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 single-chain chimeric polypeptides that include: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a second target-binding domain. Also provided herein are compositions that include any of the single-chain chimeric polypeptides described herein, nucleic acids that encode any of the single-chain chimeric polypeptides described herein, and cells that include any of the nucleic acids that encode any of the single-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 single-chain chimeric

polypeptides described herein. Also provided herein are methods of producing any of the single-chain chimeric polypeptides described herein. Additional uses of any of the single-chain chimeric polypeptides are described herein.

Accordingly, in one aspect, provided herein is a single-chain chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a second target-binding domain. In some examples, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some examples, the first target-binding domain and the second target-binding domain directly abut each other. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the second target-binding domain. In some examples, the second target-binding domain and the soluble tissue factor domain directly abut each other. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between the second target-binding domain and the soluble tissue factor domain. 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 an antigen-binding domain. 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 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 soluble cell surface protein. In some examples, the soluble interleukin, soluble cytokine protein, or soluble cell surface 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, FLT3L, MICA, MICB, and a ULP16-binding protein. In some examples, 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 soluble cell surface receptor. In some examples, the soluble interleukin receptor, soluble cytokine receptor, or soluble cell surface 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. 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: 9. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to

SEQ ID NO: 9. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9. In some examples, the single-chain chimeric polypeptide of 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 Factor VIIa. In some examples, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some examples, the single-chain chimeric polypeptide does not stimulate blood coagulation in a mammal. In some examples, the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus. In some examples, the

single-chain chimeric polypeptide comprises one or more additional target-binding domains at its N-terminus. In some examples, one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its C-terminus. In some examples, one of the one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, the single-chain chimeric polypeptide comprises one or more additional target binding domains at its N-terminus and the C-terminus. In some examples, one of the one or more additional antigen binding domains at the N-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the N-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, one of the one or more additional antigen binding domains at the C-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some examples, the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the C-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. 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, the antigen-binding domain comprises a scFv or a single domain antibody. 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-D, 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 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. In some examples, the soluble interleukin or cytokine 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, FLT3L, MICA, MICB, and a ULP16-binding protein. 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-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, a soluble CD3, or a soluble CD28. In some examples, the single-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end. In some examples, the single-chain chimeric polypeptide further comprises a peptide tag positioned at the N-terminal end or the C-terminal end of the single-chain chimeric polypeptide.

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

(i) a first target-binding domain;

(ii) a soluble tissue factor domain that does not have coagulation activity; and

(iii) a second target-binding domain,

wherein the single-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

In one aspect, the present disclosure refers to a single-chain chimeric polypeptide comprising a sequence at least 80% identical to SEQ ID NO: 108.

In one aspect, the present disclosure refers to a single-chain chimeric polypeptide comprising a sequence of SEQ ID NO: 108.

In one aspect, the present disclosure refers to a single-chain chimeric polypeptide comprising a sequence of SEQ ID NO: 110.

In one aspect, also provided herein is a composition comprising any of the single-chain chimeric polypeptides discussed above. In some examples, the composition is a pharmaceutical composition. In some examples, the single-chain chimeric polypeptides comprise at least one dose of the composition within a kit. In one aspect, the present disclosure refers to a kit comprising at least one dose of the composition discussed above.

In one aspect, also 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 single-chain chimeric polypeptides or compositions discussed above. In some examples, the method comprises contacting the immune 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 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 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.

In one aspect, 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 single-chain chimeric polypeptides or compositions discussed herein. 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, 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 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 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, after the contacting step, introducing into the immune cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. 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.

In one aspect, 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 single-chain chimeric polypeptides or compositions discussed 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 method comprises genetically 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.

In one aspect, 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 single-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 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 one aspect, also provided herein is a method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the single-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, also provided herein are nucleic acids encoding any of the single-chain chimeric polypeptides discussed above. Some examples include a vector comprising the nucleic acid 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 include 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 or the vector discussed above.

In one aspect, also provided herein is a method of producing a single-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 single-chain chimeric polypeptide; and recovering the single-chain chimeric polypeptide from the cell and/or the culture medium. Some examples comprise single-chain chimeric polypeptide produced by the discussed above. In one aspect, the present disclosure refers to a single-chain chimeric polypeptide produced by the method discussed above. 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 mutant soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 96. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 96. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 96. In some examples, the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 96. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97. In some

examples, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97. In some examples, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97. In some examples, the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

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., a 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.

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 are schematic diagrams of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide.

Figure 2 is a chromatograph showing the elution of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide from an anti-tissue factor antibody affinity column.

Figure 3 is a chromatograph showing the elution of a Superdex 200 Increase 10/300 GL gel filtration column loaded with an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide.

Figure 4 is a sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide purified using an anti-tissue factor antibody affinity column.

Figure 5 is a graph showing the ELISA quantitation of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide performed using the methods described in Example 1. Purified tissue factor was used as the control.

Figure 6 is a graph showing the ability of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide to stimulate CD25 expression in $CD4^+$ T-cells isolated from blood from two donors. The experiments were performed as described in Example 2.

Figure 7 is a graph showing the ability of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide to stimulate CD25 expression in $CD8^+$ T-cells isolated from blood from two donors. The experiments were performed as described in Example 2.

Figure 8 is a graph showing the ability of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide to stimulate CD69

WHAT IS CLAIMED IS:

1. A single-chain chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a second target-binding domain.

2. The single-chain chimeric polypeptide of claim 1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

3. The single-chain chimeric polypeptide of claim 1, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

4. The single-chain chimeric polypeptide of any one of claims 1-3, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

5. The single-chain chimeric polypeptide of any one of claims 1-3, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

6. The single-chain chimeric polypeptide of any one of claims 1-5, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

7. The single-chain chimeric polypeptide of claim 6, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

8. The single-chain chimeric polypeptide of claim 7, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

9. The single-chain chimeric polypeptide of any one of claims 1-5, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

10. The single-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 single-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 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, PDGFDD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, 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 single-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, a soluble cytokine protein, or a soluble cell surface protein.

13. The single-chain chimeric polypeptide of claim 12, wherein the soluble interleukin, soluble cytokine protein, or soluble cell surface 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-D, SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

14. The single-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 receptor, a soluble cytokine receptor, or a soluble cell surface receptor.

15. The single-chain chimeric polypeptide of claim 14, wherein the soluble interleukin receptor, the soluble cytokine receptor, or the soluble cell surface 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 single-chain chimeric polypeptide of any one of claims 1-15, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

17. The single-chain chimeric polypeptide of claim 16, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9.

18. The single-chain chimeric polypeptide of any one of claims 1-17, wherein the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus.

19. A composition comprising any of the single-chain chimeric polypeptides of claims 1-18.

20. The composition of claim 19, wherein the composition is a pharmaceutical composition.

21. A kit comprising at least one dose of the composition of claim 19 or 20.

22. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the single-chain chimeric polypeptides of claims 1-18 or the composition of claim 19 or 20.

23. 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 single-chain chimeric polypeptides of claims 1-18 or the composition of claim 19 or 20.

24. 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 single-chain chimeric polypeptides of claims 1-18 or the composition of claim 19 or 20.

25. The method of any one of claims 22-24, wherein the immune cell is contacted in vitro.

26. The method of any one of claims 22-24, wherein the immune cell is contacted in vivo.

27. The method of any one of claims 22-26, 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.

28. The method of any one of claims 22-27, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

29. The method of any one of claims 22-28, wherein the method further comprises administering the immune cell to a subject in need thereof.

30. 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 single-chain chimeric polypeptides of claims 1-18 or the composition of claim 19 or 20.

31. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the single-chain chimeric polypeptides of claims 1-18 or the composition of claim 19 or 20.

32. The method of claim 30 or 31, wherein the subject has been identified or diagnosed as having a cancer, an aging-related disease or condition, or an infectious disease.

33. The method of claim 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, gastic 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 method of claim 32, 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.

35. The method of claim 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 single-chain chimeric polypeptides of any one of claims 1-18.

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 single-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 single-chain chimeric polypeptide; and

recovering the single-chain chimeric polypeptide from the cell and/or the culture medium.

40. A single-chain chimeric polypeptide produced by the method of claim 39.

41. The single-chain chimeric polypeptide of claim 16, wherein the human soluble tissue factor domain does not initiate blood coagulation.

42. The single-chain chimeric polypeptide of any one of claims 1-18, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

43. A single-chain chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain that does not have coagulation activity; and

(iii) a second target-binding domain,

wherein the single-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

44. The single-chain chimeric polypeptide of claim 43, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

45. The single-chain chimeric polypeptide of claim 43, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

46. The single-chain chimeric polypeptide of any one of claims 43-45, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

47. The single-chain chimeric polypeptide of any one of claims 43-45, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

48. The single-chain chimeric polypeptide of any one of claims 43-47, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

49. The single-chain chimeric polypeptide of any one of claims 43-47, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

50. The single-chain chimeric polypeptide of any one of claims 43-49, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

51. The single-chain chimeric polypeptide of any one of claims 43-50, wherein one or both of the first target-binding domain and the second target-binding domain bind 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, PDGFDD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, 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.

52. The single-chain chimeric polypeptide of any one of claims 43-51, wherein 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 soluble cell surface protein.

53. The single-chain chimeric polypeptide of claim 52, wherein the soluble interleukin, soluble cytokine protein, or soluble cell surface 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-D, SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

54. The single-chain chimeric polypeptide of any one of claims 43-53, wherein 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 soluble cell surface receptor.

55. The single-chain chimeric polypeptide of claim 54, wherein the soluble interleukin receptor, the soluble cytokine receptor, or the soluble cell surface 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.

56. The single-chain chimeric polypeptide of any one of claims 43-55, wherein the soluble tissue factor domain is a soluble human tissue factor domain that does not have coagulation activity.

57. A composition comprising any of the single-chain chimeric polypeptides of any one of claims 43-56 or 59.

58. A kit comprising at least one dose of the composition of claim 57.

59. The single-chain chimeric polypeptide of any one of claims 43-56, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

60. The single-chain chimeric polypeptide of any one of claims 43-47, wherein the first target-binding domain and the second target-binding domain are human IL-2.

61. The single-chain chimeric polypeptide of claim 60, wherein the human IL-2 comprises SEQ ID NO: 28.

62. The single-chain chimeric polypeptide of claim 61, wherein the soluble tissue factor domain comprises SEQ ID NO: 9.

63. A single-chain chimeric polypeptide comprising a sequence at least 80% identical to SEQ ID NO: 108.

64. The single-chain chimeric polypeptide of claim 63, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108.

65. The single-chain chimeric polypeptide of claim 63, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 108.

66. The single-chain chimeric polypeptide of claim 63, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 99% identical to SEQ ID NO: 108.

67. A composition comprising any of the single-chain chimeric polypeptide of claim 63.

68. A kit comprising at least one dose of the composition of claim 67.

69. A single-chain chimeric polypeptide comprising a sequence of SEQ ID NO: 108.

70. A composition comprising any of the single-chain chimeric polypeptide of claim 69.

71. A kit comprising at least one dose of the composition of claim 70.

72. A single-chain chimeric polypeptide comprising a sequence of SEQ ID NO: 110.

73. A composition comprising any of the single-chain chimeric polypeptide of claim 72.

74. A kit comprising at least one dose of the composition of claim 73.

75. A method of stimulating an immune cell, the method comprising:

contacting an immune cell with an effective amount of any of the single-chain chimeric polypeptides of any one of claims 43-56, 59-66, 69, and 72, or the composition of any one of claims 57, 67, 70, and 73.

76. 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 single-chain chimeric polypeptides of any one of claims 43-56, 59-66, 69, and 72, or the composition of any one of claims 57, 67, 70, and 73.

77. 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 single-chain chimeric polypeptides of any one of claims 43-56, 59-66, 69, and 72, or the composition of any one of claims 57, 67, 70, and 73.

78. The method of any one of claims 75-77, wherein the immune cell is contacted in vitro.

79. The method of any one of claims 75-77, wherein the immune cell is contacted in vivo.

80. The method of any one of claims 75-79, 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.

81. The method of any one of claims 75-80, wherein the immune cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

82. The method of any one of claims 75-81, wherein the method further comprises administering the immune cell to a subject in need thereof.

83. 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 the single-chain chimeric polypeptide of any one of claims 43-56, 59-66, 69, and 72, or the composition of any one of claims 57, 67, 70, and 73.

84. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the single-chain chimeric polypeptides of any one of claims 43-56, 59-66, 69, and 72, or the composition of any one of claims 57, 67, 70, and 73.

85. The method of claim 83 or 84, wherein the subject has been identified or diagnosed as having a cancer, an aging-related disease or condition, or an infectious disease.

86. The method of claim 85, 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, gastic 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.

87. The method of claim 85, 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.

88. The method of claim 85, 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.

89. Nucleic acid encoding any of the single-chain chimeric polypeptides of any one of claims 43-56, 59-66, 69, and 72.

90. A vector comprising the nucleic acid of claim 89.

91. A cell comprising the nucleic acid of claim 89 or the vector of claim 90.

92. A method of producing a single-chain chimeric polypeptide, the method comprising:

culturing the cell of claim 91 in a culture medium under conditions sufficient to result in the production of the single-chain chimeric polypeptide; and

recovering the single-chain chimeric polypeptide from the cell and/or the culture medium.

93. A single-chain chimeric polypeptide produced by the method of claim 92.