method of embodiment E274, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment E278. The method of embodiment E277, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment E279. The method of embodiment E274, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment E280. The method of embodiment E279, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment E281. A method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments E1-E262 or the composition of embodiment E263 or E264.

Embodiment E282. The method of embodiment E281, wherein the immune cell is contacted in vitro.

Embodiment E283. The method of embodiment E282, wherein the immune cell was previously obtained from a subject.

Embodiment E284. The method of embodiment E283, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment E285. The method of embodiment E281, wherein the immune cell is contacted in vivo.

Embodiment E286. The method of any one of embodiments E281-E285, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a memory T cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment E287. The method of any one of embodiments E281-E286, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment E288. The method of any one of embodiments E281-E286, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment E289. The method of any one of embodiments E281-E288, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment E290. The method of embodiment E289, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment E291. The method of embodiment E290, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment E292. The method of embodiment E289, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment E293. The method of embodiment E292, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer,

colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment E294. The method of embodiment E289, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment E295. The method of embodiment E289, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment E296. A method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of embodiments E1-E262 or the composition of embodiment E263 or E264.

Embodiment E297. The method of embodiment E296, wherein the immune cell is contacted in vitro.

Embodiment E298. The method of embodiment E297, wherein the immune cell was previously obtained from a subject.

Embodiment E299. The method of embodiment E298, wherein the method further comprises obtaining the immune cell from the subject prior to the contacting step.

Embodiment E300. The method of embodiment E296, wherein the immune cell is contacted in vivo.

Embodiment E301. The method of any one of embodiments E296-E300, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Embodiment E302. The method of any one of embodiments E296-E301, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Embodiment E303. The method of any one of embodiments E296-E301, wherein the method further comprises, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Embodiment E304. The method of any one of embodiments E296-E303, wherein the method further comprises administering the immune cell to a subject in need thereof.

Embodiment E305. The method of embodiment E304, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

Embodiment E306. The method of embodiment E305, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia,

glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment E307. The method of embodiment E304, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment E308. The method of embodiment E307, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment E309. The method of embodiment E304, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment E310. The method of embodiment E309, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment E311. A method of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments E1-E262 or the composition of embodiment E263 or E264.

Embodiment E312. The method of embodiment E311, wherein the subject has been identified or diagnosed as having a cancer.

Embodiment E313. The method of embodiment E312, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment E314. The method of embodiment E311, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment E315. The method of embodiment E314, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment E316. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the multi-chain chimeric polypeptides of embodiments E1-E262 or the composition of embodiment E263 or E264.

Embodiment E317. The method of embodiment E316, wherein the subject has been identified or diagnosed as having a cancer or infectious disease.

Embodiment E318. The method of embodiment E317, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment E319. The method of embodiment E316, wherein the subject has been identified or diagnosed as having age-related disease or condition.

Embodiment E320. The method of embodiment E319, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia,

wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

Embodiment E321. The method of embodiment E316, wherein the subject has been diagnosed or identified as having an infectious disease.

Embodiment E322. The method of embodiment E321, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Embodiment E323. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments E1-E262.

Embodiment E324. A vector comprising the nucleic acid of embodiment E323.

Embodiment E325. The vector of embodiment E324, wherein the vector is an expression vector.

Embodiment E326. A cell comprising the nucleic acid of embodiment E323 or the vector of embodiment E324 or E325.

Embodiment E327. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment E326 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment E328. A multi-chain chimeric polypeptide produced by the method of embodiment E327.

Embodiment E329. The multi-chain chimeric polypeptide of embodiment E8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 3.

Embodiment E330. The multi-chain chimeric polypeptide of embodiment E329, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 3.

Embodiment E331. The multi-chain chimeric polypeptide of embodiment E330, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

Embodiment E332. The multi-chain chimeric polypeptide of embodiment E331, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 3.

Embodiment E333. The multi-chain chimeric polypeptide of embodiment E8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

Embodiment E334. The multi-chain chimeric polypeptide of embodiment E333, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

Embodiment E335. The multi-chain chimeric polypeptide of embodiment E334, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 4.

Embodiment E336. The multi-chain chimeric polypeptide of embodiment E335, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 4.

F. Exemplary Embodiments

Embodiment F1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a linker domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

Embodiment F2. The multi-chain chimeric polypeptide of embodiment F1, wherein the first target-binding domain and the linker domain directly abut each other in the first chimeric polypeptide.

Embodiment F3. The multi-chain chimeric polypeptide of embodiment F1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the linker domain in the first chimeric polypeptide.

Embodiment F4. The multi-chain chimeric polypeptide of any one of embodiments F1-F3, wherein the linker domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment F5. The multi-chain chimeric polypeptide of any one of embodiments F1-F3, wherein the first chimeric polypeptide further comprises a linker sequence between the linker domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F6. The multi-chain chimeric polypeptide of any one of embodiments F1-F5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment F7. The multi-chain chimeric polypeptide of any one of embodiments F1-F5, wherein the second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment F8. The multi-chain chimeric polypeptide of any one of embodiments F1-F7, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment F9. The multi-chain chimeric polypeptide of embodiment F8, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment F10. The multi-chain chimeric polypeptide of embodiment F9, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment F11. The multi-chain chimeric polypeptide of any one of embodiments F1-F7, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment F12. The multi-chain chimeric polypeptide of any one of embodiments F1-F11, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment F13. The multi-chain chimeric polypeptide of embodiment F12, wherein the first target-binding domain and the second target-binding domain are each antigen-binding domains.

Embodiment F14. The multi-chain chimeric polypeptide of embodiment F12 or F13, wherein the antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment F15. The multi-chain chimeric polypeptide of any one of embodiments F1-F14, wherein one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, CD80, CD86, PD-L2, B7-H4, HVEM, ILT3, ILT4, TIGIT, MHCII, LAG3, CD272, VISTA, CD137, CD40, CD47, CD70, OX40, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

Embodiment F16. The multi-chain chimeric polypeptide of any one of embodiments F1-F14, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment F17. The multi-chain chimeric polypeptide of embodiment F16, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

Embodiment F18. The multi-chain chimeric polypeptide of any one of embodiments F1-F14, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment F19. The multi-chain chimeric polypeptide of embodiment F18, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28.

Embodiment F20. The multi-chain chimeric polypeptide of any one of embodiments F1-F19, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more target-binding domain(s) is positioned between the linker domain and the first domain of the pair of affinity domains.

Embodiment F21. The multi-chain chimeric polypeptide of embodiment F20, wherein the first chimeric polypeptide further comprises a linker sequence between the linker domain and the at least one of the one or more target antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more target antigen-binding domain(s) and the first domain of the pair of affinity domains.

Embodiment F22. The multi-chain chimeric polypeptide of any one of embodiments F1-F19, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment F23. The multi-chain chimeric polypeptide of embodiment F22, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F24. The multi-chain chimeric polypeptide of embodiment F22, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment F25. The multi-chain chimeric polypeptide of embodiment F22, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment F26. The multi-chain chimeric polypeptide of embodiment F22, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment F27. The multi-chain chimeric polypeptide of embodiment F22, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the linker domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F28. The multi-chain chimeric polypeptide of embodiment F27, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F29. The multi-chain chimeric polypeptide of embodiment F27, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F30. The multi-chain chimeric polypeptide of embodiment F27, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F31. The multi-chain chimeric polypeptide of embodiment F27, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F32. The multi-chain chimeric polypeptide of embodiment F27, wherein the at least one of the one or more additional target-binding domains positioned between the linker domain and the first domain of the pair of affinity domains, directly abuts the linker domain and/or the first domain of the pair of affinity domains.

Embodiment F33. The multi-chain chimeric polypeptide of embodiment F27, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the linker domain and the at least one of the one or more additional target-binding domains positioned between the linker domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the linker domain and the first domain of the pair of affinity domains.

Embodiment F34. The multi-chain chimeric polypeptide of any one of embodiments F1-F33, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end and/or the C-terminal end of the second chimeric polypeptide.

Embodiment F35. The multi-chain chimeric polypeptide of embodiment F34, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment F36. The multi-chain chimeric polypeptide of embodiment F34, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment F37. The multi-chain chimeric polypeptide of embodiment F34, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment F38. The multi-chain chimeric polypeptide of embodiment F34, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment F39. The multi-chain chimeric polypeptide of any one of embodiments F20-F38, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment F40. The multi-chain chimeric polypeptide of embodiment F39, wherein two or more of the first target-binding domain, the second target-binding

domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment F41. The multi-chain chimeric polypeptide of embodiment F40, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment F42. The multi-chain chimeric polypeptide of embodiment F39, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen.

Embodiment F43. The multi-chain chimeric polypeptide of embodiment F42, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope.

Embodiment F44. The multi-chain chimeric polypeptide of embodiment F43, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence.

Embodiment F45. The multi-chain chimeric polypeptide of any one of embodiments F20-F38, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment F46. The multi-chain chimeric polypeptide of any one of embodiments F20-F45, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain.

Embodiment F47. The multi-chain chimeric polypeptide of embodiment F46, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain.

Embodiment F48. The multi-chain chimeric polypeptide of embodiment F46 or F47, wherein the antigen-binding domain comprises a scFv.

Embodiment F49. The multi-chain chimeric polypeptide of any one of embodiments F20-F48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, CD80, CD86, PD-L2, B7-H4, HVEM, ILT3, ILT4, TIGIT, MHCII, LAG3, CD272, VISTA, CD137, CD40, CD47, CD70, OX40, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD3, and a receptor for CD28.

Embodiment F50. The multi-chain chimeric polypeptide of any one of embodiments F20-F48, wherein one or more of the first target-binding domain, the

second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment F52. The multi-chain chimeric polypeptide of any one of embodiments F20-F48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment F53. The multi-chain chimeric polypeptide of embodiment F52, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

Embodiment F54. The multi-chain chimeric polypeptide of any one of embodiments F1-F53, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment F55. The multi-chain chimeric polypeptide of any one of embodiments F1-F53, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment F56. The multi-chain chimeric polypeptide of any one of embodiments F1-F55, wherein the linker domain is a soluble tissue factor domain.

Embodiment F57. The multi-chain chimeric polypeptide of embodiment F56, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment F58. The multi-chain chimeric polypeptide of embodiment F57, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

Embodiment F59. The multi-chain chimeric polypeptide of embodiment F58, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

Embodiment F60. The multi-chain chimeric polypeptide of embodiment F59, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 1.

Embodiment F61. The multi-chain chimeric polypeptide of any one of embodiments F57-F60, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment F62. The multi-chain chimeric polypeptide of embodiment F61, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment F63. The multi-chain chimeric polypeptide of any one of embodiments F56-F62, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment F64. The multi-chain chimeric polypeptide of any one of embodiments F56-F63, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment F65. The multi-chain chimeric polypeptide of any one of embodiments F56-F64, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment F66. The multi-chain chimeric polypeptide of any one of embodiments F1-F55, wherein the linker domain is selected from the group consisting of: a kappa chain and a lambda chain.

Embodiment F67. The multi-chain chimeric polypeptide of any one of embodiments F1-F66, wherein the pair of affinity domains is a sushi domain from an

alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15.

Embodiment F68. The multi-chain chimeric polypeptide of embodiment F67, wherein the soluble IL15 has a D8N or D8A amino acid substitution.

Embodiment F69. The multi-chain chimeric polypeptide of embodiment F67 or F68, wherein the human IL15Rα is a mature full-length IL15Rα.

Embodiment F70. The multi-chain chimeric polypeptide of any one of embodiments F1-F66, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment F71. The multi-chain chimeric polypeptide of any one of embodiments F1-F70, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment F72. The multi-chain chimeric polypeptide of any one of embodiments F1-F70, wherein the first chimeric polypeptide and/or the second chimeric polypeptide lacks a signal sequence at its N-terminal end.

Embodiment F73. A composition comprising any of the multi-chain chimeric polypeptides of embodiments F1-F72.

Embodiment F74. The composition of embodiment F73, wherein the composition is a pharmaceutical composition.

Embodiment F75. A kit comprising at least one dose of the composition of embodiment F73 or F74.

Embodiment F76. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments F1-F72.

Embodiment F77. A vector comprising the nucleic acid of embodiment F76.

Embodiment F78. The vector of embodiment F77, wherein the vector is an expression vector.

Embodiment F79. A cell comprising the nucleic acid of embodiment F76 or the vector of embodiment F77 or F78.

Embodiment F80. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment F79 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment F81. A multi-chain chimeric polypeptide produced by the method of embodiment F80.

WHAT IS CLAIMED IS:

1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

2. The multi-chain chimeric polypeptide of claim 1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

3. The multi-chain chimeric polypeptide of claim 1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

4. The multi-chain chimeric polypeptide of any one of claims 1-3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

5. The multi-chain chimeric polypeptide of any one of claims 1-3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide are connected through the linker sequence.

6. The multi-chain chimeric polypeptide of any one of claims 1-5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

7. The multi-chain chimeric polypeptide of any one of claims 1-5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

8. The multi-chain chimeric polypeptide of any one of claims 1-7, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

9. The multi-chain chimeric polypeptide of any one of claims 1-7, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

10. The multi-chain chimeric polypeptide of any one of claims 1-9, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

11. The multi-chain chimeric polypeptide of any one of claims 1-10, wherein one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26a, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3,

PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

12. The multi-chain chimeric polypeptide of any one of claims 1-10, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

13. The multi-chain chimeric polypeptide of claim 12, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L.

14. The multi-chain chimeric polypeptide of any one of claims 1-10, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

15. The multi-chain chimeric polypeptide of claim 14, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28.

16. The multi-chain chimeric polypeptide of any one of claims 1-15, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s).

17. The multi-chain chimeric polypeptide of any one of claims 1-16, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains.

18. The multi-chain chimeric polypeptide of any one of claims 1-17, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

19. The multi-chain chimeric polypeptide of claim 18, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

20. The multi-chain chimeric polypeptide of any one of claims 1-19, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15.

21. The multi-chain chimeric polypeptide of any one of claims 1-19, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

22. The multi-chain chimeric polypeptide of any one of claims 1-21, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

23. A composition comprising any of the multi-chain chimeric polypeptides of claims 1-22.

24. The composition of claim 23, wherein the composition is a pharmaceutical composition.

25. A kit comprising at least one dose of the composition of claim 23 or 24.

26. An in vitro method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of claims 1-22 or the composition of claim 23 or 24.

27. An in vitro method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of claims 1-22 or the composition of claim 23 or 24.

28. An in vitro method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the multi-chain chimeric polypeptides of claims 1-22 or the composition of claim 23 or 24.

29. The method of any one of claims 26-28, wherein the immune cell is selected from the group consisting of: an immature thymocyte, a peripheral blood lymphocyte, a naïve T cell, a pluripotent Th cell precursor, a lymphoid progenitor cell, a Treg cell, a Th17 cell, a Th22 cell, a Th9 cell, a Th2 cell, a Th1 cell, a Th3 cell, γδ T cell, an αβ T cell, a tumor-infiltrating T cell, a CD8+ T cell, a CD4+ T cell, a natural killer T cell, a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

30. The method of any one of claims 26-29, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

31. Use of the multi-chain chimeric polypeptide of any one of claims 1-22 in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject having a cancer, an aging-related disease or condition, or an infectious disease.

32. Use of the multi-chain chimeric polypeptide of any one of claims 1-22 in the manufacture of a medicament for treating a cancer, an aging-related disease or condition, or an infectious disease.

33. The use of claim 31 or 32, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

34. The use of claim 31 or 32, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis,

NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

35. The use of claim 31 or 32, wherein the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

36. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of claims 1-22.

37. A vector comprising the nucleic acid of claim 36.

38. A cell comprising the nucleic acid of claim 36 or the vector of claim 37.

39. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of claim 38 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

40. The multi-chain chimeric polypeptide of claim 18, wherein the human soluble tissue factor domain does not initiate blood coagulation.

41. The multi-chain chimeric polypeptide of any one of claims 1-22, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

42. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain binds specifically to a ligand of TGF-β receptor II (TGF-βRII) and the second target-binding domain binds specifically to a ligand of TGF-βRII;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

43. The multi-chain chimeric polypeptide of claim 42, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

44. The multi-chain chimeric polypeptide of claim 42, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

45. The multi-chain chimeric polypeptide of any one of claims 42-44, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

46. The multi-chain chimeric polypeptide of any one of claims 42-44, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide are connected through the linker sequence.

47. The multi-chain chimeric polypeptide of any one of claims 42-46, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

48. The multi-chain chimeric polypeptide of any one of claims 42-46, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

49. The multi-chain chimeric polypeptide of any one of claims 42-48, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

50. The multi-chain chimeric polypeptide of any one of claims 42-49, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

51. The multi-chain chimeric polypeptide of any one of claims 42-50, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s).

52. The multi-chain chimeric polypeptide of any one of claims 42-51, wherein the second chimeric polypeptide further comprises one or more additional target-binding domain(s).

53. The multi-chain chimeric polypeptide of any one of claims 42-52, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

54. The multi-chain chimeric polypeptide of claim 53, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

55. The multi-chain chimeric polypeptide of any one of claims 42-54, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

56. The multi-chain chimeric polypeptide of claim 42, wherein the first target-binding domain comprises a soluble TGF-βRII.

57. The multi-chain chimeric polypeptide of claim 42, wherein the first target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID NO: 81 and a second sequence that is at least 80% identical to SEQ ID NO: 81, wherein the first and second sequence are separated by a linker.

58. The multi-chain chimeric polypeptide of claim 57, wherein the first target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 81 and a second sequence that is at least 90% identical to SEQ ID NO: 81

59. The multi-chain chimeric polypeptide of claim 58, wherein the first target-binding domain comprises a first sequence of SEQ ID NO: 81 and a second sequence of SEQ ID NO: 81.

60. The multi-chain chimeric polypeptide of claim 57, wherein the linker comprises a sequence of SEQ ID NO: 7.

61. The multi-chain chimeric polypeptide of claim 56, wherein the first target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 85.

62. The multi-chain chimeric polypeptide of claim 61, wherein the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 85.

63. The multi-chain chimeric polypeptide of claim 62, wherein the first target-binding domain comprises a sequence of SEQ ID NO: 85.

64. The multi-chain chimeric polypeptide of claim 42, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 133.

65. The multi-chain chimeric polypeptide of claim 64, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 133.

66. The multi-chain chimeric polypeptide of claim 65, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 133.

67. The multi-chain chimeric polypeptide of claim 66, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 135.

68. The multi-chain chimeric polypeptide of claim 42, wherein the second target-binding domain comprises a soluble TGF-βRII.

69. The multi-chain chimeric polypeptide of claim 68, wherein the second target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID NO: 81 and a second sequence that is at least 80% identical to SEQ ID NO: 81, wherein the first and second sequence are separated by a linker.

70. The multi-chain chimeric polypeptide of claim 69, wherein the second target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 81 and a second sequence that is at least 90% identical to SEQ ID NO: 81.

71. The multi-chain chimeric polypeptide of claim 70, wherein the second target-binding domain comprises a first sequence of SEQ ID NO: 81 and a second sequence of SEQ ID NO: 81.

72. The multi-chain chimeric polypeptide of claim 69, wherein the linker comprises a sequence of SEQ ID NO: 7.

73. The multi-chain chimeric polypeptide of claim 68, wherein the second target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 85.

74. The multi-chain chimeric polypeptide of claim 69, wherein the second target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 85.

75. The multi-chain chimeric polypeptide of claim 70, wherein the second target-binding domain comprises a sequence of SEQ ID NO: 85.

76. The multi-chain chimeric polypeptide of claim 42, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 177.

77. The multi-chain chimeric polypeptide of claim 76, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 133.

78. The multi-chain chimeric polypeptide of claim 76, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 177.

79. The multi-chain chimeric polypeptide of claim 78, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 177.

80. The multi-chain chimeric polypeptide of claim 79, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 133.

81. The multi-chain chimeric polypeptide of claim 79, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 92.

82. A nucleic acid encoding the multi-chain chimeric polypeptide of any one of claims 42-81.

83. A cell comprising the nucleic acid of claim 82.

84. A method of producing the multi-chain chimeric polypeptide comprising:

culturing a cell of claim 83 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

85. The multi-chain chimeric polypeptide of claim 53, wherein the human soluble tissue factor domain does not initiate blood coagulation.

86. A composition comprising the multi-chain chimeric polypeptide of any one of claims 42-81 and 85.

87. An in vitro method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 42-81 and 85, or the composition of claim 86.

88. An in vitro method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 42-81 and 85, or the composition of claim 86.

89. Use of the multi-chain chimeric polypeptide of any one of claims 42-81 and 85 in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject having a cancer, an aging-related disease or condition, or an infectious disease.

90. Use of the multi-chain chimeric polypeptide of any one of claims 42-81 and 85 in the manufacture of a medicament for treating a cancer, an aging-related disease or condition, or an infectious disease.

91. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain binds specifically to a receptor for IL-18 and the second target-binding domain binds specifically to a receptor for IL-12, or the first target-binding domain binds specifically to a receptor for IL-12 and the second target-binding domain binds specifically to a receptor for IL-18;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

92. The multi-chain chimeric polypeptide of claim 91, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

93. The multi-chain chimeric polypeptide of claim 91, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

94. The multi-chain chimeric polypeptide of any one of claims 91-93, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

95. The multi-chain chimeric polypeptide of any one of claims 91-93, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide are connected through the linker sequence.

96. The multi-chain chimeric polypeptide of any one of claims 91-95, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

97. The multi-chain chimeric polypeptide of any one of claims 91-95, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

98. The multi-chain chimeric polypeptide of any one of claims 91-97, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

99. The multi-chain chimeric polypeptide of any one of claims 91-97, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

100. The multi-chain chimeric polypeptide of any one of claims 91-99, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s).

101. The multi-chain chimeric polypeptide of any one of claims 91-100, wherein the second chimeric polypeptide further comprises one or more additional target-binding domain(s).

102. The multi-chain chimeric polypeptide of any one of claims 91-101, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

103. The multi-chain chimeric polypeptide of claim 102, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

104. The multi-chain chimeric polypeptide of claim 102, wherein the human soluble tissue factor domain does not initiate blood coagulation.

105. The multi-chain chimeric polypeptide of any one of claims 91-104, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

106. The multi-chain chimeric polypeptide of any one of claims 91-105, wherein the first target-binding domain specifically binds to a receptor for IL-18 and the second target-binding domain specifically binds to a receptor for IL-12.

107. The multi-chain chimeric polypeptide of claim 106, wherein the first target-binding domain comprises a soluble IL-18.

108. The multi-chain chimeric polypeptide of claim 107, wherein the first target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 16.

109. The multi-chain chimeric polypeptide of claim 108, wherein the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 16.

110. The multi-chain chimeric polypeptide of claim 109, wherein the first target-binding domain comprises a sequence of SEQ ID NO: 16.

111. The multi-chain chimeric polypeptide of claim 91, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 70.

112. The multi-chain chimeric polypeptide of claim 111, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 70.

113. The multi-chain chimeric polypeptide of claim 112, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 70.

114. The multi-chain chimeric polypeptide of claim 113, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 72.

115. The multi-chain chimeric polypeptide of claim 106, wherein the second target-binding domain comprises a soluble IL-12.

116. The multi-chain chimeric polypeptide of claim 115, wherein the second target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID

NO: 66 and a second sequence that is at least 80% identical to SEQ ID NO: 68, wherein the first and second sequence are separated by a linker.

117. The multi-chain chimeric polypeptide of claim 116, wherein the second target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 66 and a second sequence that is at least 90% identical to SEQ ID NO: 68.

118. The multi-chain chimeric polypeptide of claim 117, wherein the second target-binding domain comprises a first sequence of SEQ ID NO: 66 and a second sequence of SEQ ID NO: 68.

119. The multi-chain chimeric polypeptide of claim 116, wherein the linker comprises a sequence of SEQ ID NO: 7.

120. The multi-chain chimeric polypeptide of claim 91, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 74.

121. The multi-chain chimeric polypeptide of claim 111, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 74.

122. The multi-chain chimeric polypeptide of claim 120, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 74.

123. The multi-chain chimeric polypeptide of claim 122, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 74.

124. The multi-chain chimeric polypeptide of claim 123, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 70.

125. The multi-chain chimeric polypeptide of claim 123, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 76.

126. A composition comprising the multi-chain chimeric polypeptide of any one of claims 91-125 and 131.

127. The composition of claim 126, wherein the composition is a pharmaceutical composition.

128. A nucleic acid encoding the multi-chain chimeric polypeptide of any one of claims 91-125 and 131.

129. A cell comprising the nucleic acid of claim 128.

130. A method of producing the multi-chain chimeric polypeptide comprising:

culturing a cell of claim 129 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

131. The multi-chain chimeric polypeptide of any one of claims 91-105, wherein the first target-binding domain binds specifically to a receptor for IL-12 and the second target-binding domain binds specifically to a receptor for IL-18.

132. An in vitro method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 91-125 and 131, or the composition of claim 126 or 127.

133. An in vitro method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 91-125 and 131, or the composition of claim 126 or 127.

134. An in vitro method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 91-125 and 131, or the composition of claim 126 or 127.

135. Use of the multi-chain chimeric polypeptide of any one of claims 91-125 and 131 in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject having a cancer, an aging-related disease or condition, or an infectious disease.

136. Use of the multi-chain chimeric polypeptide of any one of claims 91-125 and 131 in the manufacture of a medicament for treating a cancer, an aging-related disease or condition, or an infectious disease.

137. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain binds specifically to a receptor for IL-7 and the second target-binding domain binds specifically to a receptor for IL-21, or the first target-binding domain binds specifically to a receptor for IL-21 and the second target-binding domain binds specifically to a receptor for IL-7;

the first target-binding domain, the second target-binding domain, the first domain of the pair of affinity domains, and the second domain of the pair of affinity domains are protein domains; and

the soluble tissue factor domain directly abuts or is connected to the first domain of the pair of affinity domains in the first chimeric polypeptide through a linker sequence.

138. The multi-chain chimeric polypeptide of claim 137, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

139. The multi-chain chimeric polypeptide of claim 137, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

140. The multi-chain chimeric polypeptide of any one of claims 137-139, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

141. The multi-chain chimeric polypeptide of any one of claims 137-139, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide are connected through the linker sequence.

142. The multi-chain chimeric polypeptide of any one of claims 137-141, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

143. The multi-chain chimeric polypeptide of any one of claims 137-141, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

144. The multi-chain chimeric polypeptide of any one of claims 137-143, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

145. The multi-chain chimeric polypeptide of any one of claims 137-144, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

146. The multi-chain chimeric polypeptide of any one of claims 137-145, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s).

147. The multi-chain chimeric polypeptide of any one of claims 137-146, wherein the second chimeric polypeptide further comprises one or more additional target-binding domain(s).

148. The multi-chain chimeric polypeptide of any one of claims 137-147, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

149. The multi-chain chimeric polypeptide of claim 148, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

150. The multi-chain chimeric polypeptide of claim 149, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

151. The multi-chain chimeric polypeptide of any one of claims 137-150, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

152. The multi-chain chimeric polypeptide of any one of claims 137-151, wherein the first target-binding domain specifically binds to a receptor for IL-7 and the second target-binding domain specifically binds to a receptor for IL-21.

153. The multi-chain chimeric polypeptide of claim 152, wherein the first target-binding domain comprises a soluble IL-7.

154. The multi-chain chimeric polypeptide of claim 153, wherein the first target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 11.

155. The multi-chain chimeric polypeptide of claim 154, wherein the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 11.

156. The multi-chain chimeric polypeptide of claim 155, wherein the first target-binding domain comprises a sequence of SEQ ID NO: 11.

157. The multi-chain chimeric polypeptide of claim 137, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 104.

158. The multi-chain chimeric polypeptide of claim 157, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104.

159. The multi-chain chimeric polypeptide of claim 158, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 104.

160. The multi-chain chimeric polypeptide of claim 159, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 106.

161. The multi-chain chimeric polypeptide of claim 152, wherein the second target-binding domain comprises a soluble IL-21.

162. The multi-chain chimeric polypeptide of claim 161, wherein the second target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 78.

163. The multi-chain chimeric polypeptide of claim 162, wherein the second target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 78.

164. The multi-chain chimeric polypeptide of claim 163, wherein the second target-binding domain comprises a sequence of SEQ ID NO: 78.

165. The multi-chain chimeric polypeptide of claim 137, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 108.

166. The multi-chain chimeric polypeptide of claim 157, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 108.

167. The multi-chain chimeric polypeptide of claim 165, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108.

168. The multi-chain chimeric polypeptide of claim 167, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 108.

169. The multi-chain chimeric polypeptide of claim 168, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 104.

170. The multi-chain chimeric polypeptide of claim 168, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 110.

171. A composition comprising the multi-chain chimeric polypeptide of any one of claims 137-170 and 176.

172. The composition of claim 171, wherein the composition is a pharmaceutical composition.

173. A nucleic acid encoding the multi-chain chimeric polypeptide of any one of claims 137-170 and 176.

174. A cell comprising the nucleic acid of claim 173.

175. A method of producing the multi-chain chimeric polypeptide comprising:

culturing a cell of claim 174 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

176. The multi-chain chimeric polypeptide of any one of claims 137-151, wherein the first target-binding domain binds specifically to a receptor for IL-21 and the second target-binding domain binds specifically to a receptor for IL-7.

177. An in vitro method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 137-170 and 176, or the composition of claim 171 or 172.

178. An in vitro method of inducing or increasing proliferation of an immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 137-170 and 176, or the composition of claim 171 or 172.

179. An in vitro method of inducing differentiation of an immune cell into a memory or memory-like immune cell, the method comprising:

contacting an immune cell with an effective amount of the multi-chain chimeric polypeptide of any one of claims 137-170 and 176, or the composition of claim 171 or 172.

180. Use of the multi-chain chimeric polypeptide of any one of claims 137-170 and 176 in the manufacture of a medicament for killing a cancer cell, an infected cell, or a senescent cell in a subject having a cancer, an aging-related disease or condition, or an infectious disease.

181. Use of the multi-chain chimeric polypeptide of any one of claims 137-170 and 176 in the manufacture of a medicament for treating a subject having a cancer, an aging-related disease or condition, or an infectious disease.