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~~example, 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

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; and

the first target-binding domain and the second target-binding domain are protein domains.

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

In one ~~aspect~~example, 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 one 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~~example, 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

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, 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 single-chain chimeric polypeptides described herein or the composition described herein.

In one ~~aspect~~example, 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, 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 single-chain chimeric polypeptides described herein or the composition described herein.

In one ~~aspect~~example, 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

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, 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 single-chain chimeric polypeptides described herein or the composition described herein.

In one ~~aspect~~example, 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, the present disclosure refers to use of the single-chain chimeric polypeptide described herein 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 one ~~aspect~~example, 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, the present disclosure refers to use of the single-chain chimeric polypeptide described herein in the manufacture of a medicament for treating cancer, an aging-related disease or condition, or an infectious disease.

In one aspect, also provided herein are nucleic acids encoding any one 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~~example, 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

WHAT IS CLAIMED IS:

1. 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; and

the first target-binding domain and the second target-binding domain are protein domains.

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 claim 17, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9.

19. The single-chain chimeric polypeptide of claim 18, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9.

20. The single-chain chimeric polypeptide of any one of claims 1-15, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

~~18~~21. The single-chain chimeric polypeptide of any one of claims 1-~~17~~20, wherein the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus.

22. The single-chain chimeric polypeptide of any one of claims 1-5, wherein the first target-binding domain and the second target-binding domain are human IL-2.

23. The single-chain chimeric polypeptide of claim 22, wherein the human IL-2 comprises a sequence at least 90% identical to SEQ ID NO: 28.

24. The single-chain chimeric polypeptide of claim 23, wherein the human IL-2 comprises a sequence at least 95% identical to SEQ ID NO: 28.

25. The single-chain chimeric polypeptide of claim 24, wherein the human IL-2 comprises a sequence of SEQ ID NO: 28.

26. The single-chain chimeric polypeptide of any one of claims 22-25, wherein the soluble tissue factor domain comprises a sequence at least 90% identical to SEQ ID NO: 9.

27. The single-chain chimeric polypeptide of claim 26, wherein the soluble tissue factor domain comprises a sequence at least 95% identical to SEQ ID NO: 9.

28. The single-chain chimeric polypeptide of claim 27, wherein the soluble tissue factor domain comprises a sequence of SEQ ID NO: 9.

29. The single-chain chimeric polypeptide of claim 1, wherein the single-chain chimeric polypeptide comprises a sequence at least 80% identical to SEQ ID NO: 108.

30. The single-chain chimeric polypeptide of claim 29, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108.

31. The single-chain chimeric polypeptide of claim 30, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 108.

32. The single-chain chimeric polypeptide of claim 31, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 99% identical to SEQ ID NO: 108.

33. The single-chain chimeric polypeptide of claim 32, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 108.

34. The single-chain chimeric polypeptide of claim 33, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 110.

~~19~~35. A composition comprising ~~any of~~ the single-chain chimeric polypeptide~~s~~ of any one of claims 1-~~18~~34.

~~20~~36. The composition of claim ~~19~~35, wherein the composition is a pharmaceutical composition.

~~21~~37. A kit comprising at least one dose of the composition of claim ~~19 or 20~~35 or 36.

~~22~~38. An in vitro 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~~ 34 or the composition of claim ~~19~~ 35 or ~~20~~36.

~~23~~39. 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 single-chain chimeric polypeptides of claims 1-~~18~~ 34 or the composition of claim ~~19~~ 35 or ~~20~~36.

~~24~~40. 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 single-chain chimeric polypeptides of claims 1-~~18~~ 34 or the composition of claim ~~19~~ 35 or ~~20~~36.

~~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~~41. The method of any one of claims ~~22-26~~38-40, 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.

2842. The method of any one of claims 22-2738-41, 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.

3043. 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 anyUse of the single-chain chimeric polypeptides of any one of claims 1-18 34 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 diseaseor the composition of claim 19 or 20.

3144. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any ofUse of the single-chain chimeric polypeptides of any one of claims 1-18 34 in the manufacture of a medicament for treating cancer, an aging-related disease or condition, or an infectious diseaseor 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.

3345. The method use of claim 3243 or 44, 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~~46. The ~~method~~ use of claim ~~32~~43 or 44, 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~~47. The ~~method~~ use of claim ~~32~~43 or 44, 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~~48. Nucleic acid encoding any of the single-chain chimeric polypeptides of any one of claims 1-~~18~~34.

~~37~~49. A vector comprising the nucleic acid of claim ~~36~~48.

~~38~~50. A cell comprising the nucleic acid of claim ~~36~~ 48 or the vector of claim ~~37~~49.

~~39~~51. A method of producing a single-chain chimeric polypeptide, the method comprising:

culturing the cell of claim ~~38~~ 50 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.~~