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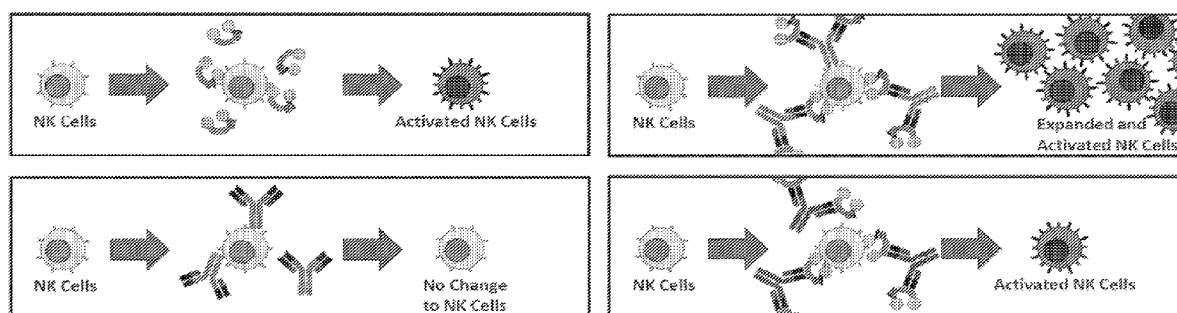


Figure 28

(57) Abstract: The present disclosure relates to methods that include the use of a first multi-chain chimeric polypeptide and a second multi-chain chimeric polypeptide for stimulating the NK cells, inducing or increasing proliferation of the NK cells, inducing differentiation of the NK cells, and treating a subject in need thereof using activated NK cells.



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MULTI-CHAIN CHIMERIC POLYPEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims priority to U.S. Provisional Patent Application Serial No. 62/864,996, filed on June 21, 2019; the entire contents of which are herein incorporated by reference.

TECHNICAL FIELD

10 The present disclosure relates to the field of biotechnology, and more specifically, to the use of a combination of two multi-chain chimeric polypeptides, each having a linker domain positioned between two target-binding domains.

BACKGROUND

15 Adoptive immunotherapy or cellular therapy requires the culture of immune cells obtained from a subject *in vivo* (and optionally genetic manipulation of the immune cells to express a T-cell or a chimeric antigen receptor) before administration back into the subject. A sufficient number of immune cells is necessary in order to provide a therapeutic effect in the subject. In many examples, immune cells obtained from a subject need to be cultured for three or more weeks before a therapeutically effective number of immune cells can be obtained. In addition, many methods of
20 culturing immune cells obtained from a subject *in vitro* require a layer of feeder cells, which requires subsequent purification or isolation of the immune cells before administration back to the subject.

SUMMARY

25 The present invention is based on the discovery that a specific two-step method of contacting NK cells results in a significant expansion of NK cells *in vitro* and the expanded NK cells are highly activated with a long persistence and a high degree of cytotoxicity against diseased cells in a recipient host.

30 In one aspect, provided herein is a method of promoting the activation and proliferation of a natural killer cell, the method includes: (a) contacting a natural killer cell in a liquid culture medium that includes an effective amount of a first multi-chain chimeric polypeptide for a first period of time under conditions that allow for the

differentiation of the natural killer cell; and (b) contacting the natural killer cell in a liquid culture medium that includes an effective amount of (i) a second multi-chain chimeric polypeptide, and (ii) an IgG1 antibody construct, for a second period of time under conditions that allow for the activation and proliferation of the natural killer cell, wherein the first multi-chain chimeric polypeptide includes: a first chimeric polypeptide that includes a first target-binding domain, a soluble tissue factor domain, and a first domain of a pair of affinity domains, and a second chimeric polypeptide that includes a second domain of a pair of affinity domains and a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide in the first multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the first multi-chain chimeric polypeptide, and the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12; and wherein the second multi-chain chimeric polypeptide includes: a first chimeric polypeptide that includes a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and a second chimeric polypeptide that includes a second domain of a pair of affinity domains, and a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide in the second multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the second multi-chain chimeric polypeptide; the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide each independently bind specifically to a receptor for IL-7 or a receptor for IL-21, and the IgG1 antibody construct includes at least one antigen-binding domain that binds specifically to the linker domain.

In some embodiments, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide in the first multi-chain chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some embodiments, the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric

polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments, the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide in the first multi-chain chimeric polypeptide further includes a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide. In some embodiments, the soluble tissue factor domain is a soluble human tissue factor domain. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain includes SEQ ID NO: 10. In some embodiments, 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 embodiments, 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 embodiments, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some embodiments, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments, the first multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some embodiments, the first chimeric polypeptide and/or the second chimeric polypeptide in the first multi-chain chimeric polypeptide further includes a signal sequence at its N-terminal end. In some embodiments, the signal sequence includes SEQ ID NO: 28. In some embodiments, the signal sequence is SEQ ID NO: 28. In some embodiments, the pair of affinity domains in the first multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. In some embodiments, the soluble IL-15 has a D8N or D8A amino acid substitution. In some embodiments, the soluble IL-15 includes a sequence that is 80% identical to SEQ ID NO: 26. In some embodiments,

the soluble IL-15 includes a sequence that is 90% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 includes a sequence that is 95% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 includes SEQ ID NO: 26. In some embodiments, the sushi domain of IL15R α includes a sushi domain from human IL15R α . In some embodiments, the sushi domain from human IL15R α includes a sequence that is 80% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α includes a sequence that is 90% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α includes a sequence that is 95% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α includes SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α is a mature full-length IL15R α .

In some embodiments, the pair of affinity domains in the first multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some embodiments, the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide are each independently a soluble IL-15 or a soluble IL-18. In some embodiments, the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18. In some embodiments, the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12. In some embodiments, the first target-binding domain in the first multi-chain chimeric polypeptide includes a soluble IL-18. In some embodiments, the soluble IL-18 is a soluble human IL-18. In some embodiments, the soluble human IL-18 includes a sequence at least 80% identical to SEQ ID NO: 20. In some embodiments, the soluble human IL-18 includes a sequence at least 90% identical to SEQ ID NO: 20. In some embodiments, the soluble human IL-18 includes a sequence at least 95% identical to SEQ ID NO: 20. In some embodiments, the soluble human IL-18 includes a sequence of SEQ ID NO: 20. In some embodiments, the second target-binding domain in the first multi-chain chimeric

polypeptide includes a soluble IL-12. In some embodiments, the soluble IL-12 is a soluble human IL-12. In some embodiments, the soluble human IL-12 includes a sequence of soluble human IL-12 β (p40) and a sequence of soluble human IL-12 α (p35). In some embodiments, the soluble human IL-15 further includes a linker

5 sequence between the sequence of soluble IL-12 β (p40) and the sequence of soluble human IL-12 α (p35). In some embodiments, the linker sequence includes SEQ ID NO: 11. In some embodiments, the sequence of soluble human IL-12 β (p40) includes a sequence that is at least 80% identical to SEQ ID NO: 14. In some

10 embodiments, the sequence of soluble human IL-12 β (p40) includes a sequence that is at least 90% identical to SEQ ID NO: 14. In some embodiments, the sequence of soluble human IL-12 β (p40) includes a sequence that is at least 95% identical to SEQ ID NO: 14. In some embodiments, the sequence of soluble human IL-12 α (p35) includes a sequence that is at least 80% identical to SEQ ID NO: 16. In

15 some embodiments, the sequence of soluble human IL-12 α (p35) includes a sequence that is at least 90% identical to SEQ ID NO: 16. In some embodiments, the sequence of soluble human IL-12 α (p35) includes a sequence that is at least 95% identical to SEQ ID NO: 16. In some embodiments, the sequence of soluble human IL-12 α (p35) includes SEQ ID NO: 16. In some embodiments, the first chimeric polypeptide in the

20 first multi-chain chimeric polypeptide includes a sequence that is at least 80% identical to

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKD
 SQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGH
 DNKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDSGTTN

25 TVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTEC
 DLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQ
 PTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLYTLYYWKSSSS
 GKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQKEG
 EFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQV

30 ISLESGDASIHTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFV
 HIVQMFINTS (SEQ ID NO: 63). In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 63. In some embodiments, the first chimeric

polypeptide in the first multi-chain chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO: 63. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide includes SEQ ID NO: 63. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide includes

MKWVTFISLLFLFSSAYSFYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD
SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPD
NIKDTKSDIIFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDEL
GDRSIMFTVQNEDESGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQIS
TKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGE
PLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLR
DVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESD
VHPCKVTAMKCFLELQVISLESGLASIHDTVENLIILANNSLSSNGNVTESG

CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 64). In some embodiments, the second chimeric polypeptide includes a sequence that is at least 80% identical to

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK
TLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE
RVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIR
DIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKR
EKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCSGGGGSGG
GGSGGGGSRNLPVATPDPGMFPCLLHHSQNLLRAVSNMLQKARQTLEFYPTCS
EEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALN
FNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPM
SVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTP
SLKCIR (SEQ ID NO: 65). In some embodiments, the second chimeric polypeptide

includes a sequence that is at least 90% identical to SEQ ID NO: 65. In some embodiments, the second chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO: 65. In some embodiments, the second chimeric polypeptide includes SEQ ID NO: 65. In some embodiments, the second chimeric

polypeptide includes

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEED
 GITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHLLLLHKKE
 DGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRG
 5 SSDPQGVTCGAATLSAERVGRDNKEYEYSVEQCEDSACPAAEESLPIEVMVD
 AVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPH
 SYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS
 WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLLHHSQNLLRAV
 SNMLQKARQTLEFYPTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRET
 10 SFITNGSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFL
 DQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTI
 DRVMSYLNASITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLT
 ECVLNKATNVAHWTPSLKCIR (SEQ ID NO: 66). In some embodiments, the
 first target-binding domain and the linker domain in the first chimeric polypeptide in
 15 the second multi-chain chimeric polypeptide directly abut each other in the first
 chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the
 second multi-chain chimeric polypeptide further includes a linker sequence between
 the first target-binding domain and the linker domain in the first chimeric polypeptide.
 In some embodiments, the linker domain and the first domain of the pair of affinity
 20 domains directly abut each other in the first chimeric polypeptide in the second multi-
 chain chimeric polypeptide. In some embodiments, the first chimeric polypeptide in
 the second multi-chain chimeric polypeptide further includes a linker sequence
 between the linker domain and the first domain of the pair of affinity domains in the
 first chimeric polypeptide. In some embodiments, the second domain of the pair of
 25 affinity domains and the second target-binding domain in the second chimeric
 polypeptide of the second multi-chain chimeric polypeptide directly abut each other in
 the second chimeric polypeptide. In some embodiments, the second chimeric
 polypeptide in the second multi-chain chimeric polypeptide further includes a linker
 sequence between the second domain of the pair of affinity domains and the second
 30 target-binding domain in the second chimeric polypeptide. In some embodiments, the
 linker domain is a soluble tissue factor domain. In some embodiments, the soluble
 tissue factor domain is a soluble human tissue factor domain. In some embodiments,
 the soluble human tissue factor domain includes a sequence that is at least 80%

identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain includes SEQ ID NO: 10.

In some embodiments, 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 embodiments, 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 embodiments, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some embodiments, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments, the second multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some embodiments, the first chimeric polypeptide and/or the second chimeric polypeptide in the second multi-chain chimeric polypeptide further includes a signal sequence at its N-terminal end. In some embodiments, the signal sequence includes SEQ ID NO: 28. In some embodiments, the signal sequence is SEQ ID NO: 28. In some embodiments, the pair of affinity domains in the second multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. In some embodiments, the soluble IL-15 has a D8N or D8A amino acid substitution. In some embodiments, the soluble IL-15 includes a sequence that is 80% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 includes a sequence that is 90% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 includes a sequence that is 95% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 includes SEQ ID NO: 26. In some embodiments, the sushi domain of IL15R α includes a sushi domain from human IL15R α . In some embodiments, the sushi domain from human IL15R α includes a sequence that is 80% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α includes a sequence that is 90% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α includes a sequence that is 95% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α includes SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α is a mature full-length IL15R α . In some embodiments, the pair of affinity domains in the second multi-chain chimeric

polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some embodiments, the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide are each independently a soluble IL-7 or a soluble IL-21. In some embodiments, the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7. In some embodiments, the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21. In some embodiments, the first target-binding domain in the second multi-chain chimeric polypeptide includes a soluble IL-21. In some embodiments, the soluble IL-21 is a soluble human IL-21. In some embodiments, the soluble human IL-21 includes a sequence at least 80% identical to SEQ ID NO: 22. In some embodiments, the soluble human IL-21 includes a sequence at least 90% identical to SEQ ID NO: 22. In some embodiments, the soluble human IL-21 includes a sequence at least 95% identical to SEQ ID NO: 22. In some embodiments, the soluble human IL-21 includes a sequence of SEQ ID NO: 22. In some embodiments, the second target-binding domain includes a soluble IL-7. In some embodiments, the soluble IL-7 is a soluble human IL-7. In some embodiments, the soluble human IL-7 includes a sequence at least 80% identical to SEQ ID NO: 23. In some embodiments, the soluble human IL-7 includes a sequence at least 90% identical to SEQ ID NO: 23. In some embodiments, the soluble human IL-7 includes a sequence at least 95% identical to SEQ ID NO: 23. In some embodiments, the soluble human IL-7 includes a sequence of SEQ ID NO: 23.

In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 80% identical to

DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTT
NTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTE

CDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
 QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSS
 SGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEK
 GEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ
 5 VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
 VHIVQMFINTS (SEQ ID NO: 67). In some embodiments, the first chimeric
 polypeptide in the second multi-chain chimeric polypeptide includes a sequence that
 is at least 90% identical to SEQ ID NO: 67. In some embodiments, the first chimeric
 polypeptide in the second multi-chain chimeric polypeptide includes a sequence that
 10 is at least 95% identical to SEQ ID NO: 67. In some embodiments, the first chimeric
 polypeptide in the second multi-chain chimeric polypeptide includes SEQ ID NO: 67.
 In some embodiments, the first chimeric polypeptide in the second multi-chain
 chimeric polypeptide includes
 MKWVTFISLLFLFSSAYSDCDIEGKDQKQYESVLMVSIQQLDSMKEIGSNCL
 15 NNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTT
 ILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKT
 CWNKILMGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQIST
 KSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEP
 LYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRD
 20 VFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTV
 NRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDV
 HPSCCKVTAMKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC
 KECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 68). In some
 embodiments, the second chimeric polypeptide in the second multi-chain chimeric
 25 polypeptide includes a sequence that is at least 80% identical to
 QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
 AQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
 EFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLSYRER
 YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 69). In
 30 some embodiments, the second chimeric polypeptide in the second multi-chain
 chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO:
 69. In some embodiments, the second chimeric polypeptide in the second multi-chain
 chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO:

69. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide includes SEQ ID NO: 69. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide includes

5 MKWVTFISLLFLFSSAYSQGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAP
EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQK
HRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPMSV
EHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNV AHWTTPSL
KCIR (SEQ ID NO: 70). In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 80%

10 identical to

QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
AQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDSGGTTNTVAAYNLTWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFS
15 YPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVDEDE
RTLVRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKG
ENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLI
QSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:

20 71). In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 90% identical to SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes a sequence that is at least 95% identical to SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain

25 chimeric polypeptide includes SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide includes

MGVKVLFALICIAVAEAAQGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAP
EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQK
HRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSGGTTNTVA
30 AYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLT
DEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTI
QSFEQVGTKVNVTVEDERTLVRNNTFLSLRDVFGKDLIYTLYYWKSSSSGK
KTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEF

RENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVIS
 LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHI
 VQMFINTS (SEQ ID NO: 72). In some embodiments, the second chimeric

polypeptide in the second multi-chain chimeric polypeptide includes a sequence that
 5 is at least 80% identical to

DCDIEGKDGKQYESVLMVSIQQLLDSTMKEIGSNCLNNEFNFFKRHICDANKE
 GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
 GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHITCPP
 PMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWT

10 TPSLKCIR (SEQ ID NO: 73). In some embodiments, the second chimeric
 polypeptide in the second multi-chain chimeric polypeptide includes a sequence that
 is at least 90% identical to SEQ ID NO: 73. In some embodiments, the second
 chimeric polypeptide in the second multi-chain chimeric polypeptide includes a
 sequence that is at least 95% identical to SEQ ID NO: 73. In some embodiments, the
 15 second chimeric polypeptide in the second multi-chain chimeric polypeptide includes
 SEQ ID NO: 73. In some embodiments, the second chimeric polypeptide in the
 second multi-chain chimeric polypeptide includes

MGVKVLFALICIAVAEADCDIEGKDGKQYESVLMVSIQQLLDSTMKEIGSNCL
 NNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTT
 20 ILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKT
 CWNKILMGTKEHITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSS
 LTECVLNKATNVAHWTTTPSLKCIR (SEQ ID NO: 74). In some embodiments, the

IgG1 antibody construct is a monoclonal IgG1 antibody, where both antigen-binding
 domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In
 25 some embodiments, the IgG1 antibody construct is a bispecific IgG1 antibody, where
 one of the two antigen-binding domains in the bispecific IgG1 antibody binds
 specifically to the linker domain. In some embodiments, the first period of time is
 about 15 minutes to about 4 hours. In some embodiments, the first period of time is
 about 30 minutes to about 3.5 hours. In some embodiments, the first period of time is
 30 about 1 hour to about 3.5 hours. In some embodiments, the first period of time is
 about 2 hours to about 3.5 hours. In some embodiments, the liquid culture medium in
 step (a) and/or step (b) is a serum-containing liquid culture medium.

In some embodiments, the NK cells are in the liquid culture medium in step (a) at a density of about 4.0×10^7 cells/mL to about 1.5×10^8 cells/mL. In some embodiments, the NK cells are in the liquid culture medium in step (a) at a density of about 5.0×10^7 cells/mL to about 2.0×10^8 cells/mL. In some embodiments, the second period of time is about 1 day to about 30 days. In some embodiments, the second period of time is about 7 days to about 30 days. In some embodiments, the second period of time is about 14 days to about 21 days. In some embodiments, the liquid culture medium in step (b) includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:1 to about 2:1. In some embodiments, the liquid culture medium in step (b) includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.8:1 to about 1.2:1. In some embodiments, the NK cells are in the liquid culture medium in step (b) are maintained at a density of about 0.5×10^6 cells/mL to about 2.0×10^6 cells/mL. In some embodiments, the NK cells are in the liquid culture medium in step (b) are maintained at a density of about 1.0×10^6 cells/mL to about 2.0×10^6 cells/mL. In some embodiments, the NK cell was previously obtained from a subject. In some embodiments, the NK cell was previously obtained from umbilical cord blood or induced pluripotent stem cells. In some embodiments, the method further includes obtaining the NK cell from the subject prior to step (a). In some embodiments, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. In some embodiments, the method further includes, between step (a) and step (b), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. In some embodiments, the method further includes, prior to step (a), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. In some embodiments, the method further includes, during step (b), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. In some embodiments, the method further includes, after step (b), isolating the NK cell. In some embodiments, after step (b), the NK cell has an increased level of expression or secretion of one or more proteins selected from the group consisting of: TNF- α , IFN- γ , granzyme A, granzyme B, perforin, 2B4, CD8, CD11a, CD16, CD25, CD27, CD48, CD49d, CD54, CD56, CD58, CD62L, CD69, CD70, CD94, CD137, CD158a, CD158b, CD158e, CD178,

CD226, CD253, NKG2C, NKG2D, LIR-1, LILR-B1, KIR2DL1, KIR3DL1, KIR2DL2, KIR2DL3, CXCR3, NKp30, NKp44, NKp46, NKG2D, DNAM-1, TRAIL, FasL, CXCR3, CXCR4, LTB, MX1, BAX, TNF- α , and IFN- γ as compared to the level of expression or secretion of the one or more proteins prior to step (a). In

5 some embodiments, the method further includes, after the contacting step, administering the NK cell to a subject in need thereof. In some embodiments, the subject has been identified or diagnosed as having an age-related disease or condition. In some embodiments, the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis,

10 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

15 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 embodiments, the subject has been identified or diagnosed as having a cancer. In

20 some embodiments, 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),

25 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

30 carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodiments, the subject has been diagnosed or identified as having an infectious disease. In some embodiments, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus,

rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Also provided herein is an activated NK cell produced by the method of any of the above embodiments. Also provided is a pharmaceutical composition comprising the activated NK cell of the above embodiment. Also provided is a kit that includes a pharmaceutical composition that includes the activated NK cell of any of the above
5 embodiments. In another aspect, 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 includes administering to the subject a therapeutically effective amount of the activated NK
10 cell of any of the above embodiments or the pharmaceutical composition of any of the above embodiments. In some embodiments, the subject has been identified or diagnosed as having a cancer. In some embodiments, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma,
15 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
20 carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodiments, the subject has been identified or diagnosed as having an aging-related disease or condition. In some embodiments, the
25 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
30 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 embodiments, the subject has been diagnosed or identified as having an infectious disease. In some embodiments, the infectious disease is infection with human
5 immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

In some embodiments, provided herein is a method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically
10 effective amount of the activated NK cell or the activated T cell of any of the above embodiments or the pharmaceutical composition of any of the above embodiment. In some embodiments, the subject has been identified or diagnosed as having a cancer. In some embodiments, the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma,
15 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,
20 stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodiments, the subject has been identified or diagnosed as
25 having an aging-related disease or condition. In some embodiments, 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,
30 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 embodiments, the subject has been diagnosed or identified as having an infectious disease. In some embodiments, 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 another aspect, provided herein is a kit comprising: (a) a first multi-chain chimeric polypeptide comprises: a first chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a first domain of a pair of affinity domains, and a second chimeric polypeptide comprising a second domain of a pair of affinity domains and a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide in the first multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the first multi-chain chimeric polypeptide, and the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12; a second multi-chain chimeric polypeptide comprises: a first chimeric polypeptide comprising a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and a second chimeric polypeptide comprising a second domain of a pair of affinity domains, and a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide in the second multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the second multi-chain chimeric polypeptide; and the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide each independently bind specifically to a receptor for IL-7 or a receptor for IL-21; and (c) an IgG1 antibody that comprises at least one antigen-binding domain that binds specifically to the linker domain. In some embodiments, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide in the first multi-chain chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric

polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some embodiments, the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments, the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide. In some embodiments, the soluble tissue factor domain is a soluble human tissue factor domain. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 10. In some embodiments, the soluble human

tissue factor domain comprises SEQ ID NO: 10. In some embodiments, 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 embodiments, 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 embodiments, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some embodiments, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments, the first multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some embodiments, the first chimeric polypeptide and/or the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end. In some embodiments, the signal sequence comprises SEQ ID NO: 28. In some embodiments, the signal sequence is SEQ ID NO: 28. In some embodiments, the pair of affinity

domains in the first multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. In some embodiments, the soluble IL-15 has a D8N or D8A amino acid substitution. In some embodiments, the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 comprises SEQ ID NO: 26. In some embodiments, the sushi domain of IL15R α comprises a sushi domain from human IL15R α . In some embodiments, the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α is a mature full-length IL15R α . In some embodiments, the pair of affinity domains in the first multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some embodiments, the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide are each independently a soluble IL-15 or a soluble IL-18. In some embodiments, the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18. In some embodiments, the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12. In some embodiments, the first target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-18. In some embodiments, the soluble IL-18 is a soluble human IL-18. In some embodiments, the soluble human IL-18 comprises a sequence at least 80% identical to SEQ ID NO: 20. In some embodiments, the soluble human IL-18

comprises a sequence at least 90% identical to SEQ ID NO: 20. In some embodiments, the soluble human IL-18 comprises a sequence at least 95% identical to SEQ ID NO: 20. In some embodiments, the soluble human IL-18 comprises a sequence of SEQ ID NO: 20. In some embodiments, the second target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-12. In some embodiments, the soluble IL-12 is a soluble human IL-12. In some embodiments, the soluble human IL-12 comprises a sequence of soluble human IL-12 β (p40) and a sequence of soluble human IL-12 α (p35). In some embodiments, the soluble human IL-15 further comprises a linker sequence between the sequence of soluble IL-12 β (p40) and the sequence of soluble human IL-12 α (p35). In some embodiments, the linker sequence comprises SEQ ID NO: 11. In some embodiments, the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 80% identical to SEQ ID NO: 14. In some embodiments, the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 90% identical to SEQ ID NO: 14. In some embodiments, the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 95% identical to SEQ ID NO: 14. In some embodiments, the sequence of soluble human IL-12 β (p40) comprises SEQ ID NO: 14. In some embodiments, the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 80% identical to SEQ ID NO: 16. In some embodiments, the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 90% identical to SEQ ID NO: 16. In some embodiments, the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 95% identical to SEQ ID NO: 16. In some embodiments, the sequence of soluble human IL-12 α (p35) comprises SEQ ID NO: 16. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 63. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 63. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 63. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 63. In some embodiments, the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 64. In some embodiments, the second chimeric polypeptide comprises a

sequence that is at least 80% identical to SEQ ID NO: 65. In some embodiments, the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 65. In some embodiments, the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 65. In some embodiments, the second chimeric polypeptide comprises SEQ ID NO: 65. In some embodiments, the second chimeric polypeptide comprises SEQ ID NO: 66. In some embodiments, the first target-binding domain and the linker domain in the first chimeric polypeptide in the second multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the linker domain in the first chimeric polypeptide. In some embodiments, the linker domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide in the second multi-chain chimeric polypeptide. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the linker domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments, the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide of the second multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide. In some embodiments, the linker domain is a soluble tissue factor domain. In some embodiments, the soluble tissue factor domain is a soluble human tissue factor domain. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises SEQ ID NO: 5. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain

comprises a sequence that is at least 90% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises SEQ ID NO: 9. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10. In some embodiments, the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3. In some embodiments, the soluble human tissue factor domain comprises SEQ ID NO: 10. In some embodiments, 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 embodiments, 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 embodiments, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some embodiments, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments, the second multi-chain chimeric polypeptide does not stimulate coagulation in a mammal. In some embodiments, the first chimeric polypeptide and/or the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end. In some embodiments, the signal sequence comprises SEQ ID NO: 28. In some embodiments, the signal sequence is SEQ ID NO: 28. In some embodiments, the pair of affinity domains in the second multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. In some embodiments, the soluble IL-15 has a D8N or D8A amino acid substitution. In some embodiments, the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26. In some embodiments, the soluble IL-15 comprises SEQ ID NO: 26. In some embodiments, the sushi domain of IL15R α comprises a sushi domain from human IL15R α . In some embodiments, the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α comprises SEQ ID NO: 24. In some embodiments, the sushi domain from human IL15R α is a mature full-length IL15R α . In some embodiments, the pair of affinity domains in the second multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some embodiments, the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide are each independently a soluble IL-7 or a soluble IL-21. In some embodiments, the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21, and the

second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7. In some embodiments, the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21. In some embodiments, the first target-binding domain in the second multi-chain chimeric polypeptide comprises a soluble IL-21. In some embodiments, the soluble IL-21 is a soluble human IL-21. In some embodiments, the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 22. In some embodiments, the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 22. In some embodiments, the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 22. In some embodiments, the soluble human IL-21 comprises a sequence of SEQ ID NO: 22. In some embodiments, the second target-binding domain comprises a soluble IL-7. 323. In some embodiments, the soluble IL-7 is a soluble human IL-7. In some embodiments, the soluble human IL-7 comprises a sequence at least 80% identical to SEQ ID NO: 23. In some embodiments, the soluble human IL-7 comprises a sequence at least 90% identical to SEQ ID NO: 23. In some embodiments, the soluble human IL-7 comprises a sequence at least 95% identical to SEQ ID NO: 23. In some embodiments, the soluble human IL-7 comprises a sequence of SEQ ID NO: 23. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 67. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 67. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 67. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 67. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 68. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 69. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ

ID NO: 69. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 69. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 69. In some
5 embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 70. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that
10 is at least 90% identical to SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 71. In some embodiments, the first chimeric polypeptide in the second multi-chain
15 chimeric polypeptide comprises SEQ ID NO: 72. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 73. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some
20 embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 73. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 73. In some embodiments, the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ
25 ID NO: 74. In some embodiments, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some embodiments, the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

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

A “multi-chain chimeric polypeptide” means a complex of two or more chimeric polypeptides.

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
10 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 embodiments, the antigen-binding domain can be an antibody or a fragment thereof. In some embodiments, an
15 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
20 90% identity, at least 95% identity, at least 99% identity, or 100% identical) to a segment of a wildtype mammalian tissue factor protein (e.g., a wildtype human tissue factor protein) that lacks the transmembrane domain and the intracellular domain. Non-limiting examples of soluble tissue factor domains are described herein.

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

The term “soluble cytokine protein” is used herein to refer to a mature and secreted cytokine protein or a biologically active fragment thereof. In some

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

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

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

The term “ligand of a co-stimulatory molecule” is used herein in the broadest sense to refer to a polypeptide that is capable of binding and activating a co-stimulatory receptor molecule on an immune cell (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a ligand of a co-stimulatory molecule can include a sequence that is at least 70%

identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to a ligand of a co-stimulatory molecule that retains its ability to specifically bind to one or more of its natural receptors. Non-limiting examples of ligands of co-stimulatory molecules are described herein.

The term “antibody” is used herein in its broadest sense and includes certain types of immunoglobulin molecules that include one or more antigen-binding domains that specifically bind to an antigen or epitope. An antibody specifically includes, e.g., intact antibodies (e.g., intact immunoglobulins), antibody fragments, and multi-specific antibodies. One example of an antigen-binding domain is an antigen-binding domain formed by a VH -VL dimer. Additional examples of an antibody are described herein. Additional examples of an antibody are known in the art.

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

The term “pair of affinity domains” is two different protein domain(s) that bind specifically to each other with a K_D of less than of less than 1×10^{-7} M (e.g., less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, or less than 1×10^{-11} M). In some examples, a pair of affinity domains can be a pair of naturally-occurring proteins. In some embodiments, 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
5 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
10 death of a pathogenic cell (e.g., a cancer cell) in the mammal. Non-limiting examples of immune effector cells include macrophages, natural killer cells, T-lymphocytes (e.g., cytotoxic T-lymphocytes and T-helper 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.
15 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
20 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 invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also
25 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.

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

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows a schematic diagram of an exemplary IL-12/IL-15R α Su DNA construct.

Figure 2 shows a schematic diagram of an exemplary IL-18/TF/IL-15 DNA
5 construct.

Figure 3 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15R α Su and IL-18/TF/IL-15 DNA constructs.

Figure 4 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15R α Su and IL-18/TF/IL-15 fusion proteins resulting in IL-18/TF/IL-15:IL-
10 12/IL-15R α Su complex (18t15-12s).

Figure 5 shows a schematic diagram of an exemplary IL-7/IL-15R α Su DNA construct.

Figure 6 shows a schematic diagram of an exemplary IL-21/TF/IL-15 DNA construct.

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

Figure 8 shows a schematic diagram of the interaction between the exemplary IL-7/IL-15R α Su and IL-21/TF/IL-15 fusion proteins resulting in an IL-21/TF/IL-
15:IL-7/IL-15R α Su complex (21t15-7s).

Figure 9 shows a schematic diagram of an exemplary IL-21/IL-15R α Su DNA
20 construct.

Figure 10 shows a schematic diagram of an exemplary IL-7/TF/IL-15 DNA construct.

Figure 11 shows a schematic diagram of the interaction between the
25 exemplary IL-21/IL-15R α Su and IL-7/TF/IL-15 DNA constructs.

Figure 12 shows a schematic diagram of the interaction between the exemplary IL-21/IL-15R α Su and IL-7/TF/IL-15 fusion proteins resulting in an IL-
7/TF/IL-15:IL-21/IL-15R α SU complex (7t15-21s).

Figure 13 shows a graph showing the fold-increase in STAT4 and STAT5
30 expression following in unstimulated NK cells (US) or NK cells contacted with a combination of an exemplary second multi-chain chimeric polypeptide and an exemplary IgG1 antibody construct (7t15-21s:anti-TF Ab), an exemplary first multi-chain chimeric polypeptide (18t15-12s), a combination of recombinant IL-12 and IL-

18 (single cytokines), recombinant IL-12 (IL-12), or recombinant IL-18 (IL-18), for 5 minutes, 15 minutes, 30 minutes, 60 minutes, or overnight.

Figure 14 is graph showing the extracellular acidification rate (ECAR) in unstimulated NK cells and NK cells contacted with an exemplary first multi-chain chimeric polypeptide (18t15-12s) at 10 nM or 100 nM, or with a combination of recombinant cytokines (IL-12, IL-15, and IL-18) for 0 to 4 hours. The NK cells were obtained from three different donors.

Figure 15 is graph showing the extracellular acidification rate (ECAR) in unstimulated NK cells and NK cells contacted with an exemplary first multi-chain chimeric polypeptide (18t15-12s) or with a combination of recombinant cytokines (IL-12, IL-15, and IL-18) for 0 to 3 hours. The NK cells were obtained from three different donors. The NK cells were further contacted with an addition of glucose, oligomycin, and 2-deoxyglucose where indicated.

Figure 16 is a graph of the fold-change in gene expression of mTORC1, MYC and SREBP in unstimulated NK cells or NK cells contacted with a combination of recombinant cytokines (IL-12, IL-15, and IL-18), an exemplary first multi-chain chimeric polypeptide (18t15-12s), or a control multi-chain chimeric polypeptide (7t15-21s) for 1 hour or 3 hours.

Figure 17 is a graph showing the expression of exemplary activation markers, mTORC1, MYC, and SREBP, in NK cells that were unstimulated or contacted with a combination of recombinant cytokines (IL-12, IL-15, and IL-18) or 100 nM of an exemplary first multi-chain chimeric polypeptide (18t15-12s) for 30 minutes to 3 hours.

Figure 18 shows the percentage of CD69 positive and interferon-gamma positive NK cells following NK cell following no stimulation or stimulation with a mixture of recombinant cytokines (IL-12, IL-15, and IL-18) or an exemplary first multi-chain chimeric polypeptide (18t15-12s) for 3 hours or overnight.

Figure 19 shows the percentage of 41BB positive and IL-21 receptor α -positive NK cells following NK cell following no stimulation or stimulation with a mixture of recombinant cytokines (IL-12, IL-15 and IL-18) or an exemplary first multi-chain chimeric polypeptide (18t15-12s) for 3 hours or overnight.

Figure 20 is a graph showing the fold expansion of NK cells over time that received the following treatments: (i) received no initial stimulation and subsequently

contacted with a combination of an exemplary second multi-chain chimeric polypeptide (7t15-21s) and an exemplary IgG1 antibody construct (18t15-12s); (ii) initial stimulation with a combination of recombinant cytokines (IL-12, IL-15, and IL-18) and subsequently contacted with a combination of an exemplary second multi-chain chimeric polypeptide (7t15-21s) and an exemplary IgG1 antibody construct (18t15-12s); or (iii) initial stimulation with an exemplary first multi-chain chimeric polypeptide and subsequently contacted with a combination of an exemplary second multi-chain chimeric polypeptide (7t15-21s) and an exemplary IgG1 antibody construct (18t15-12s).

Figure 21 is a graph showing the percentage of CD107a positive cells that received the following treatments: (i) no initial stimulation and subsequent treatment with a combination of an exemplary second multi-chain chimeric polypeptide (7t15-21s) and an exemplary IgG1 antibody construct (α TF Ab); (ii) initial stimulation with a combination of cytokines (IL-12, IL-15, and IL-18) and subsequent treatment with a combination of an exemplary second multi-chain chimeric polypeptide (7t15-21s) and an exemplary IgG1 antibody construct (α TF Ab); (iii) initial stimulation with an exemplary first multi-chain chimeric polypeptide (18t15-12s) and subsequent treatment with combination of an exemplary second multi-chain chimeric polypeptide (7t15-21s) and an exemplary IgG1 antibody construct (α TF Ab); (iv) no initial stimulation and subsequent treatment with recombinant IL-15; (v) initial stimulation with a combination of cytokines (IL-12, IL-15 and IL-18) and subsequent treatment with recombinant IL-15; or (vi) initial stimulation with an exemplary first multi-chain chimeric polypeptide.

Figure 22 is a graph showing the oxygen consumption rate (OCR) analysis of stimulated human NK cells, where the OCR analysis consists of four stages: basal respiration (without drugs), ATP-linked respiration/proton leak (1.5 μ M oligomycin), maximal respiration (2 μ M FCCP), and spare respiration (0.5 μ M rotenone). The OCR graph is a combined representation of NK cells obtained three donors. The NK cells were stimulated as shown and as described in the corresponding Example.

Figure 23 is a graph showing IFN- γ mRNA and NKG2D mRNA levels after stimulation with 18t15-12s or a combination of individual cytokines, where cells were divided into a short-term activation (180 mins) or long-term activation (overnight stimulation for 12-16 hours) groups, or were not stimulated.

Figure 24 is a graph showing persistence of adoptively transferred NK cells (CD56⁺ cells) that were expanded with 7t15-21s- α TF Ab in an NSG mouse model, where prior to the expansion, the cells were either unstimulated with media as a control or stimulated (Kick) with 100 nM of 18t15-12s for either 180 minutes or for 12-16 hours (overnight).

Figure 25 is a graph showing epigenetic analysis of “Kick and Expansion” on DNA methylation of IFN γ CNS-1 CpG region, where cells were transferred in a 24 well flat bottom plate, and subjected to either: no treatment, or stimulation with a mixture of single cytokines or 18t15-12s for a short-term exposure of either 180 minutes or 12-16 hours (overnight). Then, the NK cells were washed and resuspended in complete RPMI 1640 medium supplemented with 7t15-21s plus the anti-tissue factor antibody (α TF Ab) and incubated for 14 days (long-term). DNA isolated from the cells was subjected to pyrosequencing analysis of DNA methylation of CpG sites located within the IFN γ CNS-1 region.

Figure 26 is a graph showing epigenetic analysis of memory-like NK cells *in vivo*, where cells were subjected to either: stimulation with mixture of single cytokines or 18t15-12s for 12-16 hours. Then, the NK cells were washed and resuspended in complete RPMI 1640 medium supplemented with 7t15-21s + anti-tissue factor antibody (α TF Ab), and incubated for 14 days. After 14 days of expansion, the activated NK cells were either processed for pyrosequencing analysis (pre-infusion samples) or infused in NSG mice (five groups, five mice per group). At day 9 post-infusion, the spleen of each mice was collected, and NK cells purified and combined for each group (five group, five mice per groups). The samples (post-infusion) were subjected to pyrosequencing analysis of DNA methylation of CpG sites located within the IFN γ CNS-1 region.

Figure 27 is a graph showing the fold expansion of NK cells on day 5 of expansion, where the data shows that NK cells require IgG1 signal for expansion.

Figure 28 is an exemplary embodiment of the presently claimed methods.

Figure 29 is a set of graphs showing expansion of primary NK cells using the methods described herein.

Figure 30 is an exemplary illustration showing the multi-chain chimeric polypeptides that can be used in the methods described herein.

Figure 31 is a schematic of an exemplary embodiment of the presently claimed methods.

Figure 32 is a schematic of an exemplary embodiment of the presently claimed methods.

5 Figure 33 is a set of graphs showing the percentage of surface markers CD25 (upper panel) and CD26 (lower panel) after day 10.

Figure 34 is a set of graphs showing the percentage of surface markers NKp44 (upper panel) and NKp30 (lower panel) after day 10.

10 Figure 35 is a set of graphs showing the percentage of surface markers CD62L (upper panel) and CD16 (lower panel) after day 10. The data show that activation with 18t15-12s and expansion with 7t15-21s and anti-tissue factor IgG1 antibody resulted in an increase in expression of CD62L.

Figure 36 is a graph showing the cytotoxicity in cells following no stimulation or stimulation with a mixture of recombinant cytokines (IL-12, IL-15, and IL-18) or
15 an exemplary first multi-chain chimeric polypeptide (18t15-12s).

Figure 37A is a set of histograms showing the expansion of cord blood-derived NK cells without treatment or following stimulation for 180 minutes or 12-16 hours with 18t15-12s (250 nM), then expanded using 7t15-21s (200 nM) with anti-tissue factor antibody (α TF Ab, 100 nM) for 7 days.

20 Figure 37B is a graph showing the percentage of cell proliferation of cord blood-derived NK cells without treatment or following stimulation for 180 minutes or 12-16 hours with 18t15-12s (250 nM), then expanded using 7t15-21s (200 nM) with anti-tissue factor antibody (α TF Ab, 100 nM) for 7 days.

DETAILED DESCRIPTION

25 Provided herein are a specific combination of two multi-chain chimeric polypeptides, uses of the same, and kits including the same. The multi-chain chimeric polypeptides include a linker domain positioned between two target-binding domains. In some embodiments, the linker domain is or includes a soluble tissue factor domain. In some embodiments, the linker domain can be recognized by a cognate IgG1
30 antibody (e.g., a monoclonal antibody). In some embodiments, a combination of two of the multi-chain chimeric polypeptides described herein can be used to stimulate a NK cell, induce or increase proliferation of a NK cell, and/or induce differentiation of a NK cell, and the NK cells can thereafter be used to treat a subject.

Provided herein are methods of promoting the activation and proliferation of a natural killer cell that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of a first multi-chain chimeric polypeptide for a first period of time under
5 conditions that allow for the differentiation of the natural killer cell, and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of (i) a second multi-chain chimeric polypeptide, and (ii) an IgG1 antibody construct that includes at least one antigen-binding domain that binds specifically to the linker domain (e.g., any of the exemplary IgG1 antibody constructs described
10 herein), for a second period of time, under conditions that allow for activation and proliferation of the NK cell. Also provided are NK cells produced by any of these methods, and methods of using these NK cells to treat a subject. Also provided herein are compositions that include any of the NK cells generated using these methods. Also provided are kits that include (a) a first multi-chain chimeric polypeptide that
15 includes a first and a second chimeric polypeptide (e.g., any of the exemplary first multi-chain chimeric polypeptides described herein), (b) a second multi-chain chimeric polypeptide that includes a first and a second chimeric polypeptide (e.g., any of the exemplary second multi-chain chimeric polypeptides described herein), and (c) an IgG1 antibody construct that includes at least one antigen-binding domain that
20 binds specifically to the linker domain (e.g., any of the IgG1 antibody constructs described herein). Exemplary embodiments of the methods provided herein are depicted in Figures 30-32.

Current *ex vivo* NK expansion methods may include feeder-cell-based expansion methods and cytokine-based expansion methods. Exemplary
25 disadvantages of feeder-cell-based expansion methods include, the development and maintenance of GMP-grade feeder cells and methods showing effective irradiation of the feeder cells, the final product must be depleted of all feeder-cell contaminants, including DNA, before administration to the patient, and the manufacturing process can be tedious and expensive. Exemplary disadvantages of cytokine-based expansion
30 methods include, suboptimal purity of final NK cell product, low final NK cell number compared to feeder-cell expansion, and the expense of recombinant cytokines and the lack of commercially available GMP-grade cytokines, except IL-2. The methods described herein provide advantages such as, generation of highly activated

NK cells from patients, large-scale expansion being comparable or similar to feeder-cell methods, cost effective expansion and activation with limited processing requirements, and the cells can be injected into subjects, thereby allowing expansion of cells *in vivo*.

5 In some embodiments, the methods described herein can induce the generation and proliferation of NK cells that are highly activated with a long persistence and a high degree of cytotoxicity against diseased cells in a recipient host (e.g., a cell having any of the exemplary aspects of a memory-like natural killer cell described herein). In some embodiments of any of the methods described herein, the contacting
10 step that includes contacting a NK cell with a first multi-chain chimeric polypeptide results in less than 50% cell death (e.g., apoptosis) (e.g., less than 48% cell death, less than 46% cell death, less than 44% cell death, less than 42% cell death, less than 40% cell death, less than 38% cell death, less than 36% cell death, less than 34% cell death, less than 32% cell death, less than 30% cell death, less than 28% cell death, less than
15 26% cell death, less than 24% cell death, less than 22% cell death, less than 20% cell death, less than 18% cell death, less than 16% cell death, less than 14% cell death, less than 12% cell death, less than 10% cell death, less than 8% cell death, less than 6% cell death, less than 4% cell death, or less than 2% cell death).

In some embodiments, any of the methods described herein can result in an
20 increase (e.g., about a 1% to about a 5,000% increase, about a 1% to about a 4,000% increase, about a 1% to about a 3,000% increase, about a 1% to about a 2,000% increase, about a 1% to about a 1,000% increase, about a 1% to about a 800% increase, about a 1% to about a 600% increase, about a 1% to about a 500% increase, about a 1% to about a 400% increase, about a 1% to about a 300% increase, about a
25 1% to about a 200% increase, about a 1% to about a 180% increase, about a 1% to about a 160% increase, about a 1% to about a 140% increase, about a 1% to about a 120% increase, about a 1% to about a 100% increase, about a 1% to about a 80% increase, about a 1% to about a 60% increase, about a 1% to about a 40% increase, about a 1% to about a 30% increase, about a 1% to about a 20% increase, about a 1%
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3,000% to about a 5,000% increase, about a 3,000% to about a 4,000% increase, or

about a 4,000% to about a 5,000% increase, in one or more of glycolysis, aerobic metabolism, oxidative phosphorylation, glucose consumption, and extracellular acidification rate (ECAR) as compared to the NK cell prior to performance of any of methods described herein (e.g., prior to contacting the NK cell with any of the
5 exemplary first multi-chain chimeric polypeptides described herein).

In some embodiments of any of the methods described herein, the method results in, e.g., about a 5-fold to about a 5,000-fold, about a 5-fold to about a 4,500-fold, about a 5-fold to about a 4,000-fold, about a 5-fold to about a 3,500-fold, about a
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4,000-fold, about a 100-fold to about a 3,500-fold, about a 100-fold to about a 3,000-fold, about a 100-fold to about a 2,500-fold, about a 100-fold to about a 2,000-fold, about a 100-fold to about a 1,500-fold, about a 100-fold to about a 1,000-fold, about a 100-fold to about a 800-fold, about a 100-fold to about a 600-fold, about a 100-fold to about a 500-fold, about a 100-fold to about 450-fold, about a 100-fold to about a 400-fold, about a 100-fold to about a 350-fold, about a 100-fold to about a 300-fold, about a 100-fold to about a 250-fold, about a 100-fold to about a 200-fold, about a 100-fold to about a 150-fold, about a 150-fold to about a 5,000-fold, about a 150-fold to about a 4,500-fold, about a 150-fold to about a 4,000-fold, about a 150-fold to about a 3,500-fold, about a 150-fold to about a 3,000-fold, about a 150-fold to about a 2,500-fold, about a 150-fold to about a 2,000-fold, about a 150-fold to about a 1,500-fold, about a 150-fold to about a 1,000-fold, about a 150-fold to about a 800-fold, about a 150-fold to about a 600-fold, about a 150-fold to about a 500-fold, about a 150-fold to about 450-fold, about a 150-fold to about a 400-fold, about a 150-fold to about a 350-fold, about a 150-fold to about a 300-fold, about a 150-fold to about a 250-fold, about a 150-fold to about a 200-fold, about a 200-fold to about a 5,000-fold, about a 200-fold to about a 4,500-fold, about a 200-fold to about a 4,000-fold, about a 200-fold to about a 3,500-fold, about a 200-fold to about a 3,000-fold, about a 200-fold to about a 2,500-fold, about a 200-fold to about a 2,000-fold, about a 200-fold to about a 1,500-fold, about a 200-fold to about a 1,000-fold, about a 200-fold to about a 800-fold, about a 200-fold to about a 600-fold, about a 200-fold to about a 500-fold, about a 200-fold to about 450-fold, about a 200-fold to about a 400-fold, about a 200-fold to about a 350-fold, about a 200-fold to about a 300-fold, about a 200-fold to about a 250-fold, about a 250-fold to about a 5,000-fold, about a 250-fold to about a 4,500-fold, about a 250-fold to about a 4,000-fold, about a 250-fold to about a 3,500-fold, about a 250-fold to about a 3,000-fold, about a 250-fold to about a 2,500-fold, about a 250-fold to about a 2,000-fold, about a 250-fold to about a 1,500-fold, about a 250-fold to about a 1,000-fold, about a 250-fold to about a 800-fold, about a 250-fold to about a 600-fold, about a 250-fold to about a 500-fold, about a 250-fold to about 450-fold, about a 250-fold to about a 400-fold, about a 250-fold to about a 350-fold, about a 250-fold to about a 300-fold, about a 300-fold to about a 5,000-fold, about a 300-fold to about a 4,500-fold, about a 300-fold to about a 4,000-fold, about a 300-fold to about a 3,500-fold, about a 300-fold to about a 3,000-fold, about a 300-fold to about a

2,500-fold, about a 300-fold to about a 2,000-fold, about a 300-fold to about a 1,500-fold, about a 300-fold to about a 1,000-fold, about a 300-fold to about a 800-fold, about a 300-fold to about a 600-fold, about a 300-fold to about a 500-fold, about a 300-fold to about 450-fold, about a 300-fold to about a 400-fold, about a 300-fold to
5 about a 350-fold, about a 350-fold to about a 5,000-fold, about a 350-fold to about a 4,500-fold, about a 350-fold to about a 4,000-fold, about a 350-fold to about a 3,500-fold, about a 350-fold to about a 3,000-fold, about a 350-fold to about a 2,500-fold, about a 350-fold to about a 2,000-fold, about a 350-fold to about a 1,500-fold, about a 350-fold to about a 1,000-fold, about a 350-fold to about a 800-fold, about a 350-fold
10 to about a 600-fold, about a 350-fold to about a 500-fold, about a 350-fold to about 450-fold, about a 350-fold to about a 400-fold, about a 400-fold to about a 5,000-fold, about a 400-fold to about a 4,500-fold, about a 400-fold to about a 4,000-fold, about a 400-fold to about a 3,500-fold, about a 400-fold to about a 3,000-fold, about a 400-fold to about a 2,500-fold, about a 400-fold to about a 2,000-fold, about a 400-fold to
15 about a 1,500-fold, about a 400-fold to about a 1,000-fold, about a 400-fold to about a 800-fold, about a 400-fold to about a 600-fold, about a 400-fold to about a 500-fold, about a 400-fold to about 450-fold, about a 450-fold to about a 5,000-fold, about a 450-fold to about a 4,500-fold, about a 450-fold to about a 4,000-fold, about a 450-fold to about a 3,500-fold, about a 450-fold to about a 3,000-fold, about a 450-fold to
20 about a 2,500-fold, about a 450-fold to about a 2,000-fold, about a 450-fold to about a 1,500-fold, about a 450-fold to about a 1,000-fold, about a 450-fold to about a 800-fold, about a 450-fold to about a 600-fold, about a 450-fold to about a 500-fold, about a 500-fold to about a 5,000-fold, about a 500-fold to about a 4,500-fold, about a 500-fold to about a 4,000-fold, about a 500-fold to about a 3,500-fold, about a 500-fold to
25 about a 3,000-fold, about a 500-fold to about a 2,500-fold, about a 500-fold to about a 2,000-fold, about a 500-fold to about a 1,500-fold, about a 500-fold to about a 1,000-fold, about a 500-fold to about a 800-fold, about a 500-fold to about a 600-fold, about a 600-fold to about a 5,000-fold, about a 600-fold to about a 4,500-fold, about a 600-fold to about a 4,000-fold, about a 600-fold to about a 3,500-fold, about a 600-fold to
30 about a 3,000-fold, about a 600-fold to about a 2,500-fold, about a 600-fold to about a 2,000-fold, about a 600-fold to about a 1,500-fold, about a 600-fold to about a 1,000-fold, about a 600-fold to about a 800-fold, about a 800-fold to about a 5,000-fold, about a 800-fold to about a 4,500-fold, about a 800-fold to about a 4,000-fold, about a

800-fold to about a 3,500-fold, about a 800-fold to about a 3,000-fold, about a 800-fold to about a 2,500-fold, about a 800-fold to about a 2,000-fold, about a 800-fold to about a 1,500-fold, about a 800-fold to about a 1,000-fold, about a 1,000-fold to about a 5,000-fold, about a 1,000-fold to about a 4,500-fold, about a 1,000-fold to about a 4,000-fold, about a 1,000-fold to about a 3,500-fold, about a 1,000-fold to about a 3,000-fold, about a 1,000-fold to about a 2,500-fold, about a 1,000-fold to about a 2,000-fold, about a 1,000-fold to about a 1,500-fold, about a 1,500-fold to about a 5,000-fold, about a 1,500-fold to about a 4,500-fold, about a 1,500-fold to about a 4,000-fold, about a 1,500-fold to about a 3,500-fold, about a 1,500-fold to about a 3,000-fold, about a 1,500-fold to about a 2,500-fold, about a 1,500-fold to about a 2,000-fold, about a 2,000-fold to about a 5,000-fold, about a 2,000-fold to about a 4,500-fold, about a 2,000-fold to about a 4,000-fold, about a 2,000-fold to about a 3,500-fold, about a 2,000-fold to about a 3,000-fold, about a 2,000-fold to about a 2,500-fold, about a 2,500-fold to about a 5,000-fold, about a 2,500-fold to about a 4,500-fold, about a 2,500-fold to about a 4,000-fold, about a 2,500-fold to about a 3,500-fold, about a 2,500-fold to about a 3,000-fold, about a 3,000-fold to about a 5,000-fold, about a 3,000-fold to about a 4,500-fold, about a 3,000-fold to about a 4,000-fold, about a 3,000-fold to about a 3,500-fold, about a 3,500-fold to about a 5,000-fold, about a 3,500-fold to about a 4,500-fold, about a 3,500-fold to about a 4,000-fold, about a 4,000-fold to about a 5,000-fold, about a 4,000-fold to about a 4,500-fold, or about a 4,500-fold to about a 5,000-fold, expansion in the number of viable NK cells as compared to the number of NK cells prior to the step of contacting the NK cells with any of the exemplary first multi-chain chimeric polypeptides described herein.

In some embodiments, the methods provide NK cells that have improved persistence and cytotoxicity against diseased cells in a subject as compared to untreated control NK cells.

Multi-Chain Chimeric Polypeptides

The methods described herein include the use of two different the multi-chain chimeric polypeptides.

The first multi-chain chimeric polypeptides provided herein include: (a) a first chimeric polypeptide including: a first target-binding domain; a soluble tissue factor

domain; and a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including: a second domain of a pair of affinity domains; and a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain
5 of the pair of affinity domains, and the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor of IL-18 or a receptor of IL-12.

The second multi-chain chimeric polypeptides provided herein include: (a) first chimeric polypeptide including: a first target-binding domain, a linker domain, a
10 first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including a second domain of a pair of affinity domains, and a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains, and the first target-binding domain and the second target-binding
15 domain each independently bind specifically to a receptor for IL-7 or a receptor for IL-21.

In some examples of any of the first and second multi-chain chimeric polypeptides described herein the total length of first chimeric polypeptide and/or the second chimeric polypeptide can each independently be about 50 amino acids to about
20 3000 amino acids, about 50 amino acids to about 2500 amino acids, about 50 amino acids to about 2000 amino acids, about 50 amino acids to about 1500 amino acids, about 50 amino acids to about 1000 amino acids, about 50 amino acids to about 950 amino acids, about 50 amino acids to about 900 amino acids, about 50 amino acids to about 850 amino acids, about 50 amino acids to about 800 amino acids, about 50
25 amino acids to about 750 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 480 amino acids, about 50 amino acids to about 460 amino acids, about 50 amino acids to about 440 amino
30 acids, about 50 amino acids to about 420 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 380 amino acids, about 50 amino acids to about 360 amino acids, about 50 amino acids to about 340 amino acids, about 50 amino acids to about 320 amino acids, about 50 amino acids to about 300 amino

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

1000 amino acids to about 2000 amino acids, about 1000 amino acids to about 1500 amino acids, about 1500 amino acids to about 3000 amino acids, about 1500 amino acids to about 2500 amino acids, about 1500 amino acids to about 2000 amino acids, about 2000 amino acids to about 3000 amino acids, about 2000 amino acids to about 2500 amino acids, or about 2500 amino acids to about 3000 amino acids. Diagrams of exemplary first multi-chain chimeric polypeptides provided herein are depicted in Figures 1-4. Diagrams of exemplary second multi-chain chimeric polypeptides are depicted in Figure 5-12.

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

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

In some embodiments of any of the first and second multi-chain chimeric polypeptides described herein, the second domain of the pair of affinity domains (e.g.,

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

Tissue Factor

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

The extracellular domain of tissue factor, composed of two fibronectin type III domains, is connected to the transmembrane domain through a six-amino acid linker. This linker provides conformational flexibility to decouple the tissue extracellular domain from its transmembrane and cytoplasmic domains. Each tissue factor fibronectin type III module is composed of two overlapping β sheets with the top sheet domain containing three antiparallel β -strands and the bottom sheet containing four β -strands. The β -strands are connected by β -loops between strand β A and β B, β C and β D, and β E and β F, all of which are conserved in conformation in the two modules. There are three short α -helix segments connecting the β -strands. A unique

feature of tissue is a 17-amino acid β -hairpin between strand β 10 and strand β 11, which is not a common element of the fibronectin superfamily. The N-terminal domain also contains a 12 amino acid loop between β 6F and β 7G that is not present in the C-terminal domain and is unique to tissue factor. Such a fibronectin type III domain structure is a feature of the immunoglobulin-like family of protein folds and is conserved among a wide variety of extracellular proteins.

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

FVII is a ~50 kDa, single-chain polypeptide consisting of 406 amino acid residues, with an N-terminal γ -carboxyglutamate-rich (GLA) domain, two epidermal growth factor-like domains (EGF1 and EGF2), and a C-terminal serine protease domain. FVII is activated to FVIIa by a specific proteolytic cleavage of the Ile¹⁵⁴-Arg¹⁵² bond in the short linker region between the EGF2 and the protease domain. This cleavage results in the light and heavy chains being held together by a single disulfide bond of Cys¹³⁵ and Cys²⁶². FVIIa binds phospholipid membrane in a Ca^{2+} -dependent manner through its N-terminal GLA-domain. Immediately C-terminal to the GLA domain is an aromatic stack and two EGF domains. The aromatic stack connects the GLA to EGF1 domain which binds a single Ca^{2+} ion. Occupancy of this Ca^{2+} -binding site increases FVIIa amidolytic activity and tissue factor association. The catalytic triad consist of His¹⁹³, Asp²⁴², and Ser³⁴⁴, and binding of a single Ca^{2+} ion within the FVIIa protease domain is critical for its catalytic activity. Proteolytic

activation of FVII to FVIIa frees the newly formed amino terminus at Ile¹⁵³ to fold back and be inserted into the activation pocket forming a salt bridge with the carboxylate of Asp³⁴³ to generate the oxyanion hole. Formation of this salt bridge is critical for FVIIa activity. However, oxyanion hole formation does not occur in free FVIIa upon proteolytic activation. As a result, FVIIa circulates in a zymogen-like state that is poorly recognized by plasma protease inhibitors, allowing it to circulate with a half-life of approximately 90 minutes.

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

Alanine scanning mutagenesis has been used to assess the role of specific amino acid side chains in the tissue factor extracellular domain for interaction with FVIIa (Gibbs et al., *Biochemistry* 33(47): 14003-14010, 1994; Schullek et al., *J Biol Chem* 269(30): 19399-19403, 1994). Alanine substitution identified a limited number of residue positions at which alanine replacements cause 5- to 10-fold lower affinity for FVIIa binding. Most of these residue side chains were found to be well-exposed to solvent in the crystal structure, concordant with macromolecular ligand interaction. The FVIIa ligand-binding site is located over an extensive region at the boundary between the two modules. In the C-module, residues Arg¹³⁵ and Phe¹⁴⁰ located on the protruding B-C loop provide an independent contact with FVIIa. Leu¹³³ is located at the base of the fingerlike structure and packed into the cleft between the two modules.

This provides continuity to a major cluster of important binding residues consisting of Lys²⁰, Thr⁶⁰, Asp⁵⁸, and Ile²². Thr⁶⁰ is only partially solvent-exposed and may play a local structural role rather than making a significant contact with ligand. The binding site extends onto the concave side of the intermodule angle involving Glu²⁴ and Gln¹¹⁰, and potentially the more distant residue Val²⁰⁷. The binding region extends from Asp⁵⁸ onto a convex surface area formed by Lys⁴⁸, Lys⁴⁶, Gln³⁷, Asp⁴⁴, and Trp⁴⁵. Trp⁴⁵ and Asp⁴⁴ do not interact independently with FVIIa, indicating that the mutational effect at the Trp⁴⁵ position may reflect a structural importance of this side chain for the local packing of the adjacent Asp⁴⁴ and Gln³⁷ side chain. The interactive area further includes two surface-exposed aromatic residues, Phe⁷⁶ and Tyr⁷⁸, which form part of the hydrophobic cluster in the N-module.

The known physiologic substrates of tissue factor-FVIIa are FVII, FIX, and FX and certain proteinase-activated receptors. Mutational analysis has identified a number of residues that, when mutated, support full FVIIa amidolytic activity towards small peptidyl substrates but are deficient in their ability to support macromolecular substrate (i.e., FVII, FIX, and FX) activation (Ruf et al., *J Biol Chem* 267(31): 22206-22210, 1992; Ruf et al., *J Biol Chem* 267(9): 6375-6381, 1992; Huang et al., *J Biol Chem* 271(36): 21752-21757, 1996; Kirchhofer et al., *Biochemistry* 39(25): 7380-7387, 2000). The tissue factor loop region at residues 159-165, and residues in or adjacent to this flexible loop have been shown to be critical for the proteolytic activity of the tissue factor-FVIIa complex. This defines the proposed substrate-binding exosite region of tissue factor that is quite distant from the FVIIa active site. A substitution of the glycine residue by a marginally bulkier residue alanine, significantly impairs tissue factor-FVIIa proteolytic activity. This suggests that the flexibility afforded by glycine is critical for the loop of residues 159-165 for tissue factor macromolecular substrate recognition.

The residues Lys¹⁶⁵ and Lys¹⁶⁶ have also been demonstrated to be important for substrate recognition and binding. Mutation of either of these residues to alanine results in a significant decrease in the tissue factor co-factor function. Lys¹⁶⁵ and Lys¹⁶⁶ face away from each other, with Lys¹⁶⁵ pointing towards FVIIa in most tissue factor-FVIIa structures, and Lys¹⁶⁶ pointing into the substrate binding exosite region in the crystal structure. Putative salt bridge formation between Lys¹⁶⁵ of and Gla³⁵ of FVIIa would support the notion that tissue factor interaction with the GLA domain of

FVIIa modulates substrate recognition. These results suggest that the C-terminal portion of the tissue factor ectodomain directly interacts with the GLA-domain, the possible adjacent EGF1 domains, of FIX and FX, and that the presence of the FVIIa GLA-domain may modulate these interactions either directly or indirectly.

5

Exemplary Linker Domains and IgG1 Antibody Constructs

In some examples of any of the methods, compositions, or kits described herein, the linker domain in the second multi-chain chimeric polypeptide can be or include any antigen that is recognized by a cognate IgG1 antibody (e.g., a monoclonal antibody). IgG1 antibodies are distinguished from other IgG classes of antibodies by their constant region, particularly in hinge regions and upper CH2 domains (see, e.g., Vadarsson et al., *IgG Subclasses and Allotypes: From Structure to Effector Functions*, *Front Immunol.*, 5, Article 520, 2014). Human IgG1 is the only known IgG subclass which binds to human CD16a and activates the signaling of human CD16a.

Any of a variety of linker domains can be used in the second multi-chain chimeric polypeptides provided herein. In certain embodiments, a linker domain is recognized by an antibody (e.g., an IgG1 antibody) or antibody fragment. In some embodiments, the linker domain is a kappa light chain of an antibody that is recognized by a cognate anti-kappa light chain IgG1 antibody. In some embodiments, the linker domain is a lambda light chain of an antibody that is recognized by a cognate anti-lambda light chain human IgG1 antibody. A variety of kappa light chains and lambda light chains are known in the art (see, e.g., Smith et al., *Antigen Nature and Complexity Influence Human Antibody Light Chain Usage and Specificity*, *Vaccine*, 34(25): 2813–2820, 2016). In some embodiments, the linker domain is or comprises a human polypeptide (e.g., a human kappa light chain of an antibody or a human lambda light chain of an antibody) that is recognized by a cognate human IgG1 antibody (e.g., a monoclonal antibody). In some embodiments, the cognate human IgG1 antibody includes at least two antigen-binding domains, and each antigen-binding domain binds specifically to the linker domain. In some embodiments, the cognate human IgG1 antibody includes at least two antigen-binding domains, and only a subset (e.g., one) of the antigen-binding domains bind specifically to the linker domain in the second multi-chain chimeric polypeptide. In

some embodiments, a linker domain can be any of the soluble tissue factor domains described herein.

In some embodiments of any of the methods, compositions, and kits described herein, an IgG1 antibody construct can be an IgG1 antibody (e.g., a monoclonal or a polyclonal IgG1 antibody that binds specifically to the linker domain). In some
5 embodiments of any of the methods, compositions, and kits described herein, an IgG1 antibody construct can be an antibody or an antibody fragment that includes an IgG1 Fc region (e.g., a human IgG1 Fc region) and that binds specifically to the linker domain in the second multi-chain chimeric polypeptide. As is known in the art, the
10 IgG1 Fc region binds to CD16a (FcRgammaIII) (e.g., human CD16a) and induces its intracellular signaling. In some embodiments, an IgG1 antibody construct can be a single chain or a multi-chain polypeptide that includes an Fc region that is capable of binding specifically to CD16a (FcRgammaIII) (e.g., human CD16a) and is capable of inducing its intracellular signaling in a natural killer cell (e.g., a human natural killer
15 cell), and specifically binds to the linker domain. In some embodiments, an IgG1 antibody construct can be a single chain or a multi-chain polypeptide that includes an Fc region that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at
20 least 98% identical, or at least 99% identical) to a wildtype IgG1 Fc domain (e.g., a wildtype human IgG1 Fc domain, e.g., SEQ ID NO: 1) and is capable of binding specifically to CD16a (FcRgammaIII) (e.g., human CD16a), and is capable of inducing its intracellular signaling in a natural killer cell (e.g., a human natural killer cell), and specifically binds to the linker domain. In some embodiments of any of the
25 methods, compositions, or kits described herein, the IgG1 antibody construct can be an antibody or antibody fragment that specifically binds to the linker domain and includes a non-IgG1 Fc region (e.g., an IgG2, IgG3, or IgG4 Fc region) that has been altered (e.g., by substituting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in the wildtype non-IgG1 Fc region) such that the non-IgG1 Fc
30 region is capable of binding to CD16a (FcRgammaIII) (e.g., human CD16a) and inducing its intracellular signaling in a natural killer cell (e.g., a human natural killer cell). In some embodiments of any of the methods, compositions, or kits described herein, the IgG1 antibody construct binds specifically to the linker domain in the

second multi-chain chimeric polypeptide and includes a non-human Fc region (e.g., a Fc region from a non-human antibody) that has been altered (e.g., by substituting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in the wildtype non-human Fc region) such that the non-human Fc region is capable of

5 binding to human CD16a (human FcRgammaIII) and inducing its intracellular signaling in a natural killer cell (e.g., a human natural killer cell).

Wildtype Human IgG1 Fc Region (SEQ ID NO: 1)

PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR

10 EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE

LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV

FSCSVMHEALHNHYTQKSLSLSPGK

Wildtype Human IgG2 Fc Region (SEQ ID NO: 2)

15 APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQF

NSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN

QVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS

VMHEALHNHYTQKSLSLSPGK

20 Wildtype Human IgG3 Fc Region (SEQ ID NO: 3)

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQ

YNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTK

NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDSDGSFFLYSKLTVDKSRWQQGNIFSC

SVMHEALHNRFTQKSLSLSPGK

25

Wildtype Human IgG4 Fc Region (SEQ ID NO: 4)

APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ

FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK

NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC

30 SVMHEALHNHYTQKSLSLGLGK

In some embodiments, the linker domain can have a total length of about 20 amino acids to about 220 amino acids, about 20 amino acids to about 215 amino acids, about 20 amino acids to about 210 amino acids, about 20 amino acids to about

35 205 amino acids, about 20 amino acids to about 200 amino acids, about 20 amino acids to about 195 amino acids, about 20 amino acids to about 190 amino acids, about

[illegible]

amino acids to about 70 amino acids, about 30 amino acids to about 60 amino acids,
about 30 amino acids to about 50 amino acids, about 30 amino acids to about 40
amino acids, about 40 amino acids to about 220 amino acids, about 40 amino acids to
about 215 amino acids, about 40 amino acids to about 210 amino acids, about 40
5 amino acids to about 205 amino acids, about 40 amino acids to about 200 amino
acids, about 40 amino acids to about 195 amino acids, about 40 amino acids to about
190 amino acids, about 40 amino acids to about 185 amino acids, about 40 amino
acids to about 180 amino acids, about 40 amino acids to about 175 amino acids, about
40 amino acids to about 170 amino acids, about 40 amino acids to about 165 amino
10 acids, about 40 amino acids to about 160 amino acids, about 40 amino acids to about
155 amino acids, about 40 amino acids to about 150 amino acids, about 40 amino
acids to about 145 amino acids, about 40 amino acids to about 140 amino acids, about
40 amino acids to about 135 amino acids, about 40 amino acids to about 130 amino
acids, about 40 amino acids to about 125 amino acids, about 40 amino acids to about
15 120 amino acids, about 40 amino acids to about 115 amino acids, about 40 amino
acids to about 110 amino acids, about 40 amino acids to about 105 amino acids, about
40 amino acids to about 100 amino acids, about 40 amino acids to about 95 amino
acids, about 40 amino acids to about 90 amino acids, about 40 amino acids to about
85 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids
20 to about 75 amino acids, about 40 amino acids to about 70 amino acids, about 40
amino acids to about 60 amino acids, about 40 amino acids to about 50 amino acids,
about 50 amino acids to about 220 amino acids, about 50 amino acids to about 215
amino acids, about 50 amino acids to about 210 amino acids, about 50 amino acids to
about 205 amino acids, about 50 amino acids to about 200 amino acids, about 50
25 amino acids to about 195 amino acids, about 50 amino acids to about 190 amino
acids, about 50 amino acids to about 185 amino acids, about 50 amino acids to about
180 amino acids, about 50 amino acids to about 175 amino acids, about 50 amino
acids to about 170 amino acids, about 50 amino acids to about 165 amino acids, about
50 amino acids to about 160 amino acids, about 50 amino acids to about 155 amino
30 acids, about 50 amino acids to about 150 amino acids, about 50 amino acids to about
145 amino acids, about 50 amino acids to about 140 amino acids, about 50 amino
acids to about 135 amino acids, about 50 amino acids to about 130 amino acids, about
50 amino acids to about 125 amino acids, about 50 amino acids to about 120 amino

[illegible]

[illegible]

acids to about 135 amino acids, about 90 amino acids to about 130 amino acids, about
90 amino acids to about 125 amino acids, about 90 amino acids to about 120 amino
acids, about 90 amino acids to about 115 amino acids, about 90 amino acids to about
110 amino acids, about 90 amino acids to about 105 amino acids, about 90 amino
5 acids to about 100 amino acids, about 100 amino acids to about 220 amino acids,
about 100 amino acids to about 215 amino acids, about 100 amino acids to about 210
amino acids, about 100 amino acids to about 205 amino acids, about 100 amino acids
to about 200 amino acids, about 100 amino acids to about 195 amino acids, about 100
amino acids to about 190 amino acids, about 100 amino acids to about 185 amino
10 acids, about 100 amino acids to about 180 amino acids, about 100 amino acids to
about 175 amino acids, about 100 amino acids to about 170 amino acids, about 100
amino acids to about 165 amino acids, about 100 amino acids to about 160 amino
acids, about 100 amino acids to about 155 amino acids, about 100 amino acids to
about 150 amino acids, about 100 amino acids to about 145 amino acids, about 100
15 amino acids to about 140 amino acids, about 100 amino acids to about 135 amino
acids, about 100 amino acids to about 130 amino acids, about 100 amino acids to
about 125 amino acids, about 100 amino acids to about 120 amino acids, about 100
amino acids to about 115 amino acids, about 100 amino acids to about 110 amino
acids, about 110 amino acids to about 220 amino acids, about 110 amino acids to
20 about 215 amino acids, about 110 amino acids to about 210 amino acids, about 110
amino acids to about 205 amino acids, about 110 amino acids to about 200 amino
acids, about 110 amino acids to about 195 amino acids, about 110 amino acids to
about 190 amino acids, about 110 amino acids to about 185 amino acids, about 110
amino acids to about 180 amino acids, about 110 amino acids to about 175 amino
25 acids, about 110 amino acids to about 170 amino acids, about 110 amino acids to
about 165 amino acids, about 110 amino acids to about 160 amino acids, about 110
amino acids to about 155 amino acids, about 110 amino acids to about 150 amino
acids, about 110 amino acids to about 145 amino acids, about 110 amino acids to
about 140 amino acids, about 110 amino acids to about 135 amino acids, about 110
30 amino acids to about 130 amino acids, about 110 amino acids to about 125 amino
acids, about 110 amino acids to about 120 amino acids, about 110 amino acids to
about 115 amino acids, about 115 amino acids to about 220 amino acids, about 115
amino acids to about 215 amino acids, about 115 amino acids to about 210 amino

[illegible]

[illegible]

[illegible]

acids, about 165 amino acids to about 205 amino acids, about 165 amino acids to
about 200 amino acids, about 165 amino acids to about 195 amino acids, about 165
amino acids to about 190 amino acids, about 165 amino acids to about 185 amino
acids, about 165 amino acids to about 180 amino acids, about 165 amino acids to
5 about 175 amino acids, about 165 amino acids to about 170 amino acids, about 170
amino acids to about 220 amino acids, about 170 amino acids to about 215 amino
acids, about 170 amino acids to about 210 amino acids, about 170 amino acids to
about 205 amino acids, about 170 amino acids to about 200 amino acids, about 170
amino acids to about 195 amino acids, about 170 amino acids to about 190 amino
10 acids, about 170 amino acids to about 185 amino acids, about 170 amino acids to
about 180 amino acids, about 170 amino acids to about 175 amino acids, about 175
amino acids to about 220 amino acids, about 175 amino acids to about 215 amino
acids, about 175 amino acids to about 210 amino acids, about 175 amino acids to
about 205 amino acids, about 175 amino acids to about 200 amino acids, about 175
15 amino acids to about 195 amino acids, about 175 amino acids to about 190 amino
acids, about 175 amino acids to about 185 amino acids, about 175 amino acids to
about 180 amino acids, about 180 amino acids to about 220 amino acids, about 180
amino acids to about 215 amino acids, about 180 amino acids to about 210 amino
acids, about 180 amino acids to about 205 amino acids, about 180 amino acids to
20 about 200 amino acids, about 180 amino acids to about 195 amino acids, about 180
amino acids to about 190 amino acids, about 180 amino acids to about 185 amino
acids, about 185 amino acids to about 220 amino acids, about 185 amino acids to
about 215 amino acids, about 185 amino acids to about 210 amino acids, about 185
amino acids to about 205 amino acids, about 185 amino acids to about 200 amino
25 acids, about 185 amino acids to about 195 amino acids, about 185 amino acids to
about 190 amino acids, about 190 amino acids to about 220 amino acids, about 190
amino acids to about 215 amino acids, about 190 amino acids to about 210 amino
acids, about 190 amino acids to about 205 amino acids, about 190 amino acids to
about 200 amino acids, about 190 amino acids to about 195 amino acids, about 195
30 amino acids to about 220 amino acids, about 195 amino acids to about 215 amino
acids, about 195 amino acids to about 210 amino acids, about 195 amino acids to
about 205 amino acids, about 195 amino acids to about 200 amino acids, about 200
amino acids to about 220 amino acids, about 200 amino acids to about 215 amino

acids, about 200 amino acids to about 210 amino acids, about 200 amino acids to about 205 amino acids, about 205 amino acids to about 220 amino acids, about 205 amino acids to about 215 amino acids, about 205 amino acids to about 210 amino acids, about 210 amino acids to about 220 amino acids, about 210 amino acids to about 215 amino acids, or about 215 amino acids to about 220 amino acids.

Soluble Tissue Factor Domain

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

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

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYT
 TDTECDLTDEIVKDKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLET
 NLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWK
 SSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQ
 EKGEFRE

Exemplary Nucleic Acid Encoding Soluble Human Tissue Factor Domain (SEQ ID NO: 6)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCAC
 CAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTA

CACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTA
 TACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
 ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAG
 CACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCC
 5 TTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGT
 TGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGC
 GGAACAACACCTTTCTCAGCCTCCGGGATGTGTTTCGGCAAAGATTTAATCT
 ACACACTGTATTACTGGAAGTCCTCTTCTCCGGCAAGAAGACAGCTAAAA
 CCAACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACACTACTGTT
 10 TCAGCGTGCAAGCTGTGATCCCCCTCCCGGACCGTGAATAGGAAAAGCACC
 GATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

Exemplary Soluble Mouse Tissue Factor Domain (SEQ ID NO: 7)

agipekafnltwistdfktilwqpkptnytytvqisdrsrnwknkcfs
 15 ttdtecdltdeivkdvtwayeakvlsvprnsvhgdgdqlvihgeepf
 tnapkflpyrdtnlgqpviqqfeqdgklnvvvkdsltlvrkngtfltl
 rqvfgkdlgyiityrkgsstgkktntntnefsidveegvsycffvqam
 ifsrktnqnspsstvcteqwksflge

Exemplary Soluble Rat Tissue Factor Domain (SEQ ID NO: 8)

Agtppgkafnltwistdfktilwqpkptnytytvqisdrsrnwkykct
 gttdecdltdeivkdvnwtyearvlsvpwrnsthgketlfgthgeepf
 ftnarkflpyrdtkigqpviqkyeggtklkvtkdsftlvrkngtflt
 lrqvfgndlgyiltyrkdsstgrktnthtneflidvekgvsycffafa
 25 vifsrktnhkspsitkcteqwksvlge

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 9)

SGTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDWKSKCFY
 TTDTECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
 30 ETNLGQPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLY
 YWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVEC
 MGQEKGEFRE

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 10)

SGTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDAKSKCFY
 35 TTDTECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLAENSPEFTPYL

ETNLGQPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLY
 YWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVEC
 MGQEKGEFRE

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

As will be appreciated by those of skill in the art, mutation of amino acids that are conserved between different mammalian species is more likely to decrease the activity and/or structural stability of the protein, while mutation of amino acids that are not conserved between different mammalian species is less likely to decrease the activity and/or structural stability of the protein.

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

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

In some examples, the soluble tissue factor domain does not include one or more (e.g., two, three, four, five, six or seven) of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino

acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some embodiments, the mutant soluble tissue factor possesses the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

In some examples, the soluble tissue factor domain can be encoded by a nucleic acid including a sequence that is at least 70% identical, at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 6.

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

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acids to about 195 amino acids, about 80 amino acids to about 190 amino acids, about
80 amino acids to about 185 amino acids, about 80 amino acids to about 180 amino
acids, about 80 amino acids to about 175 amino acids, about 80 amino acids to about
170 amino acids, about 80 amino acids to about 165 amino acids, about 80 amino
5 acids to about 160 amino acids, about 80 amino acids to about 155 amino acids, about
80 amino acids to about 150 amino acids, about 80 amino acids to about 145 amino
acids, about 80 amino acids to about 140 amino acids, about 80 amino acids to about
135 amino acids, about 80 amino acids to about 130 amino acids, about 80 amino
acids to about 125 amino acids, about 80 amino acids to about 120 amino acids, about
10 80 amino acids to about 115 amino acids, about 80 amino acids to about 110 amino
acids, about 80 amino acids to about 105 amino acids, about 80 amino acids to about
100 amino acids, about 80 amino acids to about 95 amino acids, about 80 amino acids
to about 90 amino acids, about 90 amino acids to about 220 amino acids, about 90
amino acids to about 215 amino acids, about 90 amino acids to about 210 amino
15 acids, about 90 amino acids to about 205 amino acids, about 90 amino acids to about
200 amino acids, about 90 amino acids to about 195 amino acids, about 90 amino
acids to about 190 amino acids, about 90 amino acids to about 185 amino acids, about
90 amino acids to about 180 amino acids, about 90 amino acids to about 175 amino
acids, about 90 amino acids to about 170 amino acids, about 90 amino acids to about
20 165 amino acids, about 90 amino acids to about 160 amino acids, about 90 amino
acids to about 155 amino acids, about 90 amino acids to about 150 amino acids, about
90 amino acids to about 145 amino acids, about 90 amino acids to about 140 amino
acids, about 90 amino acids to about 135 amino acids, about 90 amino acids to about
130 amino acids, about 90 amino acids to about 125 amino acids, about 90 amino
25 acids to about 120 amino acids, about 90 amino acids to about 115 amino acids, about
90 amino acids to about 110 amino acids, about 90 amino acids to about 105 amino
acids, about 90 amino acids to about 100 amino acids, about 100 amino acids to about
220 amino acids, about 100 amino acids to about 215 amino acids, about 100 amino
acids to about 210 amino acids, about 100 amino acids to about 205 amino acids,
30 about 100 amino acids to about 200 amino acids, about 100 amino acids to about 195
amino acids, about 100 amino acids to about 190 amino acids, about 100 amino acids
to about 185 amino acids, about 100 amino acids to about 180 amino acids, about 100
amino acids to about 175 amino acids, about 100 amino acids to about 170 amino

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acids, about 175 amino acids to about 220 amino acids, about 175 amino acids to about 215 amino acids, about 175 amino acids to about 210 amino acids, about 175 amino acids to about 205 amino acids, about 175 amino acids to about 200 amino acids, about 175 amino acids to about 195 amino acids, about 175 amino acids to about 190 amino acids, about 175 amino acids to about 185 amino acids, about 175 amino acids to about 180 amino acids, about 180 amino acids to about 220 amino acids, about 180 amino acids to about 215 amino acids, about 180 amino acids to about 210 amino acids, about 180 amino acids to about 205 amino acids, about 180 amino acids to about 200 amino acids, about 180 amino acids to about 195 amino acids, about 180 amino acids to about 190 amino acids, about 180 amino acids to about 185 amino acids, about 185 amino acids to about 220 amino acids, about 185 amino acids to about 215 amino acids, about 185 amino acids to about 210 amino acids, about 185 amino acids to about 205 amino acids, about 185 amino acids to about 200 amino acids, about 185 amino acids to about 195 amino acids, about 185 amino acids to about 190 amino acids, about 190 amino acids to about 220 amino acids, about 190 amino acids to about 215 amino acids, about 190 amino acids to about 210 amino acids, about 190 amino acids to about 205 amino acids, about 190 amino acids to about 200 amino acids, about 190 amino acids to about 195 amino acids, about 195 amino acids to about 220 amino acids, about 195 amino acids to about 215 amino acids, about 195 amino acids to about 210 amino acids, about 195 amino acids to about 205 amino acids, about 195 amino acids to about 200 amino acids, about 200 amino acids to about 220 amino acids, about 200 amino acids to about 215 amino acids, about 200 amino acids to about 210 amino acids, about 200 amino acids to about 205 amino acids, about 205 amino acids to about 220 amino acids, about 205 amino acids to about 215 amino acids, about 205 amino acids to about 210 amino acids, about 210 amino acids to about 220 amino acids, about 210 amino acids to about 215 amino acids, or about 215 amino acids to about 220 amino acids.

30 **Linker Sequences**

In some embodiments, the linker sequence can be a flexible linker sequence. Non-limiting examples of linker sequences that can be used are described in Klein et al., Protein Engineering, Design & Selection Vol. 27, No. 10, pp. 325–330, 2014;

Priyanka et al., Protein Sci., 2013 Feb; 22(2):153–167. In some examples, the linker sequence is a synthetic linker sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first and/or second polypeptide(s) can include one, two, three, 5 four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art).

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

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[illegible]

acids to about 35 amino acids, about 35 amino acids to about 100 amino acids, about 35 amino acids to about 90 amino acids, about 35 amino acids to about 80 amino acids, about 35 amino acids to about 70 amino acids, about 35 amino acids to about 60 amino acids, about 35 amino acids to about 50 amino acids, about 35 amino acids to about 45 amino acids, about 35 amino acids to about 40 amino acids, about 40 amino acids to about 100 amino acids, about 40 amino acids to about 90 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 70 amino acids, about 40 amino acids to about 60 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 45 amino acids, about 45 amino acids to about 100 amino acids, about 45 amino acids to about 90 amino acids, about 45 amino acids to about 80 amino acids, about 45 amino acids to about 70 amino acids, about 45 amino acids to about 60 amino acids, about 45 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, about 50 amino acids to about 90 amino acids, about 50 amino acids to about 80 amino acids, about 50 amino acids to about 70 amino acids, about 50 amino acids to about 60 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to about 70 amino acids, about 70 amino acids to about 100 amino acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about 80 amino acids, about 80 amino acids to about 100 amino acids, about 80 amino acids to about 90 amino acids, or about 90 amino acids to about 100 amino acids.

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

In some embodiments, the linker sequence can comprise or consist of GGGGSGGGGSGGGGS (SEQ ID NO: 11). In some embodiments, the linker sequence can be encoded by a nucleic acid comprising or consisting of: GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT (SEQ ID NO: 12). In some embodiments, the linker sequence can comprise or consist of: GGGSGGGGS (SEQ ID NO: 13).

Target-Binding Domains

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

In some embodiments of any of the first multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor of IL-18 and a receptor of IL-12.

In some embodiments of any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor of IL-7 and a receptor of IL-21.

In some embodiments of any of the first multi-chain chimeric polypeptides or any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and/or the second target-binding domain can be a soluble interleukin or soluble cytokine protein. In some examples, the soluble interleukin or soluble cytokine protein is selected from the group of: IL-7, IL-12, IL-18, and IL-21.

In some embodiments of any of the first multi-chain chimeric polypeptides or any of the second multi-chain chimeric polypeptides described herein, the first and second target-binding domains can each independent have a total number of amino acids of about 5 amino acids to about 1000 amino acids, about 5 amino acids to about 950 amino acids, about 5 amino acids to about 900 amino acids, about 5 amino acids

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[illegible]

acids to about 300 amino acids, about 30 amino acids to about 280 amino acids, about
30 amino acids to about 260 amino acids, about 30 amino acids to about 240 amino
acids, about 30 amino acids to about 220 amino acids, about 30 amino acids to about
200 amino acids, about 30 amino acids to about 195 amino acids, about 30 amino
5 acids to about 190 amino acids, about 30 amino acids to about 185 amino acids, about
30 amino acids to about 180 amino acids, about 30 amino acids to about 175 amino
acids, about 30 amino acids to about 170 amino acids, about 30 amino acids to about
165 amino acids, about 30 amino acids to about 160 amino acids, about 30 amino
acids to about 155 amino acids, about 30 amino acids to about 150 amino acids, about
10 30 amino acids to about 145 amino acids, about 30 amino acids to about 140 amino
acids, about 30 amino acids to about 135 amino acids, about 30 amino acids to about
130 amino acids, about 30 amino acids to about 125 amino acids, about 30 amino
acids to about 120 amino acids, about 30 amino acids to about 115 amino acids, about
30 amino acids to about 110 amino acids, about 30 amino acids to about 105 amino
15 acids, about 30 amino acids to about 100 amino acids, about 30 amino acids to about
95 amino acids, about 30 amino acids to about 90 amino acids, about 30 amino acids
to about 85 amino acids, about 30 amino acids to about 80 amino acids, about 30
amino acids to about 75 amino acids, about 30 amino acids to about 70 amino acids,
about 30 amino acids to about 65 amino acids, about 30 amino acids to about 60
20 amino acids, about 30 amino acids to about 55 amino acids, about 30 amino acids to
about 50 amino acids, about 30 amino acids to about 45 amino acids, about 30 amino
acids to about 40 amino acids, about 30 amino acids to about 35 amino acids, about
35 amino acids to about 1000 amino acids, about 35 amino acids to about 950 amino
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500 amino acids, about 35 amino acids to about 450 amino acids, about 35 amino
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240 amino acids, about 35 amino acids to about 220 amino acids, about 35 amino

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35 amino acids to about 190 amino acids, about 35 amino acids to about 185 amino
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175 amino acids, about 35 amino acids to about 170 amino acids, about 35 amino
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5 40 amino acids to about 135 amino acids, about 40 amino acids to about 130 amino acids, about 40 amino acids to about 125 amino acids, about 40 amino acids to about 120 amino acids, about 40 amino acids to about 115 amino acids, about 40 amino acids to about 110 amino acids, about 40 amino acids to about 105 amino acids, about 40 amino acids to about 100 amino acids, about 40 amino acids to about 95 amino
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100 amino acids, about 50 amino acids to about 95 amino acids, about 50 amino acids
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amino acids to about 800 amino acids, about 800 amino acids to about 1000 amino acids, about 800 amino acids to about 950 amino acids, about 800 amino acids to about 900 amino acids, about 800 amino acids to about 850 amino acids, about 850 amino acids to about 1000 amino acids, about 850 amino acids to about 950 amino acids, about 850 amino acids to about 900 amino acids, about 900 amino acids to about 1000 amino acids, about 900 amino acids to about 950 amino acids, or about 950 amino acids to about 1000 amino acids.

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

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

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

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

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

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

90 pM to about 150 pM, about 90 pM to about 100 pM, about 100 pM to about 30 nM, about 100 pM to about 25 nM, about 100 pM to about 30 nM, about 100 pM to about 15 nM, about 100 pM to about 10 nM, about 100 pM to about 5 nM, about 100 pM to about 2 nM, about 100 pM to about 1 nM, about 100 pM to about 950 pM, about 100 pM to about 900 pM, about 100 pM to about 850 pM, about 100 pM to about 800 pM, about 100 pM to about 750 pM, about 100 pM to about 700 pM, about 100 pM to about 650 pM, about 100 pM to about 600 pM, about 100 pM to about 550 pM, about 100 pM to about 500 pM, about 100 pM to about 450 pM, about 100 pM to about 400 pM, about 100 pM to about 350 pM, about 100 pM to about 300 pM, about 100 pM to about 250 pM, about 100 pM to about 200 pM, about 100 pM to about 150 pM, about 150 pM to about 30 nM, about 150 pM to about 25 nM, about 150 pM to about 30 nM, about 150 pM to about 15 nM, about 150 pM to about 10 nM, about 150 pM to about 5 nM, about 150 pM to about 2 nM, about 150 pM to about 1 nM, about 150 pM to about 950 pM, about 150 pM to about 900 pM, about 150 pM to about 850 pM, about 150 pM to about 800 pM, about 150 pM to about 750 pM, about 150 pM to about 700 pM, about 150 pM to about 650 pM, about 150 pM to about 600 pM, about 150 pM to about 550 pM, about 150 pM to about 500 pM, about 150 pM to about 450 pM, about 150 pM to about 400 pM, about 150 pM to about 350 pM, about 150 pM to about 300 pM, about 150 pM to about 250 pM, about 150 pM to about 200 pM, about 200 pM to about 30 nM, about 200 pM to about 25 nM, about 200 pM to about 30 nM, about 200 pM to about 15 nM, about 200 pM to about 10 nM, about 200 pM to about 5 nM, about 200 pM to about 2 nM, about 200 pM to about 1 nM, about 200 pM to about 950 pM, about 200 pM to about 900 pM, about 200 pM to about 850 pM, about 200 pM to about 800 pM, about 200 pM to about 750 pM, about 200 pM to about 700 pM, about 200 pM to about 650 pM, about 200 pM to about 600 pM, about 200 pM to about 550 pM, about 200 pM to about 500 pM, about 200 pM to about 450 pM, about 200 pM to about 400 pM, about 200 pM to about 350 pM, about 200 pM to about 300 pM, about 200 pM to about 250 pM, about 300 pM to about 30 nM, about 300 pM to about 25 nM, about 300 pM to about 30 nM, about 300 pM to about 15 nM, about 300 pM to about 10 nM, about 300 pM to about 5 nM, about 300 pM to about 2 nM, about 300 pM to about 1 nM, about 300 pM to about 950 pM, about 300 pM to about 900 pM, about 300 pM to about 850 pM, about 300 pM to about 800 pM, about 300 pM to about 750 pM, about 300 pM to about 700 pM, about 300 pM to

about 650 pM, about 300 pM to about 600 pM, about 300 pM to about 550 pM, about 300 pM to about 500 pM, about 300 pM to about 450 pM, about 300 pM to about 400 pM, about 300 pM to about 350 pM, about 400 pM to about 30 nM, about 400 pM to about 25 nM, about 400 pM to about 30 nM, about 400 pM to about 15 nM, about 400 pM to about 10 nM, about 400 pM to about 5 nM, about 400 pM to about 2 nM, about 400 pM to about 1 nM, about 400 pM to about 950 pM, about 400 pM to about 900 pM, about 400 pM to about 850 pM, about 400 pM to about 800 pM, about 400 pM to about 750 pM, about 400 pM to about 700 pM, about 400 pM to about 650 pM, about 400 pM to about 600 pM, about 400 pM to about 550 pM, about 400 pM to about 500 pM, about 500 pM to about 30 nM, about 500 pM to about 25 nM, about 500 pM to about 30 nM, about 500 pM to about 15 nM, about 500 pM to about 10 nM, about 500 pM to about 5 nM, about 500 pM to about 2 nM, about 500 pM to about 1 nM, about 500 pM to about 950 pM, about 500 pM to about 900 pM, about 500 pM to about 850 pM, about 500 pM to about 800 pM, about 500 pM to about 750 pM, about 500 pM to about 700 pM, about 500 pM to about 650 pM, about 500 pM to about 600 pM, about 500 pM to about 550 pM, about 600 pM to about 30 nM, about 600 pM to about 25 nM, about 600 pM to about 30 nM, about 600 pM to about 15 nM, about 600 pM to about 10 nM, about 600 pM to about 5 nM, about 600 pM to about 2 nM, about 600 pM to about 1 nM, about 600 pM to about 950 pM, about 600 pM to about 900 pM, about 600 pM to about 850 pM, about 600 pM to about 800 pM, about 600 pM to about 750 pM, about 600 pM to about 700 pM, about 600 pM to about 650 pM, about 600 pM to about 30 nM, about 700 pM to about 25 nM, about 700 pM to about 30 nM, about 700 pM to about 15 nM, about 700 pM to about 10 nM, about 700 pM to about 5 nM, about 700 pM to about 2 nM, about 700 pM to about 1 nM, about 700 pM to about 950 pM, about 700 pM to about 900 pM, about 700 pM to about 850 pM, about 700 pM to about 800 pM, about 700 pM to about 750 pM, about 800 pM to about 30 nM, about 800 pM to about 25 nM, about 800 pM to about 30 nM, about 800 pM to about 15 nM, about 800 pM to about 10 nM, about 800 pM to about 5 nM, about 800 pM to about 2 nM, about 800 pM to about 1 nM, about 800 pM to about 950 pM, about 800 pM to about 900 pM, about 800 pM to about 850 pM, about 900 pM to about 30 nM, about 900 pM to about 25 nM, about 900 pM to about 30 nM, about 900 pM to about 15 nM, about 900 pM to about 10 nM, about 900 pM to about 5 nM, about 900 pM to about 2 nM, about 900 pM to about 1 nM, about 900 pM to

about 950 pM, about 1 nM to about 30 nM, about 1 nM to about 25 nM, about 1 nM to about 20 nM, about 1 nM to about 15 nM, about 1 nM to about 10 nM, about 1 nM to about 5 nM, about 2 nM to about 30 nM, about 2 nM to about 25 nM, about 2 nM to about 20 nM, about 2 nM to about 15 nM, about 2 nM to about 10 nM, about 2 nM to about 5 nM, about 4 nM to about 30 nM, about 4 nM to about 25 nM, about 4 nM to about 20 nM, about 4 nM to about 15 nM, about 4 nM to about 10 nM, about 4 nM to about 5 nM, about 5 nM to about 30 nM, about 5 nM to about 25 nM, about 5 nM to about 20 nM, about 5 nM to about 15 nM, about 5 nM to about 10 nM, about 10 nM to about 30 nM, about 10 nM to about 25 nM, about 10 nM to about 20 nM, about 10 nM to about 15 nM, about 15 nM to about 30 nM, about 15 nM to about 25 nM, about 15 nM to about 20 nM, about 20 nM to about 30 nM, and about 20 nM to about 25 nM).

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

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

Antigen-Binding Domains

In some embodiments of any of the first multi-chain chimeric polypeptides and/or any of the second multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain can be an antigen-binding domain. In some embodiments of any of the first multi-chain chimeric polypeptides or any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain can each be an antigen-binding domain. In some embodiments of any of the first multi-chain chimeric polypeptides and/or any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain can bind specifically to different antigens. In some embodiments, the first target-binding domain and/or the second target-binding domain can be an agonistic antigen-binding domain.

In some embodiments of any of the first multi-chain chimeric polypeptides or any of the second multi-chain chimeric polypeptides described herein, the antigen-binding domain can include or is a scFv or a single domain antibody (e.g., a VHH or a VNAR domain). The antigen-binding domain present in any of the multi-chain polypeptides described herein are each independently selected from the group of: a VHH domain, a VNAR domain, and a scFv. In some examples, an antigen-binding domain (e.g., any of the antigen-binding domain described herein) can bind specifically to a receptor of IL-7, a receptor of IL-12, a receptor of IL-18, or a receptor for IL-21.

In some embodiments, any of the antigen-binding domains described herein is a BiTe, a (scFv)₂, a nanobody, a nanobody-HSA, a DART, a TandAb, a scDiabody, a scDiabody-CH3, scFv-CH-CL-scFv, a HSAbody, scDiabody-HAS, or a tandem-scFv. Additional examples of antigen-binding domains that can be used in any of the multi-chain chimeric polypeptides (e.g., a first multi-chain chimeric polypeptide and a second multi-chain chimeric polypeptide) described herein are known in the art.

A VHH domain is a single monomeric variable antibody domain that can be found in camelids. A VNAR domain is a single monomeric variable antibody domain that can be found in cartilaginous fish. Non-limiting aspects of VHH domains and VNAR domains are described in, e.g., Cromie et al., *Curr. Top. Med. Chem.* 15:2543-2557, 2016; De Genst et al., *Dev. Comp. Immunol.* 30:187-198, 2006; De Meyer et al.,

Trends Biotechnol. 32:263-270, 2014; Kijanka et al., *Nanomedicine* 10:161-174, 2015; Kovaleva et al., *Expert. Opin. Biol. Ther.* 14:1527-1539, 2014; Krah et al., *Immunopharmacol. Immunotoxicol.* 38:21-28, 2016; Mujic-Delic et al., *Trends Pharmacol. Sci.* 35:247-255, 2014; Muyldermans, *J. Biotechnol.* 74:277-302, 2001; 5 Muyldermans et al., *Trends Biochem. Sci.* 26:230-235, 2001; Muyldermans, *Ann. Rev. Biochem.* 82:775-797, 2013; Rahbarizadeh et al., *Immunol. Invest.* 40:299-338, 2011; Van Audenhove et al., *EBioMedicine* 8:40-48, 2016; Van Bockstaele et al., *Curr. Opin. Investig. Drugs* 10:1212-1224, 2009; Vincke et al., *Methods Mol. Biol.* 911:15-26, 2012; and Wesolowski et al., *Med. Microbiol. Immunol.* 198:157-174, 2009.

10 In some embodiments of any of the first multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are both VHH domains, or at least one antigen-binding domain is a VHH domain. In some embodiments of any of the first multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding 15 domain are both VNAR domains, or at least one antigen-binding domain is a VNAR domain. In some embodiments of any of the first multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are both scFv domains, or at least one antigen-binding domain is a scFv domain.

20 In some embodiments of any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are both VHH domains, or at least one antigen-binding domain is a VHH domain. In some embodiments of any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding 25 domain are both VNAR domains, or at least one antigen-binding domain is a VNAR domain. In some embodiments of any of the second multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are both scFv domains, or at least one antigen-binding domain is a scFv domain. DARTs are described in, e.g., Garber, *Nature Reviews Drug Discovery* 30 13:799-801, 2014.

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

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

Soluble Interleukin or Cytokine Protein

5 In some embodiments of any of the first multi-chain chimeric polypeptides and/or the second multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain can be a soluble interleukin protein or soluble cytokine protein. In some embodiments, the soluble interleukin or soluble cytokine protein is selected from the group of: IL-7, IL-12, IL-10 18, and IL-21. Non-limiting examples of soluble IL-7, IL-12, IL-18, and IL-21 are provided below.

Human Soluble IL-12 β (p40) (SEQ ID NO: 14)

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK
15 TLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE
RVRGDNKEYEYSVEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRD
IHKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKRE
KKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

20

Nucleic Acid Encoding Human Soluble IL-12 β (p40) (SEQ ID NO: 15)

ATTTGGGAAGTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCC
GATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGA
CGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAA
25 AGACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACAT
GCCACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGA
AGGAAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCC
AAGAATAAGACCTTTTAAAGGTGTGAGGCCAAAACTACAGCGGTCGTTTC
ACTTGTTGGTGGCTGACCACCATTTCCACCGATTAACTTCTCCGTGAAA
30 AGCAGCCGGGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTAC
CCTCAGCGCTGAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCG
TGGAGTGCCAAGAAGATAGCGCTTGTCGCTGCCGAAGAATCTTTACCCA
TTGAGGTGATGGTGGACGCCGTGCACAACTCAAGTACGAGAACTACACC

TCCTCCTTCTTTATCCGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACA
 GCTGAAGCCTCTCAAAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCC
 CGACACTTGGAGCACACCCACAGCTACTTCTCTTTAACCTTTTGTGTGCA
 AGTTCAAGGTAAAAGCAAGCGGGAGAAGAAAGACCGGGTGTTTACCGAC
 5 AAAACCAGCGCCACCGTCATCTGTCTGGAAGAACGCCTCCATCAGCGTGAG
 GGCTCAAGATCGTTATTACTCCAGCAGCTGGTCCGAGTGGGCCAGCGTGCC
 TTGTTCC

Human Soluble IL-12 α (p35) (SEQ ID NO: 16)

10 RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPTSEEIDHEDIT
 KDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
 DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQK
 SSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

15 Nucleic Acid Encoding Human Soluble IL-12 α (p35) (SEQ ID NO: 17)

CGTAACCTCCCCGTGGCTACCCCGATCCCGGAATGTTCCCTTGTTTACACC
 ACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAGG
 CAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT
 ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTG
 20 ACAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAT
 GGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCA
 GCTCCATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGA
 ACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACAA
 TGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGA
 25 CCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGA
 TCAAACCTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGA
 CCGGGTCATGAGCTATTTAACGCCAGC

Exemplary Human Soluble IL-12 (SEQ ID NO: 18)

30 IWELKKDVYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK
 TLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
 KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE
 RVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIR

DIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKR
EKKDRVFTDKTSATVICRKNASISVRAQDRYSSSSWSEWASVPCSGGGGSGG
GGSGGGGSRNLPVATPDPGMFPC LHHSQNLLRAVSNMLQKARQTLEFY PCTS
EEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
5 ALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALN
FNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

Nucleic Acid Encoding Exemplary Human Soluble IL-12 (SEQ ID NO: 19)

ATTTGGGAAGTGAAGAAGGACGTCTACGTGGTCTGAACTGGACTGGTATCC
10 CGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGA
AAGACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACAC
ATGCCACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACA
AGAAGGAAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGA
15 GCCCAAGAATAAGACCTTTTTAAGGTGTGAGGCCAAAACTACAGCGGTC
GTTTCACTTGTGTTGGTGGCTGACCACCATTTCACCGATTTAACCTTCTCCGT
GAAAAGCAGCCGGGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCC
GCTACCCTCAGCGCTGAGAGGGTTCGTGGCGATAACAAGGAATACGAGTA
CAGCGTGGAGTGCCAAGAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTT
20 TACCCATTGAGGTGATGGTGGACGCCGTGCACAACTCAAGTACGAGAAC
TACACCTCCTCCTTCTTTATCCGGGACATCATTAAGCCCGATCCTCCTAAG
AATTTACAGCTGAAGCCTCTCAAAAATAGCCGGCAAGTTGAGGTCTCTTG
GGAATATCCCGACACTTGGAGCACACCCACAGCTACTTCTCTTTAACCTT
TTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGGAGAAGAAAGACCGGGTG
25 TTTACCGACAAAACCAGCGCCACCGTCATCTGTCGGAAGAACGCCTCCAT
CAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGCTGGTCCGAGTGGG
CCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGC
GGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTT
CCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGC
30 TGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGACACCAGCGAGGAG
ATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGGCTTG
TTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAAAC
CAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT

GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGT
 GGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGA
 TCTTTTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTT
 TAAACTTCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCC
 5 GATTTTTACAAGACAAAGATCAAAGTGTGCATTTTACTCCACGCCTTTAGG
 ATCCGGGCCGTGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGC

Human Soluble IL-18 (SEQ ID NO: 20)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQP
 10 RGMVTVISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHDKMQ
 FESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE

Nucleic Acid Encoding Human Soluble IL-18 (SEQ ID NO: 21)

TACTTCGGCAAAGTGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGAC
 15 CAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTTCGAGGACATGACC
 GACTCCGATTGCCGGGACAATGCCCCCGGACCATCTTCATTATCTCCATGT
 ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGT
 GAGAAAATCAGCACTTTATCTTGTGAGAACAAAGATCATCTCCTTTAAGGAA
 ATGAACCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCC
 20 AGCGGTCCGTGCCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCT
 ACGAGGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGA
 TCCTCAAGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTACCGTCC
 AAAACGAGGAT

25 In some embodiments, a soluble IL-12 protein can include a first sequence that
 is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least
 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or
 100% identical) to SEQ ID NO: 14, and a second sequence that is at least 70%
 identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at
 30 least 90% identical, at least 95% identical, at least 99% identical, or 100% identical)
 to SEQ ID NO: 16. In some embodiments, the soluble IL-12 can further include a
 linker sequence (e.g., any of the exemplary linker sequences described herein or
 known in the art) between the first sequence and the second sequence.

In some embodiments, a soluble IL-12 protein is encoded by a first nucleic acid encoding a first sequence at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to SEQ ID NO: 15, and a second
 5 nucleic acid sequence encoding a second sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to SEQ ID NO: 17. In some embodiments, the nucleic acid encoding a soluble IL-12 protein further includes a nucleic acid sequence encoding a linker sequence (e.g., any of the
 10 exemplary linker sequences described herein or known in the art) between the first nucleic acid and the second nucleic acid.

In some embodiments, a soluble IL-12 protein includes a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100%
 15 identical) to SEQ ID NO: 18. In some embodiments, a soluble IL-12 protein is encoded by a nucleic acid including a sequence at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to SEQ ID NO: 19.

In some embodiments, a soluble IL-18 protein can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100%
 20 identical) to SEQ ID NO: 20. In some embodiments, a soluble IL-18 protein is encoded by a nucleic acid including a sequence at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to SEQ ID NO: 21.
 25

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain can be a soluble IL-21 (e.g., a soluble human IL-21). For example, one or both of the first target-binding domain and the second target-binding domain
 30 can be a human soluble IL-21 including an amino acid sequence of
 QGQDRHMRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
 AQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
 EFLERFKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 22). In some

embodiments, one or both of the first target-binding domain and the second target-binding domain can include a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 22.

- 5 In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain can be a soluble IL-7 (a soluble human IL-7). For example, one or both of the first target-binding domain and the second target-binding domain can be a human IL-7 including an amino acid sequence of
- 10 DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLQEIKTCWNKILMGTKHEH (SEQ
ID NO: 23). In some embodiments, one or both of the first target-binding domain and the second target-binding domain can include a sequence that is at least 80%, at least
15 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 23.

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

20 **Pairs of Affinity Domains**

- The first and second multi-chain chimeric polypeptides include: 1) a first chimeric polypeptide that includes a first domain of a pair of affinity domains, and 2) a second chimeric polypeptide that includes a second domain of a pair of affinity domains such that the first chimeric polypeptide and the second chimeric polypeptide
25 associate through the binding of the first domain and the second domain of the pair of affinity domains. In some embodiments, the pair of affinity domains is a sushi domain from an alpha chain of an IL-15 receptor (IL15R α) (e.g., a human IL-15 receptor (IL15R α)) and a soluble IL-15 (e.g., a human soluble IL-15). A sushi domain, also known as a short consensus repeat or type 1 glycoprotein motif, is a
30 common motif in protein-protein interaction. Sushi domains have been identified on a number of protein-binding molecules, including complement components C1r, C1s, factor H, and C2m, as well as the nonimmunologic molecules factor XIII and β 2-glycoprotein. A typical Sushi domain has approximately 60 amino acid residues and

contains four cysteines (Ranganathan, *Pac. Symp Biocomput.* 2000:155-67). The first cysteine can form a disulfide bond with the third cysteine, and the second cysteine can form a disulfide bridge with the fourth cysteine. In some embodiments in which one member of the pair of affinity domains is a soluble IL-15, the soluble IL15 has a D8N or D8A amino acid substitution. In some embodiments in which one member of the pair of affinity domains is an alpha chain of human IL-15 receptor (IL15R α), the human IL15R α is a mature full-length IL15R α . In some embodiments, the pair of affinity domains is barnase and barnstar. In some embodiments, the pair of affinity domains is a PKA and an AKAP. In some embodiments, the pair of affinity domains is an adapter/docking tag module based on mutated RNase I fragments (Rossi, *Proc Natl Acad Sci USA.* 103:6841-6846, 2006; Sharkey et al., *Cancer Res.* 68:5282-5290, 2008; Rossi et al., *Trends Pharmacol Sci.* 33:474-481, 2012) or SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25 (Deyev et al., *Nat Biotechnol.* 1486-1492, 2003).

In some embodiments, a first chimeric polypeptide of a first and second multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the first and second multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a dissociation equilibrium constant (K_D) of less than 1×10^{-7} M, less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, less than 1×10^{-11} M, less than 1×10^{-12} M, or less than 1×10^{-13} M. In some embodiments, the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a K_D of about 1×10^{-4} M to about 1×10^{-6} M, about 1×10^{-5} M to about 1×10^{-7} M, about 1×10^{-6} M to about 1×10^{-8} M, about 1×10^{-7} M to about 1×10^{-9} M, about 1×10^{-8} M to about 1×10^{-10} M, about 1×10^{-9} M to about 1×10^{-11} M, about 1×10^{-10} M to about 1×10^{-12} M, about 1×10^{-11} M to about 1×10^{-13} M, about 1×10^{-4} M to about 1×10^{-5} M, about 1×10^{-5} M to about 1×10^{-6} M, about 1×10^{-6} M to about 1×10^{-7} M, about 1×10^{-7} M to about 1×10^{-8} M, about 1×10^{-8} M to about 1×10^{-9} M, about 1×10^{-9} M to about 1×10^{-10} M, about 1×10^{-10} M to about 1×10^{-11} M, about 1×10^{-11} M to about 1×10^{-12} M, or about 1×10^{-12} M to about 1×10^{-13} M (inclusive). Any of a variety of different methods known in the art can be used to determine the K_D value of the binding of the first domain of the pair of affinity

domains and the second domain of the pair of affinity domains (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

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

domains, or both is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the first and/or second domains of a pair of affinity domains disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the first and/or second domains of a pair of affinity domains remains intact. For example, a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a soluble IL-15. Additionally or alternatively, a soluble IL-15 can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a sushi domain from an alpha chain of human IL-15 receptor (IL15R α).

A non-limiting example of a sushi domain from an alpha chain of IL-15 receptor alpha (IL15R α) can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to ITCPPPMMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVA HWTTPSLKCIR (SEQ ID NO: 24). In some embodiments, a sushi domain from an alpha chain of IL15R α can be encoded by a nucleic acid including ATTACATGCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAG AGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAG AGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTAC CAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 25).

In some embodiments, a soluble IL-15 can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLLELQVISLES GDASIHDVTENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQ MFINTS (SEQ ID NO: 26). In some embodiments, a soluble IL-15 can be encoded by a nucleic acid including the sequence of AACTGGGTGAACGTCATCAGCGATTAAAGAAGATCGAAGATTTAATTCAG

TCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTT
 GTAAGGTGACCGCCATGAAATGTTTTTACTGGAGCTGCAAGTTATCTCTTT
 AGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
 AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCA
 5 AGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCC
 TTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 27).

Signal Sequence

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

In some embodiments, a first and/or second polypeptide includes a signal
 20 sequence having an amino acid sequence MKWVTFISLLFLFSSAYS (SEQ ID NO:
 28). In some embodiments, a single chain chimeric polypeptide includes a signal
 sequence encoded by the nucleic acid sequence
 ATGAAATGGGTGACCTTTATTTCTTTACTGTTCTCTTTAGCAGCGCCTACTC
 25 C (SEQ ID NO: 29),
 ATGAAGTGGGTCACATTTATCTCTTTACTGTTCTCTTCTCCAGCGCCTACA
 GC (SEQ ID NO: 30), or
 ATGAAATGGGTGACCTTTATTTCTTTACTGTTCTCTTTAGCAGCGCCTACTC
 C (SEQ ID NO: 31).

In some embodiments, a first and/or second polypeptide includes a signal
 30 sequence having an amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO:
 32). In some embodiments, a first and/or second polypeptide includes a signal
 sequence having an amino acid sequence

MGQIVTMFEALPHIIDEVINIVIIVLIIITSIKAVYNFATCGILALVSFLFLAGRSCG
(SEQ ID NO: 33). In some embodiments, a first and/or second polypeptide includes a
signal sequence having an amino acid sequence

MPNHQSGSPTGSSDLLLSGKKQRPHLALRRKRRREMRKINRKVRRMNLAPIK

5 EKTAWQHLQALISEAEEVLKTSQTPQNSLTLFLALLSVLGPPVTG (SEQ ID NO:
34). In some embodiments, a first and/or second polypeptide includes a signal
sequence having an amino acid sequence

MDSKGSSQKGSRLLLLLVVSNNLLCQGVVS (SEQ ID NO: 35). Those of

ordinary skill in the art will be aware of other appropriate signal sequences for use in
10 a single-chain chimeric polypeptide.

In some embodiments, a first and/or second polypeptide includes a signal
sequence that is about 10 to 100 amino acids in length. For example, a signal
sequence can be about 10 to 100 amino acids in length, about 15 to 100 amino acids
in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in
15 length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length,
about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about
50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to
100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100
amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino
20 acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in
length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length,
about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10
to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70
amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino
25 acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in
length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length,
about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10
to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15
amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino
30 acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in
length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length,
about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20
to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70

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

In some embodiments, any of the signal sequences disclosed herein can
5 include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the signal sequence remains intact. For example, a signal sequence having the amino acid sequence MKWVTFISLLFLFSSAYS (SEQ ID NO: 28) can include one or more additional amino acids at the N-terminus or C-terminus, while still retaining the
10 ability to direct the single-chain chimeric polypeptide to the secretory pathway.

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

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

In some embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence having an amino

acid sequence MKWVTFISLLFLFSSAYS (SEQ ID NO: 28). In some embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence encoded by the nucleic acid sequence

5 ATGAAATGGGTGACCTTTATTTCTTTACTGTTTCCTCTTTAGCAGCGCCTACT CC (SEQ ID NO: 29),

ATGAAGTGGGTCACATTTATCTCTTTACTGTTTCCTCTTCTCCAGCGCCTAC AGC (SEQ ID NO: 30), or

ATGAAATGGGTGACCTTTATTTCTTTACTGTTTCCTCTTTAGCAGCGCCTACT

10 CC (SEQ ID NO: 31).

In some embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 32). In some

15 embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence

MGQIVTMFEALPHIIDEVINIVIIVLIITSIKAVYNFATCGILALVSFLFLAGRSCG

20 (SEQ ID NO: 36). In some embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence

MPNHQSGSPTGSSDLLLSGKKQRPHLALRRKRRREMRKINRKVRRMNLAPIK

25 EKTAWQHLQALISEAEEVLKTSQTPQNSLTFLALLSVLGPPVTG (SEQ ID NO: 37). In some embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence MDSKGSSQKGSRLLLLLVVSNNLLCQGVVS (SEQ ID NO: 38).

30 Those of ordinary skill in the art will be aware of other appropriate signal sequences for use in a first chimeric polypeptide and/or a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptides described herein.

In some embodiments, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both includes a signal sequence that is about 10 to 100 amino acids in length. For example, a signal sequence can be about
5 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length,
10 about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length,
15 about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a signal sequence is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the signal sequences disclosed herein can
30 include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the signal sequence remains intact. For example, a signal sequence having the amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 32) can include one or more

additional amino acids at the N-terminus or C-terminus, while still retaining the ability to direct the a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both to the secretory pathway.

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

Peptide Tags

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

25 Exemplary peptide tags that can be included in a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both include, without limitation, AviTag (GLNDIFEAQKIEWHE; SEQ ID NO: 75), a calmodulin-tag (KRRWKKNFIAVSAANRFKKISSSGAL; SEQ ID NO: 76), a polyglutamate tag
(EEEEEE; SEQ ID NO: 77), an E-tag (GAPVPYPDPLEPR; SEQ ID NO: 78), a
30 FLAG-tag (DYKDDDDK; SEQ ID NO: 79), an HA-tag, a peptide from hemagglutinin (YPYDVPDYA; SEQ ID NO: 80), a his-tag (HHHHH (SEQ ID NO: 39); HHHHHH (SEQ ID NO: 40); HHHHHHH (SEQ ID NO: 41); HHHHHHHH

- (SEQ ID NO: 42); HHHHHHHHHH (SEQ ID NO: 43); or HHHHHHHHHH (SEQ ID NO: 44)), a myc-tag (EQKLISEEDL; SEQ ID NO: 45), NE-tag (TKENPRSNQEESYDDNES; SEQ ID NO: 46), S-tag, (KETAAAKFERQHMDS; SEQ ID NO: 47), SBP-tag
- 5 (MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP; SEQ ID NO: 48), Softag 1 (SLAELLNAGLGGS; SEQ ID NO: 49), Softag 3 (TQDPSRVG; SEQ ID NO: 50), Spot-tag (PDRVRAVSHWSS; SEQ ID NO: 51), Strep-tag (WSHPQFEK; SEQ ID NO: 52), TC tag (CCPGCC; SEQ ID NO: 53), Ty tag (EVHTNQDPLD; SEQ ID NO: 54), V5 tag (GKPIPNPLLGLDST; SEQ ID NO: 55), VSV-tag
- 10 (YTDIEMNRLGK; SEQ ID NO: 56), and Xpress tag (DLYDDDDK; SEQ ID NO: 57). In some embodiments, tissue factor protein is a peptide tag.

Peptide tags that can be included in a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both can be used in any of a

15 variety of applications related to the multi-chain chimeric polypeptide. For example, a peptide tag can be used in the purification of a first and/or second multi-chain chimeric polypeptide. As one non-limiting example, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the first and/or second

20 multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both can include a myc tag; the first and/or second multi-chain chimeric polypeptide that includes the myc-tagged first chimeric polypeptide, the myc-tagged second chimeric polypeptide, or both can be purified using an antibody that recognizes the myc tag(s). One non-limiting example of an antibody that

25 recognizes a myc tag is 9E10, available from the non-commercial Developmental Studies Hybridoma Bank. As another non-limiting example, a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide (e.g., a recombinantly

30 expressed second chimeric polypeptide), or both can include a histidine tag; the first and/or second multi-chain chimeric polypeptide that includes the histidine-tagged first chimeric polypeptide, the histidine-tagged second chimeric polypeptide, or both can be purified using a nickel or cobalt chelate. Those of ordinary skill in the art will be

aware of other suitable tags and agent that bind those tags for use in purifying a first and/or second multi-chain chimeric polypeptide. In some embodiments, a peptide tag is removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide after purification. In some embodiments, a peptide tag is not removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide after purification.

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

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

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

Peptide tags included in a first chimeric polypeptide of a first and/or second multi-chain chimeric polypeptide, a second chimeric polypeptide of the first and/or second multi-chain chimeric polypeptide, or both can be of any suitable length. For example, peptide tags can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids in length. In embodiments in which a first and/or second multi-chain chimeric polypeptide includes two or more peptide tags, the two or more peptide tags can be of the same or different lengths. In some embodiments, any of the peptide tags disclosed herein may include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at the N-terminus and/or C-terminus, so long as the function of the peptide tag remains intact. For example, a myc tag having the amino acid sequence EQKLISEEDL (SEQ ID NO: 58) can include one or more additional amino acids (e.g., at the N-terminus and/or the C-terminus of the peptide tag), while still retaining the ability to be bound by an antibody (e.g., 9E10).

Compositions/Kits

Also provided herein are compositions (e.g., pharmaceutical compositions) that include at least one of any of the single-chain chimeric polypeptides, multi-chain chimeric polypeptides, IgG1 antibody constructs, any of the cells, or any of the nucleic acids described herein. In some embodiments, the compositions include at least one of any of the single-chain chimeric polypeptides described herein. In some embodiments, the compositions include at least one of any of the multi-chain chimeric polypeptides described herein (e.g., a first multi-chain chimeric polypeptide and/or a second multi-chain chimeric polypeptide). In some embodiments, the compositions include at least one of any of the multi-chain chimeric polypeptides and any of the

IgG1 antibodies described herein. In some embodiments, the compositions include any of the NK cells (e.g., any of the NK cells described herein, e.g., any of the NK cells produced using any of the methods described herein).

In some embodiments, the pharmaceutical compositions are formulated for
5 different routes of administration (e.g., intravenous, subcutaneous). In some
embodiments, the pharmaceutical compositions can include a pharmaceutically
acceptable carrier (e.g., phosphate buffered saline).

Single or multiple administrations of pharmaceutical compositions can be
given to a subject in need thereof depending on for example: the dosage and
10 frequency as required and tolerated by the subject. The formulation should provide a
sufficient quantity of active agent to effectively treat, prevent or ameliorate
conditions, diseases or symptoms.

Also provided herein are kits that include any of multi-chain chimeric
polypeptides (e.g., any of the first multi-chain chimeric polypeptides and second
15 multi-chain polypeptides described herein), IgG1 antibody constructs (e.g., any of the
IgG1 antibody constructs described herein), compositions, nucleic acids, and/or cells
(e.g., NK cells) described herein. In some embodiments, the kits can include
instructions for performing any of the methods described herein. In some
embodiments, the kits can include at least one dose of any of the pharmaceutical
20 compositions described herein.

Also provided herein is an NK cell (e.g., an activated NK cell) produced by
any of the methods described herein. Also provided herein are pharmaceutical
compositions that include any of the activated NK cells produced by any of the
methods described herein. Also provided herein are kits that include any of the
25 pharmaceutical compositions described herein that include any of the activated NK
cells (e.g., an activated NK cell) produced by any of the methods described herein.

Also provided herein are kits that include any of the IgG1 antibody constructs
described herein and any of the multi-chain chimeric polypeptides described herein
(e.g., any of the exemplary first multi-chain chimeric polypeptides described herein
30 and/or any of the exemplary second multi-chain chimeric polypeptides described
herein). In some embodiments of any of the kits described herein, the kits further
include instructions for performing any of the methods described herein.

Nucleic Acids/Vectors

Also provided herein are nucleic acids that encode any of the first and/or second polypeptides of any of the first multi-chain chimeric polypeptides described herein. Also provided herein are vectors that include any of the nucleic acids
5 encoding any of the first and/or second polypeptides of any of the second multi-chain chimeric polypeptides described herein.

Also provided herein are vectors that include any of the nucleic acids encoding any of the multi-chain chimeric polypeptides described herein. In some embodiments, a first vector can include a nucleic acid encoding the first chimeric
10 polypeptide of the first multi-chain chimeric polypeptide and a second vector can include a nucleic acid encoding the second chimeric polypeptide of the first multi-chain chimeric polypeptide. In some embodiments, a single vector can include a first nucleic acid encoding the first chimeric polypeptide of the first multi-chain chimeric polypeptide and a second nucleic acid encoding the second chimeric polypeptide of
15 the first multi-chain chimeric polypeptide.

In some embodiments, a first vector can include a nucleic acid encoding the first chimeric polypeptide of the second multi-chain chimeric polypeptide and a second vector can include a nucleic acid encoding the second chimeric polypeptide of the second multi-chain chimeric polypeptide. In some embodiments, a single vector
20 can include a nucleic acid encoding the first chimeric polypeptide of the second multi-chain chimeric polypeptide and a second nucleic acid encoding the second chimeric polypeptide of the second multi-chain chimeric polypeptide.

Also provided herein is a single nucleic acid sequence that encode the first and second multi-chain chimeric polypeptides. In some embodiments, a single nucleic
25 acid can encode both the both of the first and second chimeric polypeptides of the first multi-chain chimeric polypeptide, and the first and second chimeric polypeptides of the second multi-chain chimeric polypeptide.

Also provided herein are the vectors that include the single nucleic acid sequences that encode both the first and second multi-chain chimeric polypeptides
30 (e.g., the first and second chimeric polypeptides of each the first and second multi-chain chimeric polypeptides).

Also provided herein are nucleic acids that encode any of the IgG1 antibody constructs described herein.

Any of the vectors described herein can be an expression vector. For example, an expression vector can include a promoter sequence operably linked to the sequence encoding the single-chain chimeric polypeptide, or the first chimeric polypeptide and the second chimeric polypeptide of a multi-chain chimeric polypeptide. In another
5 example, an expression vector can include a promoter sequence operably linked to the sequence encoding any of the first polypeptides and/or second polypeptides described herein.

Non-limiting examples of vectors include plasmids, transposons, cosmids, and viral vectors (e.g., any adenoviral vectors (e.g., pSV or pCMV vectors), adeno-
10 associated virus (AAV) vectors, lentivirus vectors, and retroviral vectors), and any Gateway® vectors. A vector can, e.g., include sufficient cis-acting elements for expression; other elements for expression can be supplied by the host mammalian cell or in an in vitro expression system. Skilled practitioners will be capable of selecting suitable vectors and mammalian cells for making any of the single-chain chimeric
15 polypeptides or multi-chain chimeric polypeptides described herein.

Cells

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) comprising any of the nucleic acids described herein that encode any of the exemplary first polypeptides and/or second polypeptides of any of
20 the first multi-chain chimeric polypeptides described herein.

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include any of the vectors described herein that encode any of the first polypeptides and/or second polypeptides of any of the first multi-chain chimeric polypeptides described herein.

25 Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) comprising any of the nucleic acids described herein that encode any of the first polypeptides and/or second polypeptides of any of the second multi-chain chimeric polypeptides described herein.

Also provided herein are cells (e.g., any of the exemplary cells described
30 herein or known in the art) that include any of the vectors described herein that encode any of the first polypeptides and/or second polypeptides of any of the second multi-chain chimeric polypeptides described herein.

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) comprising any of the nucleic acids described herein that encode any of the multi-chain chimeric polypeptides described herein (e.g., encoding both the first and second chimeric polypeptides of the first multi-chain chimeric polypeptide and/or the second multi-chain chimeric polypeptide). Also provided
5 herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include any of the vectors described herein that encode any of the multi-chain chimeric polypeptides described herein (e.g., encoding both the first and second chimeric polypeptides of the first multi-chain chimeric polypeptide and/or the second
10 multi-chain chimeric polypeptide).

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include (i) any of the nucleic acids described herein that encode a first multi-chain chimeric polypeptide, (ii) any of the nucleic acids described herein that encode a second multi-chain chimeric polypeptide, and (iii) any
15 of the nucleic acids described herein that encode an IgG1 antibody construct.

Also provided herein are cells (e.g., any of the exemplary cells described herein or known in the art) that include (i) any of the vectors described herein that encode a first multi-chain chimeric polypeptide, (ii) any of the vectors that encode a second multi-chain chimeric polypeptide, and (iii) any of the vectors that encode an
20 IgG1 antibody construct.

In some embodiments of any of the methods described herein, the cell can be a eukaryotic cell. As used herein, the term “eukaryotic cell” refers to a cell having a distinct, membrane-bound nucleus. Such cells may include, for example, mammalian (e.g., rodent, non-human primate, or human), insect, fungal, or plant cells. In some
25 embodiments, the eukaryotic cell is a yeast cell, such as *Saccharomyces cerevisiae*. In some embodiments, the eukaryotic cell is a higher eukaryote, such as mammalian, avian, plant, or insect cells. Non-limiting examples of mammalian cells include Chinese hamster ovary cells and human embryonic kidney cells (e.g., HEK293 cells).

Methods of introducing nucleic acids and expression vectors into a cell (e.g., a
30 eukaryotic cell) are known in the art. Non-limiting examples of methods that can be used to introduce a nucleic acid into a cell include lipofection, transfection, electroporation, microinjection, calcium phosphate transfection, dendrimer-based transfection, cationic polymer transfection, cell squeezing, sonoporation, optical

transfection, impalefection, hydrodynamic delivery, magnetofection, viral transduction (e.g., adenoviral and lentiviral transduction), and nanoparticle transfection.

5 **Methods of Producing Single-Chain and Multi-Chain Chimeric Polypeptides**

Also provided herein are methods of producing any of the single-chain chimeric polypeptides described herein that include culturing any of the cells described herein in a culture medium under conditions sufficient to result in the production of the single-chain chimeric polypeptide; and recovering the single-chain
10 chimeric polypeptide from the cell and/or the culture medium.

The recovery of the single-chain chimeric polypeptide from a culture medium or a cell (e.g., a eukaryotic cell) can be performed using techniques well-known in the art (e.g., ammonium sulfate precipitation, polyethylene glycol precipitation, ion-exchange chromatography (anion or cation), chromatography based on hydrophobic
15 interaction, metal-affinity chromatography, ligand-affinity chromatography, and size exclusion chromatography).

Also provided herein are single-chain chimeric polypeptides (e.g., any of the single-chain chimeric polypeptides described herein) produced by any of the methods described herein.

20 Also provided herein are methods of producing any of the multi-chain chimeric polypeptides described herein that include culturing any of the cells described herein in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

25 Also provided herein are methods of producing any of the multi-chain chimeric polypeptides described herein that include: culturing any of cells described herein in a first culture medium under conditions sufficient to result in the production of the first chimeric polypeptide; recovering the first chimeric polypeptide from the cell and/or the first culture medium; culturing any of the cells described herein in a
30 second culture medium under conditions sufficient to result in the production of the second chimeric polypeptide; recovering the second chimeric polypeptide from the cell and/or the second culture medium; and combining (e.g., mixing) the recovered first chimeric polypeptide and the recovered second chimeric polypeptide to form the

multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein).

The recovery of the multi-chain chimeric polypeptide, the first chimeric polypeptide, or the second chimeric polypeptide from a cell (e.g., a eukaryotic cell) can be performed using techniques well-known in the art (e.g., ammonium sulfate precipitation, polyethylene glycol precipitation, ion-exchange chromatography (anion or cation), chromatography based on hydrophobic interaction, metal-affinity chromatography, ligand-affinity chromatography, and size exclusion chromatography).

Also provided herein are multi-chain chimeric polypeptides (e.g., any of the multi-chain chimeric polypeptides described herein), first chimeric polypeptides (e.g., any of the first chimeric polypeptides), or second chimeric polypeptides (e.g., any of the second chimeric polypeptides described herein) produced by any of the methods described herein.

Methods of culturing cells are well known in the art. Cells can be maintained in vitro under conditions that favor proliferation, differentiation and growth. Briefly, cells can be cultured by contacting a cell (e.g., any cell) with a cell culture medium that includes the necessary growth factors and supplements to support cell viability and growth.

Methods of Promoting the Activation and Proliferation of an Immune Cell

Also provided herein are methods of promoting the activation and proliferation of a NK cell (e.g., any of the exemplary NK cells described herein or known in the art) that include contacting a NK cell in a liquid culture medium including an effective amount of a first multi-chain chimeric polypeptide described herein for a first period of time under conditions that allow for differentiation of the NK cell, and contacting the NK cell in a liquid culture medium including an effective amount of (i) a second multi-chain chimeric polypeptides described herein, and (ii) an IgG1 antibody construct, that comprises at least one antigen-binding domain that binds specifically to the linker domain (e.g., a monoclonal or polyclonal human, mouse, rabbit, or goat IgG1 antibody that binds specifically to a linker domain or any of the other exemplary IgG1 antibody constructs described herein), for a second

period of time under conditions that allow for the activation and proliferation of the NK cell.

In some embodiments of these methods, the IgG1 antibody construct includes at least one antigen-binding domain that binds specifically to the linker domain in the second multi-chain chimeric polypeptide. In some embodiments of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some embodiments of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the first period of time is about 15 minutes to about 4 hours (e.g., about 15 minutes to about 3.75 hours, about 15 minutes to about 3.5 hours, about 15 minutes to about 3.25 hours, about 15 minutes to about 3 hours, about 15 minutes to about 2.75 hours, about 15 minutes to about 2.5 hours, about 15 minutes to about 2.25 hours, about 15 minutes to about 2 hours, about 15 minutes to about 1.75 hours, about 15 minutes to about 1.5 hours, about 15 minutes to about 1.25 hours, about 15 minutes to about 1 hour, about 15 minutes to about 45 minutes, about 15 minutes to about 30 minutes, about 30 minutes to about 3.75 hours, about 30 minutes to about 3.5 hours, about 30 minutes to about 3.25 hours, about 30 minutes to about 3 hours, about 30 minutes to about 2.75 hours, about 30 minutes to about 2.5 hours, about 30 minutes to about 2.25 hours, about 30 minutes to about 2 hours, about 30 minutes to about 1.75 hours, about 30 minutes to about 1.5 hours, about 30 minutes to about 1.25 hours, about 30 minutes to about 1 hour, about 30 minutes to about 45 minutes, about 45 minutes to about 3.75 hours, about 45 minutes to about 3.5 hours, about 45 minutes to about 3.25 hours, about 45 minutes to about 3 hours, about 45 minutes to about 2.75 hours, about 45 minutes to about 2.5 hours, about 45 minutes to about 2.25 hours, about 45 minutes to about 2 hours, about 45 minutes to about 1.75 hours, about 45 minutes to about 1.5 hours, about 45 minutes to about 1.25 hours, about 45 minutes to 1 hour, about 1 hour to about 3.75 hours, about 1 hour to about 3.5 hours, about 1 hour to about 3.25 hours, about 1 hour to about 3 hours, about 1 hour to about 2.75 hours, about 1 hour to about 2.5 hours, about 1 hour to about 2.25 hours, about 1 hour to about 2 hours, about 1 hour to about 1.75 hours, about 1 hour to about 1.5

hours, about 1 hour to about 1.25 hours, about 1.25 hours to about 3.75 hours, about 1.25 hours to about 3.5 hours, about 1.25 hours to about 3.25 hours, about 1.25 hours to about 3 hours, about 1.25 hours to about 2.75 hours, about 1.25 hours to about 2.75 hours, about 1.25 hours to about 2.5 hours, about 1.25 hours to about 2.25 hours, about 1.25 hours to about 2 hours, about 1.25 hours to about 1.75 hours, about 1.25 hours to about 1.5 hours, about 1.5 hours to about 3.75 hours, about 1.5 hours to about 3.5 hours, about 1.5 hours to about 3.25 hours, about 1.5 hours to about 3 hours, about 1.5 hours to about 2.75 hours, about 1.5 hours to about 2.5 hours, about 1.5 hours to about 2.25 hours, about 1.5 hours to about 2 hours, about 1.5 hours to about 1.75 hours, about 1.75 hours to about 3.75 hours, about 1.75 hours to about 3.5 hours, about 1.75 hours to about 3.25 hours, about 1.75 hours to about 3 hours, about 1.75 hours to about 2.75 hours, about 1.75 hours to about 2.5 hours, about 1.75 hours to about 2.25 hours, or about 1.75 hours to 2 hours).

In some embodiments of these methods, the second period is about 1 day to about 30 days (e.g., about 1 day to about 28 days, about 1 day to about 26 days, about 1 day to about 24 days, about 1 day to about 22 days, about 1 day to about 20 days, about 1 day to about 18 days, about 1 day to about 16 days, about 1 day to about 14 days, about 1 day to about 12 days, about 1 day to about 10 days, about 1 day to about 8 days, about 1 day to about 7 days, about 1 day to about 6 days, about 1 day to about 5 days, about 1 day to about 4 days, about 1 day to about 3 days, about 1 day to about 2 days, about 2 days to about 28 days, about 2 days to about 26 days, about 2 days to about 24 days, about 2 days to about 22 days, about 2 days to about 20 days, about 2 days to about 18 days, about 2 days to about 16 days, about 2 days to about 14 days, about 2 days to about 12 days, about 2 days to about 10 days, about 2 days to about 8 days, about 2 days to about 7 days, about 2 days to about 6 days, about 2 days to about 5 days, about 2 days to about 4 days, about 2 days to about 3 days, about 3 days to about 28 days, about 3 days to about 26 days, about 3 days to about 24 days, about 3 days to about 22 days, about 3 days to about 20 days, about 3 days to about 18 days, about 3 days to about 16 days, about 3 days to about 14 days, about 3 days to about 12 days, about 3 days to about 10 days, about 3 days to about 8 days, about 3 days to about 7 days, about 3 days to about 6 days, about 3 days to about 5 days, about 3 days to about 4 days, about 4 days to about 28 days, about 4 days to about 26 days, about 4 days to about 24 days, about 4 days to about 22 days, about 4 days to about 20 days,

about 4 days to about 18 days, about 4 days to about 16 days, about 4 days to about 14 days, about 4 days to about 12 days, about 4 days to about 10 days, about 4 days to about 8 days, about 4 days to about 7 days, about 4 days to about 6 days, about 4 days to about 5 days, about 5 days to about 28 days, about 5 days to about 26 days, about 5 days to about 24 days, about 5 days to about 22 days, about 5 days to about 20 days, about 5 days to about 18 days, about 5 days to about 16 days, about 5 days to about 14 days, about 5 days to about 12 days, about 5 days to about 10 days, about 5 days to about 8 days, about 5 days to about 7 days, about 5 days to about 6 days, about 6 days to about 28 days, about 6 days to about 26 days, about 6 days to about 24 days, about 6 days to about 22 days, about 6 days to about 20 days, about 6 days to about 18 days, about 6 days to about 16 days, about 6 days to about 14 days, about 6 days to about 12 days, about 6 days to about 10 days, about 6 days to about 8 days, about 6 days to about 7 days, about 7 days to about 28 days, about 7 days to about 26 days, about 7 days to about 24 days, about 7 days to about 22 days, about 7 days to about 20 days, about 7 days to about 18 days, about 7 days to about 16 days, about 7 days to about 14 days, about 7 days to about 12 days, about 7 days to about 10 days, about 7 days to about 8 days, about 8 days to about 28 days, about 8 days to about 26 days, about 8 days to about 24 days, about 8 days to about 22 days, about 8 days to about 20 days, about 8 days to about 18 days, about 8 days to about 16 days, about 8 days to about 14 days, about 8 days to about 12 days, about 8 days to about 10 days, about 8 days to about 9 days, about 9 days to about 28 days, about 9 days to about 26 days, about 9 days to about 24 days, about 9 days to about 22 days, about 9 days to about 20 days, about 9 days to about 18 days, about 9 days to about 16 days, about 9 days to about 14 days, about 9 days to about 12 days, about 9 days to about 10 days, about 10 days to about 28 days, about 10 days to about 26 days, about 10 days to about 24 days, about 10 days to about 22 days, about 10 days to about 20 days, about 10 days to about 18 days, about 10 days to about 16 days, about 10 days to about 14 days, about 10 days to about 12 days, about 12 days to about 28 days, about 12 days to about 26 days, about 12 days to about 24 days, about 12 days to about 22 days, about 12 days to about 20 days, about 12 days to about 18 days, about 12 days to about 16 days, about 12 days to about 14 days, about 14 days to about 28 days, about 14 days to about 26 days, about 14 days to about 24 days, about 14 days to about 22 days, about 14 days to about 20 days, about 14 days to about 18 days, about 14 days to about 16 days,

about 16 days to about 28 days, about 16 days to about 26 days, about 16 days to about 24 hours, about 16 days to about 22 days, about 16 days to about 20 days, about 16 days to about 18 days, about 18 days to about 28 days, about 18 days to about 26 days, about 18 days to about 24 days, about 18 days to about 22 days, about 18 days to about 20 days, about 20 days to about 28 days, about 20 days to about 26 days, about 20 days to about 24 days, about 20 days to about 22 days, 22 days to about 28 days, about 22 days to about 26 days, about 22 days to about 24 days, about 24 days to about 28 days, about 24 days to about 26 days, or about 26 to about 28 days.

In some embodiments of any of the methods described herein, the liquid culture medium is a serum-free liquid culture medium. In some embodiments of any of the methods described herein, the liquid culture medium is a chemically-defined liquid culture medium.

In some embodiments of any of the methods described herein, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some embodiments of any of the methods described herein, the NK cell was previously obtained from a subject. Some embodiments of any of the methods described herein further include obtaining the NK cell from the subject prior to the contacting step.

In some embodiments, the NK cell was previously obtained from umbilical cord blood or induced pluripotent stem cells. Some embodiments of any of the methods described herein further include obtaining the NK cell from umbilical cord blood. Some embodiments further include generating the NK cell from an induced pluripotent stem cell.

In some embodiments of any of the methods described herein, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Some embodiments of any of the methods described herein further includes, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Some embodiments of any of the methods described herein further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Some embodiments of any of the methods described herein further include,
5 after the contacting step, isolating the NK cell.

In some embodiments of any of the methods described herein, after the first and/or second period of time, the NK cell is a chemokine-induced memory NK cell. In some examples, after the first and/or second period of time, the NK cell has an increased level of expression, secretion, and or activity of one or more proteins
10 selected from the group consisting of: 2B4, CD8, CD11a, CD16, CD25, CD27, CD48, CD49d, CD54, CD56, CD58, CD62L, CD69, CD70, CD94, CD137, CD158a, CD158b, CD158e, CD178, CD226, CD253, NKG2C, NKG2D, LIR-1, LILR-B1, KIR2DL1, KIR3DL1, KIR2DL2, KIR2DL3, CXCR3, NKp30, NKp44, NKp46, NKG2D, DNAM-1, TRAIL, FasL, CXCR3, CXCR4, LTB, MX1, BAX, TNF- α ,
15 STAT5, and IFN- γ as compared to the level of expression or secretion of the one or more proteins prior to the first period of time.

Some embodiments of any of the methods described herein further include, after the contacting step, administering the NK cell to a subject in need thereof. In some embodiments of any of the methods described herein, the subject has been
20 identified or diagnosed as having an age-related disease or condition. In some embodiments of any of the methods described herein, the age-related disease or condition is selected from the group of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular
25 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,
30 glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some embodiments of any of the methods described herein, the subject has been identified or diagnosed as having a cancer. In some embodiments of any of the methods described herein, the cancer is selected from the group 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 embodiments of any of the methods described herein, the subject has been diagnosed or identified as having an infectious disease. In some embodiments of any of the methods described herein, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

Also provided herein is an activated NK produced by any of the methods described herein. Also provided herein are pharmaceutical compositions that include any of the NK cells produced by any of the methods described herein. Also provided herein are kits that include any of the pharmaceutical compositions including any of the activated NK cells produced by any of the methods described herein.

Methods of Stimulating an NK Cell

Also provided herein are methods of stimulating a NK cell (e.g., any of the exemplary NK cells described herein or known in the art) that include contacting a NK cell in a liquid culture medium including an effective amount of a first multi-chain chimeric polypeptide described herein for a first period of time under conditions that allow for differentiation of the NK cell, and contacting the NK cell in a liquid culture medium including an effective amount of (i) a second multi-chain chimeric

polypeptides described herein, and (ii) an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the linker domain (e.g., a monoclonal or polyclonal human, mouse, rabbit, or goat IgG1 antibody that binds specifically to a linker domain or any of the other exemplary IgG1 antibody constructs described herein), for a second period of time under conditions that allow for stimulation of the NK cell. In some examples, the NK cell is contacted *in vitro* (e.g., in a suitable liquid culture medium under conditions sufficient to result in stimulation of the NK cell). In some examples, the contacting further includes contacting the NK cell with an effective amount of any of the IgG1 antibody constructs described herein.

In some examples, the NK cell has been previously obtained from a subject (e.g., a mammal, e.g., a human). Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step.

In some examples, the NK cell is contacted *in vivo*. In such embodiments, the first multi-chain chimeric polypeptide, the second multi-chain chimeric polypeptide, and the IgG1 antibody construct is administered to a subject (e.g., a mammal, e.g., a human) in an amount sufficient to result in stimulation of an immune cell in the subject. In some embodiments of any of the methods, compositions, and kits described herein, the IgG1 antibody construct (e.g., any of the exemplary IgG1 antibody constructs described herein) is administered to the subject in combination (e.g., simultaneously or sequentially) with administration of the first and second multi-chain chimeric polypeptides (e.g., any of the first and second multi-chain chimeric polypeptides described herein)).

In some examples, the NK cell has previously been genetically-modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Some embodiments of these methods can further include, after the contacting step, introducing into the NK cell (e.g., any of the NK cells described herein) a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods can further include administering a therapeutically effective amount of the NK cell to a subject in need thereof (e.g., any of the exemplary subjects described herein).

In some examples, the subject can be a subject identified or diagnosed as having an age-related disease or condition. Non-limiting examples of age-related

diseases or disorders include: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples, the subject can be a subject that has been identified or diagnosed as having a cancer. Non-limiting examples of cancers include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples, the subject can be a subject that has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, or influenza virus.

Activation of an NK cell can be determined using methods known in the art. For example, activation of an immune cell can be determined by detecting the levels of cytokines, chemokines, and activating receptors that are secreted or upregulated upon activation of an immune cell. Non-limiting examples of cytokines, chemokines

and activating receptors that are secreted or upregulated upon activation of an immune cell include: IL-2, interferon- γ , IL-1, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, IL-22, IL-33, leukotriene B4, CCL5, TNF α , perforin, TGF β , STAT3, ROR γ T, FOXP3, STAT6, GATA3, granzyme A, granzyme B, 2B4, CD8, CD11a, CD16, CD25, CD27, CD48, CD49d, CD54, CD56, CD58, CD62L, CD69, CD70, CD94, CD137, CD158a, CD158b, CD158e, CD178, CD226, CD253, NKG2C, NKG2D, LIR-1, LILR-B1, KIR2DL1, KIR3DL1, KIR2DL2, KIR2DL3, CXCR3, NKp30, NKp44, NKp46, NKG2D, DNAM-1, TRAIL, FasL, CXCR3, CXCR4, LTB, MX1, BAX, TNF- α , and IFN- γ . The detection of these cytokines and chemokines can be performed using an immunoassay (e.g., an enzyme-linked immunosorbent assay). For example, activation of an immune cell can result in an increase of about 1% to about 800% (e.g., about 1% to about 750%, about 1% to about 700%, about 1% to about 650%, about 1% to about 600%, about 1% to about 550%, about 1% to about 500%, about 1% to about 450%, about 1% to about 400%, about 1% to about 350%, about 1% to about 300%, about 1% to about 280%, about 1% to about 260%, about 1% to about 240%, about 1% to about 220%, about 1% to about 200%, about 1% to about 180%, about 1% to about 160%, about 1% to about 140%, about 1% to about 120%, about 1% to about 100%, about 1% to about 90%, about 1% to about 80%, about 1% to about 70%, about 1% to about 60%, about 1% to about 50%, about 1% to about 45%, about 1% to about 40%, about 1% to about 35%, about 1% to about 30%, about 1% to about 25%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 5%, about 5% to about 800%, about 5% to about 750%, about 5% to about 700%, about 5% to about 650%, about 5% to about 600%, about 5% to about 550%, about 5% to about 500%, about 5% to about 450%, about 5% to about 400%, about 5% to about 350%, about 5% to about 300%, about 5% to about 280%, about 5% to about 260%, about 5% to about 240%, about 5% to about 220%, about 5% to about 200%, about 5% to about 180%, about 5% to about 160%, about 5% to about 140%, about 5% to about 120%, about 5% to about 100%, about 5% to about 90%, about 5% to about 80%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 5% to about 10%, about 10% to about 800%, about 10% to about 750%, about 10% to about 700%, about 10% to about 650%,

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Methods of Inducing or Increasing Proliferation of a NK Cell

Also provided herein are methods of inducing or increasing proliferation of an NK cell (e.g., any of the exemplary NK cells described herein or known in the art) that include contacting a NK cell in a liquid culture medium including an effective amount of a first multi-chain chimeric polypeptide described herein for a first period of time under conditions that allow for differentiation of the NK cell, and contacting the NK cell in a liquid culture medium including an effective amount of (i) a second multi-chain chimeric polypeptides described herein, and (ii) an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the linker domain (e.g., a monoclonal or polyclonal human, mouse, rabbit, or goat IgG1 antibody that binds specifically to a linker domain or any of the other exemplary IgG1 antibody constructs described herein), for a second period of time under conditions that allow for the inducing or increasing proliferation of the NK cell.

In some examples, the NK cell is contacted *in vitro* (e.g., in a suitable liquid culture medium under conditions sufficient to result in stimulation of the immune cell)). In some examples, the NK cell is further contacted *in vitro* with any of the IgG1 antibody constructs described herein in combination (e.g., simultaneously or

sequentially) with contacting the immune cell *in vitro* with the multi-chain chimeric polypeptides (e.g., any of the first and/or second multi-chain chimeric polypeptides described herein).

In some examples, the NK cell has been previously obtained from a subject
5 (e.g., a mammal, e.g., a human). Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step.

In some examples, the NK cell is contacted *in vivo*. In such embodiments, any of the first multi-chain chimeric polypeptides, any of the second multi-chain chimeric polypeptides, and any of the IgG1 antibody constructs are administered to a subject
10 (e.g., a mammal, e.g., a human) in an amount sufficient to result in stimulation of a NK cell in the subject.

In some examples, the NK cell has previously been genetically-modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

Some embodiments of these methods can further include, after the contacting
15 step, introducing into the NK cell (e.g., any of the NK cells described herein) a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Some embodiments of these methods can further include administering a therapeutically effective amount of the NK cell to a subject in need thereof (e.g., any of the exemplary subjects described herein).

20 In some examples, the subject can be a subject identified or diagnosed as having an age-related disease or condition. Non-limiting examples of age-related diseases or disorders include: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis,
25 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
30 cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples, the subject can be a subject that has been identified or diagnosed as having a cancer. Non-limiting examples of cancers include: solid tumor,

hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML),
 5 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
 10 carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples, the subject can be a subject that has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease include infection with human immunodeficiency virus, cytomegalovirus,
 15 adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, or influenza virus.

Detection of the proliferation of an immune cell can be performed using methods known in the art, e.g., cytometry (e.g., fluorescence-assisted flow
 20 cytometry), microscopy, and immunofluorescence microscopy, e.g., by comparing the rate of increase in the concentration of the immune cell in a sample not contacted with a single-chain chimeric polypeptide or a multi-chain chimeric polypeptide to the rate of increase in the concentration of the immune cell in a similar sample contacted with any of the single-chain chimeric polypeptides described herein or any of the multi-
 25 chain chimeric polypeptides described herein).

In other examples, the proliferation of an NK cell can be indirectly detected by detecting an increase in the level of one or more cytokines, chemokines and proliferation biomarkers secreted or upregulated on the surface by proliferating immune cells (e.g., one or more of IL-2, interferon- γ , IL-1, IL-4, IL-5, IL-6, IL-7, IL-
 30 9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, IL-22, IL-33, leukotriene B4, CCL5, TNF α , TGF β , TNF- α , IFN- γ , and Ki67) (e.g., as compared to the level of the one or more cytokines and chemokines in a control not contacted with any of the single-chain chimeric polypeptides described herein).

In some embodiments, the methods provided herein can result in an increase (e.g., about 1% to about 800% increase, or any of the subranges of this range described herein) in the rate of increase in the concentration of the NK in a sample contacted with any of the first and second multi-chain chimeric polypeptides described herein and the IgG1 antibody constructs described herein (as set forth in the methods described herein) as compared to the rate of increase in a similar control sample before the first period of time.

Methods of Inducing Differentiation of an NK Cell

Also provided herein are methods of inducing differentiation of a NK cell (e.g., any of the exemplary immune cells described herein or known in the art) that include contacting a NK cell in a liquid culture medium including an effective amount of a first multi-chain chimeric polypeptide described herein for a first period of time under conditions that allow for differentiation of the NK cell, and contacting the NK cell in a liquid culture medium including an effective amount of (i) a second multi-chain chimeric polypeptides described herein, and (ii) an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the linker domain (e.g., a monoclonal or polyclonal human, mouse, rabbit, or goat IgG1 antibody that binds specifically to a linker domain or any of the other exemplary IgG1 antibody constructs described herein), for a second period of time under conditions that allow for the activation and proliferation of the NK cell. In some examples, the NK cell is contacted *in vitro* (e.g., in a suitable liquid culture medium under conditions sufficient to result in stimulation of the NK cell).

In some examples, the NK cell has been previously obtained from a subject (e.g., a mammal, e.g., a human). Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step.

In some examples, the immune cell is contacted *in vivo*. In such embodiments, any of the first multi-chain chimeric polypeptide, any of the second multi-chain chimeric polypeptides, and any of the IgG1 antibody constructs described herein can be administered to a subject (e.g., a mammal, e.g., a human) in an amount sufficient to result in stimulation of an NK cell in the subject.

In some examples, the NK cell has previously been genetically-modified to express a chimeric antigen receptor or a recombinant T-cell receptor. Some

embodiments of these methods can further include, after the contacting step, introducing into the NK cell (e.g., any of the immune cells described herein) a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

Some embodiments of these methods can further include administering a
5 therapeutically effective amount of the NK cell to a subject in need thereof (e.g., any of the exemplary subjects described herein).

In some examples, the subject can be a subject identified or diagnosed as having an age-related disease or condition. Non-limiting examples of age-related diseases or disorders include: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis
10 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,
15 cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples, the subject can be a subject that has been identified or
20 diagnosed as having a cancer. Non-limiting examples of cancers include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML),
25 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
30 carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples, the subject can be a subject that has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious

disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, or influenza virus.

5 In some examples, the immune cell is a NK cell, and the detection of NK cells that are highly activated with a long persistence and a high degree of cytotoxicity against diseased cells in a recipient host (e.g., a memory NK cell) can include, e.g., the detection of the level of one or more of IL-12, IL-18, IL-33, STAT4, Zbtb32, DNAM-1, BIM, Noxa, SOCS1, BNIP3, BNIP3L, interferon- γ , TNF α , CXCL16,
10 CXCR6, NKG2D, TRAIL, CD49, Ly49D, CD49b, and Ly79H. A description of NK memory cells and methods of detecting the same is described in O'Sullivan et al., *Immunity* 43:634-645, 2015.

Methods of Treatment

15 Also provided herein are methods of treating a subject in need thereof (e.g., any of the exemplary subjects described herein or known in the art) that include administering to the subject a therapeutically effective amount of any of the NK cells produced by any of the methods described herein or any of the compositions (e.g., pharmaceutical compositions) described herein.

20 In some embodiments of these methods, the subject has been identified or diagnosed as having a cancer. Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's
25 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,
30 ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma. In some embodiments, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the cancer in the subject

(e.g., as compared to the number, severity, or frequency of the one or more symptoms of the cancer in the subject prior to treatment). In some embodiments, these methods can result in a reduction (e.g., about 1% reduction to about 99% reduction, about 1% reduction to about 95% reduction, about 1% reduction to about 90% reduction, about 1% reduction to about 85% reduction, about 1% reduction to about 80% reduction, about 1% reduction to about 75% reduction, about 1% reduction to about 70% reduction, about 1% reduction to about 65% reduction, about 1% reduction to about 60% reduction, about 1% reduction to about 55% reduction, about 1% reduction to about 50% reduction, about 1% reduction to about 45% reduction, about 1% reduction to about 40% reduction, about 1% reduction to about 35% reduction, about 1% reduction to about 30% reduction, about 1% reduction to about 25% reduction, about 1% reduction to about 20% reduction, about 1% reduction to about 15% reduction, about 1% reduction to about 10% reduction, about 1% reduction to about 5% reduction, about 5% reduction to about 99% reduction, about 5% reduction to about 95% reduction, about 5% reduction to about 90% reduction, about 5% reduction to about 85% reduction, about 5% reduction to about 80% reduction, about 5% reduction to about 75% reduction, about 5% reduction to about 70% reduction, about 5% reduction to about 65% reduction, about 5% reduction to about 60% reduction, about 5% reduction to about 55% reduction, about 5% reduction to about 50% reduction, about 5% reduction to about 45% reduction, about 5% reduction to about 40% reduction, about 5% reduction to about 35% reduction, about 5% reduction to about 30% reduction, about 5% reduction to about 25% reduction, about 5% reduction to about 20% reduction, about 5% reduction to about 15% reduction, about 5% reduction to about 10% reduction, about 10% reduction to about 99% reduction, about 10% reduction to about 95% reduction, about 10% reduction to about 90% reduction, about 10% reduction to about 85% reduction, about 10% reduction to about 80% reduction, about 10% reduction to about 75% reduction, about 10% reduction to about 70% reduction, about 10% reduction to about 65% reduction, about 10% reduction to about 60% reduction, about 10% reduction to about 55% reduction, about 10% reduction to about 50% reduction, about 10% reduction to about 45% reduction, about 10% reduction to about 40% reduction, about 10% reduction to about 35% reduction, about 10% reduction to about 30% reduction, about 10% reduction to about 25% reduction, about 10% reduction to about 20% reduction, about 10% reduction to about 15%

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In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. Non-limiting examples of aging-related diseases and conditions include Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant

failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction. In some examples, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the aging-related disease or condition in the subject (e.g., as compared to the number, severity, or frequency of the one or more symptoms of the aging-related disease or condition in the subject prior to treatment). In some examples, the methods can result in a decrease (e.g., about 1% decrease to about 99% decrease, an about 1% decrease to about 95% decrease, about 1% decrease to about 90% decrease, about 1% decrease to about 85% decrease, about 1% decrease to about 80% decrease, about 1% decrease to about 75% decrease, about 1% to about 70% decrease, about 1% decrease to about 65% decrease, about 1% decrease to about 60% decrease, about 1% decrease to about 55% decrease, about 1% decrease to about 50% decrease, about 1% decrease to about 45% decrease, about 1% decrease to about 40% decrease, about 1% decrease to about 35% decrease, about 1% decrease to about 30% decrease, about 1% decrease to about 25% decrease, about 1% decrease to about 20% decrease, about 1% decrease to about 15% decrease, about 1% decrease to about 10% decrease, about 1% decrease to about 5% decrease, about 5% decrease to about 99% decrease, an about 5% decrease to about 95% decrease, about 5% decrease to about 90% decrease, about 5% decrease to about 85% decrease, about 5% decrease to about 80% decrease, about 5% decrease to about 75% decrease, about 5% to about 70% decrease, about 5% decrease to about 65% decrease, about 5% decrease to about 60% decrease, about 5% decrease to about 55% decrease, about 5% decrease to about 50% decrease, about 5% decrease to about 45% decrease, about 5% decrease to about 40% decrease, about 5% decrease to about 35% decrease, about 5% decrease to about 30% decrease, about 5% decrease to about 25% decrease, about 5% decrease to about 20% decrease, about 5% decrease to about 15% decrease, about 5% decrease to about 10% decrease, about 10% decrease to about 99% decrease, an about 10% decrease to about 95% decrease, about 10% decrease to about 90% decrease, about 10% decrease to about 85% decrease, about 10% decrease to about 80% decrease, about 10% decrease to about 75% decrease, about 10% to about

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In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. Non-limiting examples of infectious disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus. In some embodiments, these methods can result in a decrease in the infectious titer (e.g., viral titer) in a subject (e.g., as compared to the infectious titer in the subject prior to treatment). In some embodiments, these methods can result in a

reduction in the number, severity, or frequency of one or more symptoms of the infectious disease (e.g., viral infection) in the subject (e.g., as compared to the number, severity, or frequency of the one or more symptoms of the infectious disease in the subject prior to treatment).

- 5 The term “subject” refers to any mammal. In some embodiments, the subject or “subject in need of treatment” may be a canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), ovine, bovine, porcine, caprine, primate, e.g., a simian (e.g., a monkey (e.g., marmoset, baboon), or an ape (e.g., a gorilla, chimpanzee, orangutan, or gibbon) or a human; or rodent (e.g., a mouse, a guinea pig, a hamster, or a rat). In
- 10 some embodiments, the subject or “subject in need of treatment” may be a non-human mammal, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g., murine, lapine, porcine, canine or primate animals) may be employed.

15 **Methods of Killing a Cancer Cell, an Infected Cell, or a Senescent Cell**

- Also provided herein are methods of killing a cancer cell (e.g. any of the exemplary types of cancer described herein or known in the art), an infected cell (e.g., a cell infected with any of the exemplary viruses described herein or known in the art), or a senescent cell (e.g., a senescent cancer cell, a senescent fibroblast, or a
- 20 senescent endothelial cell) in a subject in need thereof (e.g., any of the exemplary subjects described herein or known in the art) that include administering to the subject a therapeutically effective amount of any of NK cells produced by any of the methods described herein or any of the compositions (e.g., pharmaceutical compositions) described herein.

- 25 In some embodiments of these methods, the subject has been identified or diagnosed as having a cancer. Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin’s lymphoma, Hodgkin’s
- 30 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.

5 In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. Non-limiting examples of aging-related diseases and conditions include Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular
10 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,
15 glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

 In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. Non-limiting examples of an infectious
20 disease include infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, and hepatitis C virus, papillomavirus, and influenza virus.

25 *Senescent Cells*

 Senescence is a form of irreversible growth arrest accompanied by phenotypic changes, resistance to apoptosis and activation of damage-sensing signaling pathways. Cellular senescence was first described in cultured human fibroblast cells that lost their ability to proliferate, reaching permanent arrest after about 50 population
30 doublings (referred to as the Hayflick limit). Senescence is considered a stress response that can be induced by a wide range of intrinsic and extrinsic insults, including oxidative and genotoxic stress, DNA damage, telomere attrition, oncogenic activation, mitochondrial dysfunction, or chemotherapeutic agents.

Senescent cells remain metabolically active and can influence the tissue hemostasis, disease and aging through their secretory phenotype. Senescence is considered as a physiologic process and is important in promoting wound healing, tissue homeostasis, regeneration, and fibrosis regulation. For instance, transient
5 induction of senescent cells is observed during wound healing and contributes to wound resolution. Perhaps one of the most important roles of senescence is its role in tumor suppression. However, the accumulation of senescent cells also drives aging and aging-related diseases and conditions. The senescent phenotype also can trigger chronic inflammatory responses and consequently augment chronic inflammatory
10 conditions to promote tumor growth. The connection between senescence and aging was initially based on observations that senescent cells accumulate in aged tissue. The use of transgenic models has enabled the detection of senescent cells systematically in many age-related pathologies. Strategies to selectively eliminate senescent cells have demonstrated that senescent cells can indeed play a causal role in
15 aging and related pathologies.

Senescent cells display important and unique properties which include changes in morphology, chromatin organization, gene expression, and metabolism. There are several biochemical and functional properties associated with cellular senescence, such as (i) increased expression of p16^{INK4a} and p21^{CIP1}, inhibitors of
20 cyclin-dependent kinases, (ii) presence of senescence-associated β -galactosidase, a marker of lysosomal activity, (iii) appearance of senescence-associated heterochromatin foci and downregulation of lamin B1 levels, (iv) resistance to apoptosis caused by an increased expression of anti-apoptotic BCL-family protein, and (v) upregulation of CD26 (DPP4), CD36 (Scavenger receptor), forkhead box 4
25 (FOXO4), and secretory carrier membrane protein 4 (SCAMP4). Senescent cells also express an inflammatory signature, the so-called senescence-associated secretory phenotype (SASP). Through SASP, the senescent cells produce a wide range of inflammatory cytokines (IL-6, IL-8), growth factors (TGF- β), chemokines (CCL-2), and matrix metalloproteinases (MMP-3, MMP-9) that operate in a cell-autonomous
30 manner to reinforce senescence (autocrine effects) and communicate with and modify the microenvironment (paracrine effects). SASP factors can contribute to tumor suppression by triggering senescence surveillance, an immune-mediated clearance of senescent cells. However, chronic inflammation is also a known driver of

tumorigenesis, and accumulating evidence indicates that chronic SASP can also boost cancer and aging-related diseases.

The secretion profile of senescent cells are context dependent. For instance, the mitochondrial dysfunction-associated senescence (MiDAS), induced by different
 5 mitochondrial dysfunction in human fibroblasts, led to the appearance of a SASP that was deficient in IL-1-dependent inflammatory factors. A decrease in the NAD⁺/NADH ratio activated AMPK signaling which induced MiDAS through the activation of p53. As a result, p53 inhibited NF-κB signaling which is a crucial inducer of pro-inflammatory SASP. In contrast, the cellular senescence caused by
 10 persistent DNA damage in human cells induced an inflammatory SASP, which was dependent on the activation of ataxia-telangiectasia mutated (ATM) kinase but not on that of p53. In particular, the expression and secretion levels of IL-6 and IL-8 were increased. It was also demonstrated that cellular senescence caused by the ectopic expression p16^{INK4a} and p21^{CIP1} induced the senescent phenotype in human fibroblasts
 15 without an inflammatory SASP indicating that the growth arrest itself did not stimulate SASP.

One of the most defining characteristics of senescence is stable growth arrest. This is achieved by two important pathways, the p16^{INK4a}/Rb and the p53/p21^{CIP1}, both of which are central in tumor suppression. DNA damage results in: (1) high
 20 deposition of γH2Ax (histone coding gene) and 53BP1 (involved in DNA damage response) in chromatin: this leads to activation of a kinase cascade eventually resulting in p53 activation, and (2) activation of p16^{INK4a} and ARF (both encoded by CDKN2A) and P15^{INK4b} (encoded by CDKN2B): p53 induces transcription of cyclin-dependent kinase inhibitor (p21^{CIP1}) and along with both p16^{INK4a} and p15^{INK4b} block
 25 genes for cell cycle progression (CDK4 and CDK6). This eventually leads to hypophosphorylation of Retinoblastoma protein (Rb) and cell cycle arrest at the G1 phase.

Selectively killing senescent cells has been shown to significantly improve the health span of mice in the context of normal aging and ameliorates the consequences
 30 of age-related disease or cancer therapy (Ovadya, *J Clin Invest.* 128(4):1247-1254, 2018). In nature, the senescent cells are normally removed by the innate immune cells. Induction of senescence not only prevents the potential proliferation and transformation of damaged/altered cells, but also favors tissue repair through the

production of SASP factors that function as chemoattractants mainly for Natural Killer (NK) cells (such as IL-15 and CCL2) and macrophages (such as CFS-1 and CCL2). These innate immune cells mediate the immunosurveillance mechanism for eliminating stressed cells. Senescent cells usually up-regulate the NK-cell activating
 5 receptor NKG2D and DNAM1 ligands, which belong to a family of stress-inducible ligands: an important component of the frontline immune defense against infectious diseases and malignancies. Upon receptor activation, NK cells can then specifically induce the death of senescent cells through their cytolytic machinery. A role for NK cells in the immune surveillance of senescent cells has been pointed out in liver
 10 fibrosis (Sagiv, *Oncogene* 32(15): 1971-1977, 2013), hepatocellular carcinoma (Iannello, *J Exp Med* 210(10): 2057-2069, 2013), multiple myeloma (Soriani, *Blood* 113(15): 3503-3511, 2009), and glioma cells stressed by dysfunction of the mevalonate pathway (Ciaglia, *Int J Cancer* 142(1): 176-190, 2018). Endometrial cells undergo acute cellular senescence and do not differentiate into decidual cells. The
 15 differentiated decidual cells secrete IL-15 and thereby recruit uterine NK cells to target and eliminate the undifferentiated senescent cells thus helping to re-model and rejuvenate the endometrium (Brighton, *Elife* 6: e31274, 2017). With a similar mechanism, during liver fibrosis, p53-expressing senescent liver satellite cells skewed the polarization of resident Kupfer macrophages and freshly infiltrated macrophages
 20 toward the pro-inflammatory M1 phenotype, which display senolytic activity. F4/80+ macrophages have been shown to play a key role in the clearance of mouse uterine senescent cells to maintain postpartum uterine function.

Senescent cells recruit NK cells by mainly upregulating ligands to NKG2D (expressed on NK cells), chemokines, and other SASP factors. In vivo models of
 25 liver fibrosis have shown effective clearance of senescent cells by activated NK cells (Krizhanovsky, *Cell* 134(4): 657-667, 2008). Studies have described various models to study senescence including liver fibrosis (Krizhanovsky, *Cell* 134(4): 657-667, 2008), osteoarthritis (Xu, *J Gerontol A Biol Sci Med Sci* 72(6): 780-785, 2017), and Parkinson's disease (Chinta, *Cell Rep* 22(4): 930-940, 2018). Animal models for
 30 studying senescent cells are described in: Krizhanovsky, *Cell* 134(4): 657-667, 2008; Baker, *Nature* 479(7372): 232-236, 2011; Farr, *Nat Med* 23(9): 1072-1079, 2017; Bourgeois, *FEBS Lett* 592(12): 2083-2097, 2018; Xu, *Nat Med* 24(8): 1246-1256, 2018).

Methods of Increasing Glucose Consumption of an NK Cell

Also provided herein are methods of increasing the glucose consumption of an NK cell that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount
5 of a first multi-chain chimeric polypeptide comprising a first chimeric polypeptide and a second chimeric polypeptide for a first period of time under conditions that allow for the differentiation of the natural killer cell; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of (i) a second multi-chain chimeric polypeptide comprising a first chimeric polypeptide and a
10 second chimeric polypeptide, and (ii) an IgG1 antibody construct that includes at least one antigen-binding domain that binds specifically to the linker domain (e.g., any of the exemplary IgG1 antibody constructs described herein), for a second period of time, under conditions that allow for glucose consumption in the NK cell.

In some embodiments of any of the methods described herein, the increase in
15 glucose consumption is compared to the level of glucose consumption in a similar NK cell not contacted with any of the multi-chain chimeric polypeptides described herein.

Non-limiting assays that can be used to detect glucose consumption include, e.g., assays that include the use of radiolabeled 3-O-methylglucose or radiolabeled 2-deoxy-glucose, or an enzymatic, fluorometric assay for detecting 2-deoxyglucose.
20 Additional examples of assays that can be used to detect glucose consumption are described in, e.g., Yamamoto et al., *Curr. Protoc. Pharmacol.*, Chapter 12, Unit 12.14.1-22, December 2011; Zou et al., *J. Biochem. Biophys. Methods* 64(3):207-215, September 2005; and MacKrell et al., *Diabetes* p. 1-9, published online on March 13, 2012.

25 In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid culture medium can be a chemically-defined liquid culture medium. In other examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the
30 second multi-chain chimeric polypeptide, and the IgG1 antibody construct at a molar ratio of about 0.5:1 to about 2:1 (e.g., about 0.8:1 to about 1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell

from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid
 5 encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, isolating the NK cell. Some embodiments of these methods further
 10 include, after the contacting step, administering the NK cell to a subject in need thereof.

In some examples of these methods, the subject has been identified or diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group of:
 15 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,
 20 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.
 25 In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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,
 30 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.

5 In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza
10 virus.

 Also provided herein are activated NK cells produced by any of the methods described herein. Also provided herein are pharmaceutical compositions that include any of the activated NK cells produced by any of these methods. Also provided
15 herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

 Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated NK cells described herein
20 produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

 In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma,
25 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
30 carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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

Also provided herein are methods of treating a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein. In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group 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 embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Methods of Increasing Oxidative Phosphorylation of an NK Cell

Also provided herein are methods of increasing the oxidative phosphorylation of an NK cell that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of any of the first multi-chain chimeric polypeptides described herein for a first period of time under conditions that allow for the differentiation of the natural killer cell; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of (i) any of the second multi-chain chimeric polypeptides described herein, and (ii) an IgG1 antibody construct that includes at least one antigen-binding domain that binds specifically to the linker domain (e.g., any of the exemplary IgG1 antibody constructs described herein), for a second period of time, under conditions that allow for oxidative phosphorylation in the natural killer cell.

In some embodiments of any of the methods described herein, the increase in oxidative phosphorylation is compared to the level of oxidative phosphorylation in a

similar NK cell not contacted with any of the multi-chain chimeric polypeptides described herein.

Non-limiting assays that can be used to detect oxidative phosphorylation include, e.g., immunofluorescent assays and colorimetric assays. A non-limiting commercial assay that can be used to determine the level of oxidative phosphorylation is MitoTox™ Complete OXPHOS Activity Assay (Abcam). Additional examples of assays that can be used to detect oxidative phosphorylation are described in, e.g., Chance et al., *Nature* 175:1120-1121, 1955; Pullman et al., *Science* 123(3208):1105-1107, 1956; Van Bergen et al., *Mitochondrion* 15:24-33, 2014; and Rocha et al., *Sci. Rep.* 5:15037, 2015.

In some examples of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some examples of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid culture medium can be a chemically-defined liquid culture medium. In other examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the

contacting step, isolating the NK cell. Some embodiments of these methods further include, after the contacting step, administering the NK cell to a subject in need thereof.

In some examples of these methods, the subject has been identified or
 5 diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group 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
 10 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,
 15 NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma,
 20 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,
 25 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.

30 In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus,

hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Also provided herein are activated NK cells produced by any of these methods. Also provided herein are pharmaceutical compositions that include any of
5 the activated NK cells produced by any of these methods. Also provided herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a
10 therapeutically effective amount of any of the activated NK cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is
15 selected from the group 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
20 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
25 hepatocellular carcinoma.

In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group of:
Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma,
30 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,
 5 spinocerebellar ataxia, multiple sclerosis, and renal dysfunction

Also provided herein are methods of treating a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated NK cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein. In some examples of
 10 these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group 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
 15 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,
 20 ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these
 25 methods, the aging-related disease or condition is selected from the group 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
 30 pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis,

NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

10 ***Methods of Increasing Aerobic Glycolysis of an Immune Cell***

Also provided herein are methods of increasing the aerobic glycolysis of an immune cell that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of any of the first multi-chain chimeric polypeptides described herein for a first period of time under conditions that allow for the differentiation of the natural killer cell; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of (i) any of the second multi-chain chimeric polypeptides described herein for a second period of time, under conditions that allow for aerobic glycolysis in the natural killer cell.

In some embodiments of any of the methods described herein, the increase in aerobic glycolysis is compared to the level of aerobic glycolysis in a similar NK cell not contacted with any of the multi-chain chimeric polypeptides described herein.

Non-limiting assays that can be used to detect aerobic glycolysis include, e.g., detection of ATP and/or CO₂ production, e.g., using gas chromatography/mass spectrometry, or extracellular acidification rate. Additional examples of assays that can be used to detect aerobic glycolysis are described in, e.g., Zhang et al., *Bone* 114:150-160, 2018; Zhao et al., *J. Breast Cancer* 21(2):112-123, 2018; Li et al., *Cell Comm. Signal.* 16(1):26, 2018; Cai et al., *J. Exp. Clin. Cancer Res.* 37(1):104, 2018; and Zhang et al., *Oncol. Rep.* 40(2):1156-1164, 2018.

In some examples of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some examples of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where

one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid culture medium can be a chemically-defined liquid culture medium. In other examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, isolating the NK cell. Some embodiments of these methods further include, after the contacting step, administering the NK cell to a subject in need thereof.

In some examples of these methods, the subject has been identified or diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group 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 of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Also provided herein are activated immune cells produced by any of these methods. Also provided herein are pharmaceutical compositions that include any of the activated immune cells produced by any of these methods. Also provided herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is

selected from the group 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 of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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

Also provided herein are methods of treating a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated NK cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein. In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group 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 embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Methods of Increasing Extracellular Acidification Rate (ECAR) of an Immune Cell

Also provided herein are method of increasing the extracellular acidification rate (ECAR) of a NK cell that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of any of the first multi-chain chimeric polypeptides described

herein for a first period of time under conditions that allow for the differentiation of the natural killer cell; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of any of the second multi-chain chimeric polypeptides described herein and any of the IgG1 antibody constructs described
 5 herein for a second period of time, under conditions that allow for extracellular acidification by the natural killer cell.

In some embodiments of any of the methods described herein, the increase in extracellular acidification rate (ECAR) is compared to the level of extracellular acidification rate (ECAR) in a similar NK cell not contacted with any of the multi-
 10 chain chimeric polypeptide and/or the IgG1 antibody construct that includes at least one antigen-binding domain that binds specifically to the linker domain.

Non-limiting commercial assays that can be used to detect extracellular acidification rate (ECAR) include, e.g., Glycolysis Assay (Extracellular Acidification Kit) (ab197244 or ab197245) (Abcam), MITO-ID® Extracellular pH Sensor Kit
 15 (ENZ-51048) (Enzo Life Sciences), and pH-Xtra™ Glycolysis Assay (Extracellular Acidification). Additional examples of assays that can be used to detect extracellular acidification rate (ECAR) are described in, e.g., Owicki et al., *Biosens. Bioelectron.* 7(4):255-272, 1992; Hynes et al., *Analytical Biochem.* 390(1):21-28, 2009; Trevani et al., *J. Immunol.* 162(8):4849-4857, 1999; and Mookerjee et al., *J. Vis. Exp.*
 20 106:53464, 2015.

In some examples of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some examples of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where
 25 one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid culture medium can be a chemically-defined liquid culture medium. In other
 30 examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen
 5 receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant
 10 T-cell receptor. Some embodiments of these methods further include, after the contacting step, isolating the NK cell. Some embodiments of these methods further include, after the contacting step, administering the NK cell to a subject in need thereof.

In some examples of these methods, the subject has been identified or
 15 diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group 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
 20 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,
 25 NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma,
 30 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
5 cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus,
10 adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Also provided herein are activated immune cells produced by any of these methods. Also provided herein are pharmaceutical compositions that include any of
15 the activated immune cells produced by any of these methods. Also provided herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a
20 therapeutically effective amount of any of the activated NK cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is
25 selected from the group 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
30 leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous

cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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

Also provided herein are methods of treating a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated NK cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein. In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group 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 embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

20

Methods of Increasing Mitochondrial Oxygen Consumption Rate of an Immune Cell

Also provided herein are methods of increasing the mitochondrial oxygen consumption rate of a natural killer cell that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of any of the first multi-chain chimeric polypeptides described herein; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of any of the second multi-chain chimeric polypeptides described herein and any of the IgG1 antibody constructs described herein for a second period of time, under conditions that allow for mitochondrial oxygen consumption rate by the natural killer cell.

In some embodiments of any of the methods described herein, the increase in mitochondrial oxygen consumption rate is compared to the level of mitochondrial

oxygen consumption rate in a similar NK cell not contacted with any of the multi-chain chimeric polypeptides described herein.

Non-limiting commercial assays that can be used to detect mitochondrial oxygen consumption rate include, e.g., assays that include the use of an electrode or a commercially available kit, e.g., Oxygen Consumption Rate Assay Kit (Cayman Chemical), the Seahorse assay (as described in Sakamuri et al., *Geroscience* 40(3):347-356, 2018). Additional examples of assays that can be used to detect mitochondrial oxygen consumption rate are described in, e.g., Li et al., *Mitochondrial Disorders*, pp. 63-72, December 2011; Kumagi et al., *Synapse* e22067, August 2018; Takahashi et al., *J. Physiol. Sci.* 67(6):731-737, 2017; and Iihoshi et al., *Toxicol. Lett.* 277:109-114, 2017.

In some examples of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some examples of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid culture medium can be a chemically-defined liquid culture medium. In other examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant

T-cell receptor. Some embodiments of these methods further include, after the contacting step, isolating the NK cell. Some embodiments of these methods further include, after the contacting step, administering the NK cell to a subject in need thereof.

- 5 In some examples of these methods, the subject has been identified or diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease,
- 10 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
- 15 elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.
- In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the
- 20 group 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),
- 25 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
- 30 carcinoma.

In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus,

adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Also provided herein are activated immune cells produced by any of these methods. Also provided herein are pharmaceutical compositions that include any of the activated immune cells produced by any of these methods. Also provided herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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 of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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,
 5 NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction

Also provided herein are methods of treating a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described
 10 herein or any of the pharmaceutical compositions described herein. In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms,
 15 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
 20 esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some embodiments of these methods, the subject has been identified or
 25 diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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
 30 mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue

elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

In some examples of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Methods of Increasing Expression of Activating Receptors in a NK Cell

Also provided herein are methods of increasing expression of activating receptors in a natural killer cell (e.g., any of the exemplary activating receptors of a natural killer cell described herein) that include: (a) contacting a natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of any of the first multi-chain chimeric polypeptides described herein; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of any of the second multi-chain chimeric polypeptides described herein and any of the IgG1 antibody constructs described herein for a second period of time, under conditions that allow for mitochondrial oxygen consumption rate by the natural killer cell.

In some examples of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some examples of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid culture medium can be a chemically-defined liquid culture medium. In other examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, isolating the NK cell. Some embodiments of these methods further include, after the contacting step, administering the NK cell to a subject in need thereof.

In some examples of these methods, the subject has been identified or diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group 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 of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Also provided herein are activated immune cells produced by any of these methods. Also provided herein are pharmaceutical compositions that include any of the activated immune cells produced by any of these methods. Also provided herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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
 5 hepatocellular carcinoma.

In some examples of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma,
 10 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
 15 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

Also provided herein are methods of treating a subject in need thereof that
 20 include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein. In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group of: solid
 25 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),
 30 myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck

carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Methods of Increasing Persistence of a NK Cell Upon Administration to a Subject

Also provided herein are methods of increasing persistence of a natural killer cell (e.g., any of the exemplary activating receptors of a natural killer cell described herein) upon administration to a subject that include, prior to administration of the NK cell to the subject: (a) contacting the natural killer cell in a liquid culture medium (e.g., any of the liquid culture media described herein) including an effective amount of any of the first multi-chain chimeric polypeptides described herein; and (b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of any of the second multi-chain chimeric polypeptides described herein and any of the IgG1 antibody constructs described herein for a second period of time,

under conditions that allow for mitochondrial oxygen consumption rate by the natural killer cell.

In some examples of these methods, the IgG1 antibody construct is a monoclonal IgG1 antibody, where both of the antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain. In some examples
5 of these methods, the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

In some embodiments of these methods, the liquid culture medium can be a serum-free liquid culture medium. In some embodiments of these methods, the liquid
10 culture medium can be a chemically-defined liquid culture medium. In other examples of these methods, the liquid culture medium includes serum.

In some examples of these methods, the liquid culture medium includes the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar
15 ratio of about 0.5:0.5:1 to about 2:2:1 (e.g., about 0.8:0.8:1 to about 1.2:1.2:1).

In some examples of these methods, the NK cell was previously obtained from a subject. Some embodiments of these methods further include obtaining the NK cell from the subject prior to the contacting step. In some embodiments of these methods, the NK cell has previously been genetically modified to express a chimeric antigen
20 receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, after the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor. Some embodiments of these methods further include, before the contacting step, introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant
25 T-cell receptor. Some embodiments of these methods further include, after the contacting step, isolating the NK cell. Some embodiments of these methods further include, after the contacting step, administering the NK cell to a subject in need thereof.

In some examples of these methods, the subject has been identified or
30 diagnosed as having an age-related disease or condition. In some examples of these methods, the age-related disease or condition is selected from the group 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 of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus, hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Also provided herein are activated immune cells produced by any of these methods. Also provided herein are pharmaceutical compositions that include any of the activated immune cells produced by any of these methods. Also provided herein are kits that include any of the pharmaceutical compositions described herein produced by any of the methods described herein.

Also provided herein are methods of killing a cancer cell, an infected cell, or a senescent cell in a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein.

In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some examples of these methods, the cancer is selected from the group 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 of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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

Also provided herein are methods of treating a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the

activated immune cells described herein produced by any of the methods described herein or any of the pharmaceutical compositions described herein. In some examples of these methods, the subject has been identified or diagnosed as having a cancer. In some embodiments of these methods, the cancer is selected from the group 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 embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition. In some examples of these methods, the aging-related disease or condition is selected from the group 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 of these methods, the subject has been diagnosed or identified as having an infectious disease. In some examples of these methods, the infectious disease is infection with human immunodeficiency virus, cytomegalovirus, adenovirus, coronavirus, rhinovirus, rotavirus, smallpox, herpes simplex virus,

hepatitis B virus, hepatitis A virus, hepatitis C virus, papillomavirus, or influenza virus.

Additional Therapeutic Agents

5 Some embodiments of any of the methods described herein can further include administering to a subject (e.g., any of the subjects described herein) a therapeutically effective amount of one or more additional therapeutic agents. The one or more additional therapeutic agents can be administered to the subject at substantially the same time as a single-chain chimeric polypeptide (e.g., any of the single-chain
10 chimeric polypeptides described herein), multi-chain chimeric polypeptides (e.g., any of the multi-chain chimeric polypeptides described herein), or an activated immune cell (e.g., an activated NK cell or an activated T cell) (e.g., administered as a single formulation or two or more formulations to the subject). In some embodiments, one or more additional therapeutic agents can be administered to the subject prior to
15 administration of a single-chain chimeric polypeptide (e.g., any of the single-chain chimeric polypeptides described herein), multi-chain chimeric polypeptides (e.g., any of the multi-chain chimeric polypeptides described herein), or an activated immune cell (e.g., an activated NK cell or an activated T cell). In some embodiments, one or more additional therapeutic agents can be administered to the subject after
20 administration of a single-chain chimeric polypeptide (e.g., any of the single-chain chimeric polypeptides described herein), multi-chain chimeric polypeptides (e.g., any of the multi-chain chimeric polypeptides described herein), or an activated immune cell (e.g., an activated NK cell or an activated T cell) to the subject.

Non-limiting examples of additional therapeutic agents include: anti-cancer
25 drugs, activating receptor agonists, immune checkpoint inhibitors, agents for blocking HLA-specific inhibitory receptors, Glucogen Synthase Kinase (GSK) 3 inhibitors, and antibodies.

Non-limiting examples of anticancer drugs include antimetabolic drugs (e.g., 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine, cytarabine,
30 floxuridine, fludarabine, gemcitabine, hydroxycarbamide, methotrexate, 6-thioguanine, cladribine, nelarabine, pentostatin, or pemetrexed), plant alkaloids (e.g., vinblastine, vincristine, vindesine, camptothecin, 9-methoxycamptothecin, coronaridine, taxol, nucleosides, diprenylated indole alkaloid, montamine,

schischkiniin, protoberberine, berberine, sanguinarine, chelerythrine, chelidonine, liriodenine, clivorine, β -carboline, antofine, tylophorine, cryptolepine, neocryptolepine, corynoline, sampangine, carbazole, crinamine, montanine, ellipticine, paclitaxel, docetaxel, etoposide, tenisopide, irinotecan, topotecan, or

5 acridone alkaloids), proteasome inhibitors (e.g., lactacystin, disulfiram, epigallocatechin-3-gallate, marizomib (salinosporamide A), oprozomib (ONX-0912), delanzomib (CEP-18770), epoxomicin, MG132, beta-hydroxy beta-methylbutyrate, bortezomib, carfilzomib, or ixazomib), antitumor antibiotics (e.g., doxorubicin, daunorubicin, epirubicin, mitoxantrone, idarubicin, actinomycin, plicamycin,

10 mitomycin, or bleomycin), histone deacetylase inhibitors (e.g., vorinostat, panobinostat, belinostat, givinostat, abexinostat, depsipeptide, entinostat, phenyl butyrate, valproic acid, trichostatin A, dacinostat, mocetinostat, pracinostat, nicotinamide, cambinol, tenovin 1, tenovin 6, sirtinol, ricolinostat, tefinostat, kevetrin, quisinostat, resminostat, tacedinaline, chidamide, or selisistat), tyrosine kinase

15 inhibitors (e.g., axitinib, dasatinib, encorafenib, erlotinib, imatinib, nilotinib, pazopanib, and sunitinib), and chemotherapeutic agents (e.g., all-trans retinoic acid, azacitidine, azathioprine, doxifluridine, epothilone, hydroxyurea, imatinib, teniposide, tioguanine, valrubicin, vemurafenib, and lenalidomide). Additional examples of chemotherapeutic agents include alkylating agents, e.g., mechlorethamine,

20 cyclophosphamide, chlorambucil, melphalan, ifosfamide, thiotepa, hexamethylmelamine, busulfan, altretamine, procarbazine, dacarbazine, temozolomide, carmustine, lumustine, streptozocin, carboplatin, cisplatin, and oxaliplatin.

Non-limiting examples of activating receptor agonists include any agonists for

25 activating receptors which activate and enhance the cytotoxicity of NK cells, including anti-CD16 antibodies (e.g., anti-CD16/CD30 bispecific monoclonal antibody (BiMAb)) and Fc-based fusion proteins. Non-limiting examples of checkpoint inhibitors include anti-PD-1 antibodies (e.g., MEDI0680), anti-PD-L1 antibodies (e.g., BCD-135, BGB-A333, CBT-502, CK-301, CS1001, FAZ053,

30 KN035, MDX-1105, MSB2311, SHR-1316, anti-PD-L1/CTLA-4 bispecific antibody KN046, anti-PD-L1/TGF β RII fusion protein M7824, anti-PD-L1/TIM-3 bispecific antibody LY3415244, atezolizumab, or avelumab), anti-TIM3 antibodies (e.g., TSR-022, Sym023, or MBG453) and anti-CTLA-4 antibodies (e.g., AGEN1884, MK-1308,

or an anti-CTLA-4/OX40 bispecific antibody ATOR-1015). Non-limiting examples of agents for blocking HLA-specific inhibitory receptors include monalizumab (e.g., an anti-HLA-E NKG2A inhibitory receptor monoclonal antibody). Non-limiting examples of GSK3 inhibitor include tideglusib or CHIR99021. Non-limiting examples of antibodies that can be used as additional therapeutic agents include anti-CD26 antibodies (e.g., YS110), anti-CD36 antibodies, and any other antibody or antibody construct that can bind to and activate an Fc receptor (e.g., CD16) on a NK cell. In some embodiments, an additional therapeutic agent can be insulin or metformin.

10

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

15 **Example 1: Creation of an IL-12/IL-15R α Su DNA construct**

In a non-limiting example, an IL-12/IL-15R α Su DNA construct was created (see Fig. 1) The human IL-12 subunit sequences, human IL-15R α Su sequence, human IL-15 sequence, human tissue factor 219 sequence, and human IL-18 sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-12 subunit beta (p40) to IL-12 subunit alpha (p35) with a GS (3) linker to generate a single chain version of IL-12 and then directly linking the IL-12 sequence to the IL-15R α Su sequence. The final IL-12/IL-15R α Su DNA construct sequence was synthesized by Genewiz.

25

The nucleic acid sequence of the IL12/IL-15R α Su construct (including signal peptide sequence) is as follows (SEQ ID NO: 59):

(Signal peptide)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCTTCTTTAGCAGCGC

30 CTACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAAGTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGG
TATCCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGA

AGAAGACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCT
 CCGGAAAGACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAA
 TACACATGCCACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATT
 ACACAAGAAGGAAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAG
 5 AAGGAGCCCAAGAATAAGACCTTTTTAAGGTGTGAGGCCAAAACTACA
 GCGGTTCGTTTCACTTGTGTTGGTGGCTGACCACCATTTCACCGATTTAACCT
 TCTCCGTGAAAAGCAGCCGGGGAAGCTCCGACCCTCAAGGTGTGACATGT
 GGAGCCGCTACCCTCAGCGCTGAGAGGGTTCGTGGCGATAACAAGGAATA
 CGAGTACAGCGTGGAGTGCCAAGAAGATAGCGCTTGTCCCGCTGCCGAAG
 10 AATCTTTACCCATTGAGGTGATGGTGGACGCCGTGCACAACTCAAGTAC
 GAGAACTACACCTCCTCCTTCTTTATCCGGGACATCATTAAGCCCGATCCT
 CCTAAGAATTTACAGCTGAAGCCTCTCAAAAATAGCCGGCAAGTTGAGGT
 CTCTTGGGAATATCCCGACACTTGGAGCACACCCACAGCTACTTCTCTTT
 AACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGGAGAAGAAAGAC
 15 CGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTGCGGAAGAACGC
 CTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGCTGGTCCG
 AGTGGGCCAGCGTGCCTTGTTC

(Linker)

GCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT
 20 *(Human IL-12 subunit alpha (p35))*

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTT
 ACACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAA
 GCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCA
 TGAAGATATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTC
 25 TGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTC
 ATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCT
 TTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTC
 AAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTTTTT
 AGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
 30 TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTT
 ACAAGACAAAGATCAAACGTGTCATTTTACTCCACGCCTTTAGGATCCGG
 GCCGTGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGC

(Human IL-15R α sushi domain)

ATTACATGCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGG
TGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGC
TTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCAGTGCGTGCTGAATAA
GGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

5

Example 2: Creation of an IL-18/TF/IL-15 DNA construct

In a non-limiting example, an IL-18/TF/IL-15 construct was made (see Fig. 2) linking the IL-18 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-18/TF construct with the N-terminus coding region of IL-15.

- 10 The nucleic acid sequence of the IL-18/TF/IL-15 construct (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 60):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTTCCTCTTCTCCAGCGC
CTACAGC

15 *(Human IL-18)*

TACTTCGGCAAACCTGGAATCCAAGCTGAGCGTGATCCGGAATTTAA
ACGACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGAC
ATGACCGACTCCGATTGCCGGGACAATGCCCCCGGACCATCTTCATTATC
TCCATGTACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGT
20 GAAGTGTGAGAAAATCAGCACTTTATCTTGTGAGAACAAAGATCATCTCCT
TTAAGGAAATGAACCCCCCGATAACATCAAGGACACCAAGTCCGATATC
ATCTTCTTCCAGCGGTCCGTGCCCCGGTCACGATAACAAGATGCAGTTCGA
ATCCTCCTCCTACGAGGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTT
ATTCAAGCTGATCCTCAAGAAGGAGGACGAGCTGGGCGATCGTTCCATCA
25 TGTTACCGTCCAAAACGAGGAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAG
AGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCA
AGTTTACACCGTGCAGATCAGACCAAGTCCGGCGACTGGAAGTCCAAAT
30 GTTTCTATACCAACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAA
GATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAA
TGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCG
AATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGC

TTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGA
 CTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTTCGGCA
 AAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGA
 AGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAAGGC
 5 GAAAACACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGAA
 TAGGAAAAGCACCGATAGCCCCGTTGAGTGTCATGGGCCAAGAAAAGGGC
 GAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTA
 10 ATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCA
 CCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGT
 TATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATT
 TAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAG
 TCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGT
 15 TTCTGCAATCCTTTGTGCACATTGTCCAGATGTTTCATCAATACCTCC

Example 3: Secretion of IL-12/IL-15R α Su and IL-18/TF/IL-15 fusion proteins

The IL-12/IL-15R α Su and IL-18/TF/IL-15 DNA constructs were cloned into a
 pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene*
 20 *Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector
 was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1
 cells allowed for formation and secretion of a soluble IL-18/TF/IL-15:IL-12/IL-
 15R α Su protein complex (referred to as 18t15-12s; see Figs. 3 and 4). The 18t15-12s
 protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody
 25 (hOAT) affinity chromatography and size exclusion chromatography resulting in
 soluble (non-aggregated) protein complexes consisting of IL-12/IL-15R α Su and IL-
 18/TF/IL-15 fusion proteins.

The amino acid sequence of the IL12/IL-15R α Su fusion protein (including
 signal peptide sequence) is as follows (SEQ ID NO: 61):

30 *(Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-12 subunit beta (p40))

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL
 GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQK
 EPKNKTFLRCEAKNYSGRFTCWWLTITSTDLTFSVKSSRGSSDPQGVTCGAAT
 LSAERVGRDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAVHKLKYENYTSS
 5 FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQG
 KSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCS

(Linker)

GGGGSGGGSGGGGS

(Human IL-12 subunit alpha (p35))

10 RNLPVATPDPGMFPC LHHSQNLLRAVSNMLQKARQTLEFYPTSEEID
 HEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL
 SSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSET
 VPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human IL-15R α sushi domain)

15 ITCPPPMSEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK
 ATNVAHWTTPSLKCIR

The amino acid sequence of the IL-18/TF/IL-15 fusion protein (including
 signal peptide sequence) is as follows (SEQ ID NO: 62):

(Signal peptide)

20 MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFDQGNRPLFEDMTDSDCRDNAPRTIFIIS
 MYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRS
 VPGHDNKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE

25 *(Human Tissue Factor 219)*

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
 KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEF
 TPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIY
 TLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSP
 30 VECMGQEKGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLLELQ
 VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSF
 VHIVQMFINTS

In some cases, the leader (signal sequence) peptide is cleaved from the intact
 5 polypeptide to generate the mature form that may be soluble or secreted.

Example 3: Creation of an IL-7/IL-15R α Su DNA construct

In a non-limiting example, an IL-7/IL-15R α Su DNA construct was created
 (see Figure 5). The human IL-7 sequence, human IL-15R α Su sequence, human IL-15
 10 sequence, and human tissue factor 219 sequence were obtained from the UniProt
 website and DNA for these sequences was synthesized by Genewiz. A DNA
 construct was made linking the IL-7 sequence to the IL-15R α Su sequence. The final
 IL-7/IL-15R α Su DNA construct sequence was synthesized by Genewiz.

Example 4: Creation of an IL-21/TF/IL-15 DNA construct

In a non-limiting example, an IL-21/TF/IL-15 construct was made (see Figure
 6) by linking the IL-21 sequence to the N-terminus coding region of tissue factor 219,
 and further linking the IL-21/TF construct with the N-terminus coding region of IL-
 15.

20

Example 5: Secretion of IL-7/IL-15R α Su and IL-21/TF/IL-15 fusion proteins

The IL-7/IL-15R α Su and IL-21/TF/IL-15 DNA constructs were cloned into a
 pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene*
Ther 16:457–72, 2005, hereby incorporated by reference), and the expression vector
 25 was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1
 cells allowed for formation and secretion of a soluble IL-21/TF/IL-15:IL-7/IL-
 15R α Su protein complex (referred to as 21t15-7s, see Figures 7 and 8). The 21t15-7s
 protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody
 affinity chromatography and size exclusion chromatography resulting in soluble (non-
 30 aggregated) protein complexes consisting of IL-7/IL-15R α Su and IL-21/TF/IL-15
 fusion proteins.

In some cases, the leader (signal sequence) peptide is cleaved from the intact
 polypeptide to generate the mature form that may be soluble or secreted.

Example 6: Creation of an IL-21/IL-15R α Su DNA construct

In a non-limiting example, an IL-21/IL-15R α Su DNA construct was created. The human IL-21 sequence and human IL-15R α Su sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA
 5 construct was made linking the IL-21 sequence to the IL-15R α Su sequence. The final IL-21/IL-15R α Su DNA construct sequence was synthesized by Genewiz. See Figure 9.

Example 7: Creation of an IL-7/TF/IL-15 DNA construct

10 In a non-limiting example, an IL-7/TF/IL-15 construct was made by linking the IL-7 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-7/TF construct with the N-terminus coding region of IL-15. See Figure 10.

Example 8: Secretion of IL-21/IL-15R α Su and IL-7/TF/IL-15 fusion proteins

15 The IL-21/IL-15R α Su and IL-7/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1
 20 cells allowed for formation and secretion of a soluble IL-7/TF/IL-15:IL-21/IL-15R α Su protein complex (referred to as 7t15-21s) (Figures 11 and 12). The 7t15-21s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody (IgG1) affinity chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-21/IL-15R α Su and IL-
 25 7/TF/IL-15 fusion proteins.

Example 9: Measurement of Cytokine Signaling via downstream protein phosphorylation of STAT4 and STAT5

30 A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15: 18t15-12s) on cytokine signaling on purified NK cells (Figure 13).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent

(StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in $0.5 \times 10^6/\text{mL}$ in 96 wells flat bottom plate in 0.1 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with hIL-12 (10 ng/mL) (Biolegend) and hIL-15 (50 ng/mL) (NCI) or either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or IL-12/IL-15 α Su and IL-18/IL-15 multi-chain chimeric polypeptide 100 nM for 15, 45, 90, 180 minutes or overnight at 37°C, 5% CO₂. Cells were harvested at different points and fixed in Paraformaldehyde (Sigma) to a final concentration of 1.6%. Plates were incubated in dark in room temperature for 10 minutes. 100 mL of FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma) was added in Cells and transferred to 96 wells “V” bottom plate. Cells were washed for 1500 RPM for 5 minutes at room temperature. Cell pellet was mixed in 100 mL chilled methanol and pipetting up and down gently and incubate for 30 minutes at 4°C. Cells were mixed with 100 mL of FACS buffer and washed and centrifuged for 1500 RPM for 5 minutes at room temperature. Cell pellets were mixed with 50 mL of FACS buffer containing 4 mL of pSTAT4 (BD Bioscience) and pSTAT5 (BD Bioscience) and incubated for 30 minutes at room temp in the dark. Cells were mixed with 100 mL of FACS buffer, washed and centrifuged for 1500 RPM for 5 minutes at room temperature. Cell pellets were mixed with 50 mL of FACS buffer and analyzed by Flow Cytometry (Celesta-BD Bioscience).

The data show that cytokine signaling is elevated (STAT4 and STAT5) following contact with the IL-12/IL-15 α Su and IL-18/IL-15) multi-chain chimeric polypeptide compared to the controls (Figures 13).

Example 10: Acute stimulation of Immune cells to measure Extra Cellular Acidification Rate (ECAR) a surrogate of cellular metabolism and fitness

A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15 α Su and IL-18/IL-15: 18t15-12s) on ECAR on purified NK cells (Figure 14).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 4×10^6 /mL in 96 well, 50 μ L/well, were seeded in Cell-Tak-coated Seahorse Bioanalyzer XFe96 culture plates in Seahorse XF RPMI medium, pH 7.4 supplemented with 2 mM L-glutamine. The cells were allowed to attach to the plate for 30 mins at 37°C. Additional 130 μ L of assay medium was added to each well of the plate (also the background wells). The plate was incubated in 37°C, non-CO₂ incubator for 1 hr. For Calibration plate, the 10x solution of Glucose/oligomycin/2DG were prepared in Seahorse assay media. 20 μ L volume of Glucose/oligomycin/2DG were added to ports B, C and D of the extracellular flux plate that was calibrated overnight. For the acute experiment hIL-12 (10 ng/mL) (Biolegend) and hIL-15 (50 ng/mL) (NCI) or either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or 100 nM of IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide were added directly to the port A of the calibration plate. Extracellular acidification rate (ECAR) was measured using an XFe96 Extracellular Flux Analyzer. Complete ECAR analysis consisted of four stages: basal (without drugs), glycolysis induction (10 mM glucose), maximal glycolysis induction (2 μ M oligomycin), and glycolysis inhibition (100 mM 2-DG). (Experiment representation of one donor out of 9).

The data show that ECAR is elevated following contact with an IL-12/IL-15R α Su and IL-18/IL-15) multi-chain chimeric polypeptide compared to the controls (Figure 14).

Example 11: Acute stimulation of Immune cells to measure Extra Cellular Acidification Rate (ECAR) in the presence of Glucose

A set of experiments was performed to determine the effect of the first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15: 18t15-12s) on ECAR on purified NK cells when in the presence of glucose (Figure 15).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent

(StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 4×10^6 /mL in 96 well, 50 μ L/well, were seeded in Cell-Tak-coated Seahorse Bioanalyzer XFe96 culture plates in Seahorse XF RPMI medium, pH 7.4 supplemented with 2 mM L-glutamine. The cells were allowed to attach to the plate for 30 mins at 37°C. Additional 130 μ L of assay medium was added to each well of the plate (also the background wells). The plate was incubated in 37°C, non-CO₂ incubator for 1 hr. For Calibration plate: the 10x solution of oligomycin/2DG were prepared in Seahorse assay media. 20 μ L volume of oligomycin/2DG were added to port B and C of the extracellular flux plate that was calibrated overnight. Cells were stimulated with Glucose (10 mM) mixed with single cytokines or (IL-12/IL-15R α Su and IL-18/IL-15) multi-chain polypeptide or media alone were added directly to the port A of the calibration plate. Extracellular acidification rate (ECAR) was measured using an XFe96 Extracellular Flux Analyzer. Complete ECAR analysis consisted of four stages: basal (without drugs), glycolysis induction (10 mM glucose), maximal glycolysis induction (2 μ M oligomycin), and glycolysis inhibition (100 mM 2-DG). (Experiment representation of one donor out of 4).

The data show that ECAR is elevated following contact with an IL-12/IL-15R α Su and IL-18/IL-15: 18t15-12s multi-chain chimeric polypeptide and in the presence of glucose compared to the controls (Figure 15).

Example 12: Acute stimulation of Immune cells to measure Extra Cellular Acidification Rate (ECAR) with addition (spike) of Glucose to show increased glycolysis and glycolytic capacity

A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15: 18t15-12s) on ECAR on NK cells when glucose was spiked in after the addition of the fusion protein (Figure 16).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750

(BioLegend). Cells were counted and resuspended in 4×10^6 /mL in 96 well, 50 μ L/well, were seeded in Cell-Tak-coated Seahorse Bioanalyzer XFe96 culture plates in Seahorse XF RPMI medium, pH 7.4 supplemented with 2 mM L-glutamine. The cells were allowed to attach to the plate for 30 mins at 37°C. Additional 130 μ L of assay medium was added to each well of the plate (also the background wells). The plate was incubated in 37°C, non-CO2 incubator for 1 hr. For Calibration plate: The 10x solution of Glucose/oligomycin/2DG were prepared in Seahorse assay media. 20 μ L volume of Glucose/oligomycin/2DG were added to port B, C and D of the extracellular flux plate that was calibrated overnight. For the acute experiment hIL-12 (10 ng/mL) (Biolegend) and hIL-15 (50 ng/mL) (NCI) or either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or 100 nM of IL-12/IL-15R α Su and IL-18/IL-15 were added directly to the port A. Cells were stimulated with Glucose (10 mM) after 3 reading of acute stimulation with mixture of single cytokine or IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide or media alone. Extracellular acidification rate (ECAR) was measured using an XFe96 Extracellular Flux Analyzer. Complete ECAR analysis consisted of four stages: basal (without drugs), glycolysis induction (10 mM glucose), maximal glycolysis induction (2 μ M oligomycin), and glycolysis inhibition (100 mM 2-DG). (Experiment representation of one donor out of 4).

The data show that ECAR is elevated following contact with the IL-12/IL-15R α Su and IL-18/IL-15: 18t15-12s) multi-chain chimeric polypeptide and when the glucose is spiked in after the addition of the fusion protein compared to the controls (Figure 16).

Example 13: Gene expression analysis of mTOR, MYC and SREBP after stimulation with IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide showing speed of drug effects on cellular gene programming

A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15) on gene expression in NK cells (Figure 17).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by

staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 2×10^6 /mL in 24 wells flat bottom plate in 1 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with hIL-12 (10 ng/mL) (Biolegend) and hIL-15 (50 ng/mL) (NCI) or either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or IL-12/IL-15 α Su and IL-18/IL-15 multi-chain chimeric polypeptide complex 100 nM for 60 and 180 minutes at 37°, 5% CO₂. Cells were harvested at different points and proceed for gene expression analysis. RNA extraction, cDNA synthesis and real-time PCR: Total RNA was extracted using RNeasy Mini Kit (Qiagen #74106) according to the manufacturer's instructions. 1 μ g of total RNA was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was carried out with CFX96 Detection System (Bio-Rad) using FAM labeled predesigned primers for human specific genes mTORC, Myc, SREBP and 18S were purchased from Thermo Scientific. Reactions were run in triplicate in three independent experiments. The housekeeping gene 18S ribosomal RNA was used as an internal control to normalize the variability in expression levels. The expression of each target mRNA relative to 18S rRNA was calculated based on Ct as $2^{-\Delta(\Delta Ct)}$, in which $\Delta Ct = Ct_{\text{target}} - Ct_{18S}$. The data is presented as fold change as compared to no treatment control.

The data show that gene expression is elevated following contact with the IL-12/IL-15 α Su and IL-18/IL-15) multi-chain chimeric polypeptide compared to the controls (Figure 17).

Example 14: Increase in activation and intracellular markers after stimulation with IL-12/IL-15 α Su and IL-18/IL-15 multi-chain chimeric polypeptide

A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15 α Su and IL-18/IL-15) on activation in NK cells (Figure 18).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by

staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in $0.2 \times 10^6/\text{mL}$ in 96 well flat bottom plate in 0.2 mL of complete media (Cells were cultured in RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D) and hIL-15 (50 ng/mL) (NCI) or 100 nM of IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide for 30, 60, 90 and 180 minutes at 37°C, 5% CO₂. Next day cells were treated with 10 µg/mL of Brefeldin A (Sigma) and 1x of Monensin (eBioscience) for 4 hrs. Cells were harvested and surface stained for CD56-BV421, CD16-BV510, CD25-APC, CD69-APCFire750, CD62L-PeCy7 for 30 minutes. After surface staining, cells were washed (1500 RPM for 5 minutes in room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma)) and fixed for 10 minutes at room temperature. After fixation steps, cells were washed (1500 RPM for 5 minutes in room temperature) in 1x Permeabilized Buffer (eBioscience) and stained for intracellular staining of IFN- γ - PE (Biolegend) for 30 minutes at room temperature. Cells were washed once again with 1x Permeabilized Buffer and then washed with FACS buffer. Cell pellets were resuspended in 300 µL of FACS Buffer for analysis by Flow Cytometry (Celesta-BD Bioscience).

The data show that NK activation is elevated following contact with the IL-12/IL-15R α Su and IL-18/IL-15) multi-chain chimeric polypeptide compared to the controls (Figure 18).

Example 15: Increase in 41BB and IL21R markers after stimulation with an IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide comparing short term vs overnight showing cells are primed to respond to activating stimuli

A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15) on activation in NK cells (Figure 19).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by

staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 0.2×10^6 /mL in 96 wells flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or 100 nM of IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide for either 180 minutes or overnight at 37° C, 5% CO₂. Cells were harvested, and surface stained for CD56-BV421, CD16-BV510, IL21r-PE, 41BB-APC.Cy7 (BioLegend) for 30 minutes. After surface staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1x PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma)). After two washes, cells were analyzed by Flow Cytometry (Celesta-BD Bioscience).

The data show that NK activation is elevated following contact with the IL-12/IL-15R α Su and IL-18/IL-15) multi-chain chimeric polypeptide compared to the controls (Figure 19).

Example 16: Expansion of NK cells by IL-7/IL-15R α Su and IL-21/IL-15 multi-chain chimeric polypeptide and IgG1 antibody construct after short stimulations (180 minutes) with IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide or media alone

A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15), the second multi-chain chimeric polypeptide (IL-7/IL-15R α Su and IL-21/IL-15), the IgG1 antibody construct on expansion of NK cells (Figure 20).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 1×10^8 /mL in 96 wells flat bottom plate in complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies),

streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or 100 nM of IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide for 180 minutes. Cells were diluted
 5 from 1x10⁸/mL to 2x10⁶/mL with 100 nM of IL-7/IL-15R α Su and IL-21/IL-15 and 50 nM of IgG1 antibody construct in 24 wells plate at 37°, 5% CO₂. Cells were maintained at 2x10⁶/mL with fresh complete containing 100 nM of IL-7/IL-15R α Su and IL-21/IL-15 and 50 nM of IgG1 antibody construct. Cell expansion was measured in fold expansion (3 Donors).

10 The data show that NK expand following treatment with the first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15), the second chimeric polypeptides (IL-7/IL-15R α Su and IL-21/IL-15) and the IgG1 antibody construct.

Example 17: Increase in degranulation markers after stimulation with an exemplary first multi-chain chimeric polypeptide and expansion with an exemplary second multi-chain chimeric polypeptide

15 A set of experiments was performed to determine the effect of a first multi-chain chimeric polypeptide (IL-12/IL-15R α Su and IL-18/IL-15), a second multi-chain chimeric polypeptide (IL-7/IL-15R α Su and IL-21/IL-15), and a IgG1 antibody
 20 construct on degranulation of NK cells (Figure 11).

In these experiments, fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750
 25 (BioLegend). Cells were counted and resuspended in 1x10⁸/mL in 96 wells flat bottom plate in complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50
 30 ng/mL)(R&D Systems) and hIL-15 (50 ng/mL) (NCI) or 100 nM of IL-12/IL-15R α Su and IL-18/IL-15 multi-chain chimeric polypeptide for 180 minutes. Cells were diluted from 1x10⁸/mL to 2x10⁶/mL with 100 nM of IL-7/IL-15R α Su and IL-21/IL-15 multi-chain chimeric polypeptide and 50 nM of IgG1 antibody construct in 24 wells

plate at 37°C with 5% CO₂. Cells were maintained at 2x10⁶/mL with fresh complete containing 100 nM of IL-7/IL-15RαSu and IL-21/IL-15 and 50 nM of IgG1 antibody construct or 1 ng/mL of rhIL-15 up to day 11. Cells were harvested and resuspended in 2:1 (effector : target) ratio with K562 cell lines (target) with 4 μL of CD107-FITC (Biolegend) at 37°, 5% CO₂. After four hours of incubation cells were harvested and surface stained for CD56-BV421, CD16-BV510 for 30 minutes. After surface staining, cells were washed (1500 RPM for 5 minutes in room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma). Cell pellets were resuspended in 50 μL of FACS Buffer for Flow Cytometry analysis (Celesta-BD Bioscience).

The data show the NK cells exhibit enhanced cytotoxicity against target cells after treatment with a first multi-chain chimeric polypeptide (IL-12/IL-15RαSu and IL-18/IL-15^(D8N)), a second multi-chain chimeric polypeptides (IL-7/IL-15RαSu and IL-21/IL-15^(D8N)), and a IgG1 antibody construct (Figure 21).

Example 18: Oxygen Consumption Rate (OCR)

Fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies) and CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 1x10⁸/mL in a 96-well flat-bottom plate in complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone). Cells were stimulated with either a mixture of cytokines (SCKs) [hIL-12 (10 ng/mL) (BioLegend), hIL-18 (50 ng/mL) (R&D Systems), and hIL-15 (50 ng/mL) (NCI)] or 100 nM of 18t15-12s for 180 minutes (Kick) or with media only (not stimulated). Cells were diluted from 1x10⁸/mL to 2x10⁶/mL and expanded with 100 nM of 7t15-21s and 50 nM of anti-tissue factor antibody (IgG1) (αTF Ab) in a 24-well plate at 37 °C, 5% CO₂. Cells were maintained at 2x10⁶/mL with fresh complete media containing 100 nM of 7t15-21s and 50 nM of αTF Ab or 50 ng/mL of recombinant human IL-15 for 14 days. Cells were harvested on day 14, counted and resuspended in 4 x 10⁶/mL in a 96-well plate, 50 μL/well, and were seeded in Cell-Tak-coated Seahorse Bioanalyzer XFe96 culture plates in Seahorse XF RPMI medium, pH 7.4 supplemented with 10 mM glucose and 2 mM L-glutamine.

The cells were allowed to attach to the plate for 30 mins at 37 °C. Additionally, 130 µL of the assay medium was added to each well of the plate (also the background wells). The plate was incubated in 37 °C, non-CO₂ incubator for 1 hour. For Calibration plate: The 10x solution of oligomycin/FCCP/rotenone were prepared in
 5 Seahorse assay media. 20 µL of oligomycin, FCCP and rotenone was added to each of the ports of the extracellular flux plate that was calibrated overnight. Oxygen Consumption Rate (OCR) was measured using an XFe96 Extracellular Flux Analyzer. Complete OCR analysis consisted of four stages: basal respiration (without drugs), ATP-linked respiration/proton leak (1.5 µM oligomycin), maximal respiration (2 µM
 10 FCCP), and spare respiration (0.5 µM rotenone). The OCR graph is a combined representation of three donors.

The data show that NK cells stimulated with either a mixture of cytokines (SCKs) or with 18t15-12s and then supported for expansion with 7t15-21s and anti-tissue factor antibody (αTF Ab) resulted in increased oxygen consumption rate
 15 (OCR). Addition of carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) further enhanced the oxygen consumption rate (OCR) capacity. The data also show that the unstimulated cells, cells stimulated with mixture of cytokines (SCKs) or with 18t15-12s and supported for expansion with 7t15-21s and anti-tissue factor antibody (αTF Ab) also resulted in an increase in the OCR. However, NK cells treated
 20 overnight with mixture of cytokines (SCKs) or with 18t15-12s and then supported with a low dose of IL-15 did not show much increase in the OCR. Overall, NK cells stimulated (Kick) with 18t15-12s and then expanded with 7t15-21s and anti-tissue factor antibody (αTF Ab) are more metabolically fit as compared to NK cells stimulated with 18t15-12s and then expanded with a low dose of IL-15 (Figure 22).

25

Example 19: Gene expression analysis of IFN-γ and NKG2D after stimulation with 18t15-12s

Fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell
 30 Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 1x10⁸/mL in 96-well flat bottom plate in complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life

Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were divided into a short-term activation (180 mins) or long-term activation group (overnight stimulation for 12-16 hours), or were not stimulated. Cells were stimulated (Kick) with either a mixture of cytokines (SCKs) [hIL-12 (10 ng/mL) (BioLegend), hIL-18 (50 ng/mL) (R&D Systems) and hIL-15 (50 ng/mL) (NCI)] or 100 nM of 18t15-12s for 180 minutes or for 12-16 hours. Cells were diluted from $1 \times 10^8/\text{mL}$ to $2 \times 10^6/\text{mL}$ and expanded with 100 nM of 7t15-21s and 50 nM of anti-tissue factor antibody ($\alpha\text{TF Ab}$) in a 24-well plate at 37°C , 5% CO_2 . Cells were maintained at $2 \times 10^6/\text{mL}$ with fresh complete media containing 100 nM of 7t15-21s and 50 nM of $\alpha\text{TF Ab}$ for 14 days. Every other day cells were stimulated with fresh media and 100 nM of 7t15-21s and 50 nM of $\alpha\text{TF Ab}$ up to 14 days. Cells were harvested on day 14, at different points, and then evaluated for gene expression analysis. RNA extraction, cDNA synthesis, and real-time PCR were performed. Total RNA was extracted using RNeasy Mini Kit (Qiagen #74106) according to the manufacturer's instructions. One μg of total RNA was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was carried out with CFX96 Detection System (Bio-Rad) using FAM labeled predesigned primers purchased for $\text{IFN}\gamma$ and NKG2D from Thermo Scientific. Reactions were run in triplicate in three independent experiments. The housekeeping gene 18S ribosomal RNA was used as an internal control to normalize the variability in expression levels. The expression of each target mRNA relative to 18S rRNA was calculated based on Ct as $2^{-\Delta(\Delta\text{Ct})}$, in which $\Delta\text{Ct} = \text{Ct}_{\text{target}} - \text{Ct}_{18\text{S}}$. The data is presented as fold-change as compared to no treatment control.

The data show that NK cells stimulated with 18t15-12s for 180 minutes and then expanded with 7t15-21s and anti-tissue factor antibody ($\alpha\text{TF Ab}$) were able to increase $\text{IFN}\gamma$ and NKG2D gene expression compared to NK cells stimulated with a mixture of cytokines (SCKs) and then expanded with 7t15-21s and anti-tissue factor antibody ($\alpha\text{TF Ab}$). There was no difference in the $\text{IFN}\gamma$ and NKG2D gene expression in overnight stimulated NK cells with a mixture of cytokines (SCKs) or 18t15-12s and then expanded with 7t15-21s and anti-tissue factor antibody ($\alpha\text{TF Ab}$). This implies that short-term stimulation with 18t15-12s, and then expansion with 7t15-21s and anti-tissue factor antibody ($\alpha\text{TF Ab}$) is enough to upregulate memory-like markers on NK cells (Figure 23).

Example 20: Persistence of adoptively transferred NK cells, activated with 18t15-12s and expanded with 7t15-21s and α TF IgG1 in an NSG mouse model

Fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 (BioLegend). Cells were counted and resuspended in 1×10^8 /mL in a 96-well flat bottom plate in complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone). Cells were either unstimulated with media as a control or stimulated (Kick) with 100 nM of 18t15-12s for either 180 minutes or for 12-16 hours (overnight). Cells were diluted from 1×10^8 /mL to 2×10^6 /mL with 100 nM of 7t15-21s and 50 nM of anti-tissue factor antibody (α TF Ab) in a 24-well plate at 37 °C, 5% CO₂. Cells were maintained at 2×10^6 /mL with fresh complete media containing 100 nM of 7t15-21s and 50 nM of α TF Ab for 21 days. Cells were maintained at 2×10^6 /mL with fresh complete media containing 100 nM of 7t15-21s and 50 nM of α TF Ab and as the volume increased, the cells were transferred to higher volume flasks. Cells were harvested on day 21 and counted using trypan blue to access the fold-expansion. Expanded cells were washed three times in warm HBSS Buffer (Hyclone) at 1000 RPM for 10 minutes at room temperature. Cells were resuspended in 10×10^6 /0.2 mL HBSS buffer and injected intravenously in tail-vein of NSG mouse (NOD scid gamma mouse) (Jackson Laboratories). Cells were supported with Proleukin (rhIL2) (5000 IU) by subcutaneous injection every 48 hours, up to Day 12. Engraftment of cells were measured every week in blood staining for hCD45, mCD45, hCD56, hCD3 and hCD16 by flow cytometry (Celesta-BD Bioscience). (Data represent 5 mice per group).

The data show that NK cells stimulated with 18t15-12s for either 180 minutes or overnight and supported for expansion with 7t15-21s and anti-tissue factor antibody (α TF Ab) are able to persist *in vivo* for up to 12 days after infusion in NSG mouse compared to NK cells without the stimulation with 18t15-12s (Figure 24).

Example 21: Epigenetic Analysis of DNA Methylation of IFN γ -CNS-1 CpG**Region**

Fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >85% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 (BioLegend). Cells were counted and resuspended at a density of 2×10^6 cells/mL in RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), antibiotics (penicillin, 10,000 units/mL; streptomycin, 10,000 μ g/mL; Thermo Life Technologies), and 10% FBS (Hyclone). Cells (1 mL) were transferred in a 24-well flat bottom plate, and subjected to either: no treatment, or stimulated with a mixture of single cytokines (SCKs) [hIL-12 (10 ng/mL) (BioLegend), hIL-18 (50 ng/mL) (R&D Systems) and hIL-15 (50 ng/mL) (NCI)] or 18t15-12s (100 nM) for a short-term exposure of either 180 minutes or 12-16 hours (Overnight) at 37 °C under an atmosphere of 5% CO₂. Then, the NK cells were washed and resuspended in complete RPMI 1640 medium supplemented with 7t15-21s plus the anti-tissue factor antibody (α TF Ab, 50 nM) and incubated for 14 days (Long-term). Cells were replenished with 7t15-21s plus α TF Ab (50 nM) to keep the cell density at approximately 1×10^6 cells/mL.

Treatment of NK cells with single cytokines followed by a low dose of IL-15 has been shown to promote memory-like NK cells differentiation and was used as comparative controls for memory-like NK-cell epigenetics. Unexposed/untreated NK cells from donors to treatment groups were used as positive controls for full DNA methylation levels. Genomic DNA (nDNA) was extracted from NK cells ($0.2 - 1.0 \times 10^6$) using the QIAamp UCP DNA Micro Kit (Qiagen). Purified nDNA (500 ng) was subjected to sodium bisulfite treatment using the EZ DNA Methylation-Direct kit (Zymo Research) according to the manufacturer's protocol. Bisulfite treatment introduces methylation-dependent changes in the DNA with demethylated cytosines being converted into uracil, whereas methylated cytosines remain unchanged. The bisulfite-treated nDNA (10 - 50 ng) was used as template to PCR amplify a 247-bp region of the IFN γ distal promoter conserved noncoding sequence 1 (CNS-1) enhancer region containing six characterized CpG sites (located at positions -4399, -4377, -4360 -4325, -4293, and -4278 relative to the transcription start site). This

CNS-1 region has been found to be important in T-cell and NK cells differentiation and is epigenetically regulated by DNA methylation (Dong *et al.*, *Eur. J. Immunol.* 43:793-804, 2013). The PCR were performed using the Pyromark PCR kit (Qiagen) with the forward primer IFNG-CNS1F (5'-AGAAAAGGGGGGATTTA-3') (SEQ ID NO: 81) and the biotinylated reverse primer IFNG-CNS1R-bio (5'-TAACACTCACAACCAAATTATC-3') (SEQ ID NO: 82) (GENEWIZ). The PCR conditions were 15 min at 95 °C, 50 cycles of 35 sec at 95 °C, 35 sec at 55 °C, 40 sec at 72 °C followed by 5 min at 72 °C. The integrity and quality of the 247-bp PCR amplified products were visualized on a 1.2% TAE agarose gel. The DNA methylation status of the six CpG sites was determined by pyrosequencing, which is the gold standard technique to quantitatively measure DNA methylation at single CpG-site. Pyrosequencing reactions were performed at Johns Hopkins University Genetic Resources Core Facility using the DNA sequencing primers C4399-CNS1F (5'-GGGGATTTAGAAAAAT-3' (SEQ ID NO: 83), specific to the CpG sites -4399, -4377, -4360, and -4325), and C4293-CNS1F (5'-TGTATGATGTTAGGAGTTT-3' (SEQ ID NO: 84), specific to the CpG sites -4293, -4278, and -4227). For DNA methylation controls, human non-methylated and methylated genomic DNA were used (Zymo Research). The methylation percentages of the six IFN γ CNS-1 CpG sites were averaged for each treatment.

Analysis of the DNA methylation status of these six IFN γ CNS-1 CpG sites revealed higher levels of DNA demethylation in NK cells stimulated (kick) for 180 minutes or 12-16 hours (Overnight) with either a mixture of single cytokines (SCKs) or 18t15-12s and supported for 14 days (Long-term) in culture by 7t15-21s (50 nM) plus α TF Ab (50 nM) compared to unexposed (Control) or NK cells stimulated (kick) for 180 minutes or Overnight (Short-term) with either a mixture of single cytokines (IL12 + IL18 + IL15) or 18t15-12s. Indeed, the 7t15-21s + α TF Ab supported NK cells exhibited 33.80% $\pm$ 13.20 to 40.72% $\pm$ 15.96 DNA methylation compared to 79.50% $\pm$ 14.21 for unexposed (Control), 79.25% $\pm$ 13.14 to 79.97% $\pm$ 14.13 for 180 minutes stimulated (kick), and 73.51% $\pm$ 14.08 to 73.66% $\pm$ 14.01 for overnight stimulated (kick) NK cells. Taken together, these results suggest that long-term exposure of NK cells to 7t15-21s + α TF Ab induces significant DNA demethylation of the CpG sites within the CNS-1 region, leading to epigenetic remodeling of IFN γ

gene expression and interconversion of NK cells into innate immune memory NK cells (Figure 25).

Example 22: Epigenetic Analysis of Memory-Like NK cells *in vivo*

5 Fresh human leukocytes were obtained from the blood bank and CD56⁺ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 (BioLegend). Cells were counted and resuspended at a density of 2×10^6 cells/mL in RPMI 1640 medium
10 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), antibiotics (penicillin, 10,000 units/mL; streptomycin, 10,000 µg/mL; Thermo Life Technologies), and 10% FBS (Hyclone). Cells (1 mL) were transferred in 24 wells flat bottom plate, and subjected to either: stimulated with mixture of single cytokines (labeled SCKs) [hIL-12 (10 ng/mL) (BioLegend), hIL-18 (50 ng/mL) (R&D
15 Systems), and hIL-15 (50 ng/mL) (NCI)] or 18t15-12s (100 nM) for 12-16 hours at 37 °C under an atmosphere of 5% CO₂. Then, the NK cells were washed and resuspended in complete RPMI 1640 medium supplemented with 7t15-21s + anti-tissue factor antibody (αTF Ab), and incubated for 14 days, with medium frequently replenished with fresh 7t15-21s + αTF Ab to keep the cell density at approximately 1×10^6 cells/mL. After 14 days of expansion, the activated NK cells were either
20 processed for pyrosequencing analysis (pre-infusion samples) or infused in NSG mice (five groups, five mice per group). At day 9 post-infusion, the spleen of each mice was collected, and NK cells purified and combined for each group (five group, five mice per groups). The samples (post-infusion) were subjected to pyrosequencing
25 analysis of DNA methylation of CpG sites located within the IFNγ CNS-1 region as described below.

Genomic DNA (nDNA) was extracted from NK cells ($0.2 - 1.0 \times 10^6$) using the QIAamp UCP DNA Micro Kit (Qiagen). Purified nDNA (500 ng) was subjected to sodium bisulfite treatment using the EZ DNA Methylation-Direct kit (Zymo
30 Research) according to the manufacturer's protocol. Bisulfite treatment introduces methylation-dependent changes in the DNA with demethylated cytosines being converted into uracil, whereas methylated cytosines remain unchanged. The bisulfite-treated nDNA (10-50 ng) was used as template to PCR amplify a 247-bp region of the

IFN γ distal promoter conserved noncoding sequence 1 (CNS-1) enhancer region containing six characterized CpG sites (located at positions -4399, -4377, -4360 - 4325, -4293, and -4278 relative to the transcription start site). This CNS-1 region has been found to be important in T-cell and NK cells differentiation and is epigenetically regulated by DNA methylation (Dong *et al.*, *Eur. J. Immunol.* 43:793-804, 2013). The PCR were performed using the Pyromark PCR kit (Qiagen) with the forward primer IFNG-CNS1F (5'-AGAAAAGGGGGGATTTA-3'; SEQ ID NO: 81) and the biotinylated reverse primer IFNG-CNS1R-bio (5'-TAACACTCACAACCAAATTATC-3'; SEQ ID NO: 82) (GENEWIZ). The PCR conditions were 15 min at 95 °C, 50 cycles of 35 sec at 95 °C, 35 sec at 55 °C, 40 sec at 72 °C followed by 5 min at 72 °C. The integrity and quality of the 247-bp PCR amplified products were visualized on a 1.2% TAE agarose gel. The DNA methylation status of the six CpG sites was determined by pyrosequencing, which is the gold standard technique to quantitatively measure DNA methylation at single CpG-site. Pyrosequencing reactions were performed at Johns Hopkins University Genetic Resources Core Facility using the DNA sequencing primers C4399-CNS1F (5'-GGGGATTTAGAAAAAT-3' (SEQ ID NO: 83), specific to the CpG sites -4399, -4377, -4360, and -4325), and C4293-CNS1F (5'-TGTATGATGTTAGGAGTTT-3' (SEQ ID NO: 84), specific to the CpG sites -4293, -4278, and -4227). For DNA methylation controls, human non-methylated and methylated genomic DNA were used (Zymo Research). The methylation percentages of the six IFN γ CNS-1 CpG sites were averaged for each treatment.

Analysis of the DNA methylation status of these six IFN γ CNS-1 CpG sites revealed higher levels of DNA demethylation in pre-infused activated NK cells stimulated overnight with either a mixture of single cytokines (IL12 + IL18 + IL15) or 18t15-12s and supported for 14 days in culture by 7t15-21s (50 nM) plus α TF Ab (50 nM) compared to methylated DNA controls. Indeed, the single cytokines and the 18t15-12s stimulated NK cells exhibited $26.99\% \pm 12.85$ and $24.58\% \pm 13.22$ DNA methylation, respectively, versus $87.09\% \pm 10.65$ for methylated DNA controls. More important, post-infused NK cells isolated from NSG mice at day 9 also exhibited very low levels of DNA methylation with $18.97\% \pm 11.17$ and $19.57\% \pm 10.22$ DNA methylation for single cytokines and 18t15-12s treated NK cells, respectively. Taken together, these results suggest that the memory-like phenotype of

activated NK cells in maintained *in vivo* for up 9 days, and that once the epigenetic remodeling mechanism is established, it is fixed in subsequent NK cell progeny and persist *in vivo* (Figure 26).

5 **Example 23: Expansion of NK cells by IL-7/IL-21/IL-15 and anti-TF IgG1**

In a non-limiting example, a set of experiments was performed to determine the effect of 7t15-21s alone or 7t15-21s in combination with either anti-TF IgG1 or anti-TF IgG4 on expansion of NK cells (assessed at 5 days of expansion). The data show that NK cells require IgG1 signal for expansion (Figure 27). A schematic
10 diagram depicting NK cell expansion and activation is shown in Figure 28.

Example 24: Expansion of Primary NK cells with 7t15-21s + α TF Ab

In a non-limiting example, a set of experiments was performed to determine the effect of 7t15-21s and anti-TF IgG1 on expansion of primary NK cells over a time
15 period of 15 days. The data shows NK cells expanded over the 15-day period and had increased expression of surface markers CD25 and CD69, and decreased expression of CD62L and CD57 (Figure 29).

Example 25: Expansion strategy after short term activation

In a non-limiting example, NK cells were activated for a short term of 1-3
20 hours with 18t15-12s to induce memory-like NK cells. After a short-term kick, the NK cells were expanded with 7t15-21s and anti-TF IgG1 for 14-21 days. The expansion of memory-like NK cells were characterized by measuring the percentage of surface markers (e.g., CD25, CD69, NKp44, NKp30, CD62L, and CD16)
25 expression in the kicked and expanded NK cells (Figures 33-35). The data also shows the cytotoxicity of NK cells following no stimulation or stimulation with a mixture of recombinant cytokines (IL-12, IL-15, and IL-18) or 18t15-12s (Figure 36).

Example 26. Kick and Expansion of Cord Blood-Derived NK Cells

30 Fresh human CD34⁺ cord blood were obtained (OrganaBio) and CD56⁺ NK cells were isolated with the CD56⁺CD16⁺ NK cell isolation kit (Miltenyi Biotec). The purity of NK cells was >50% and confirmed by staining with CD56-BV421, CD16-BV510, CD4-BV488, CD14-APC, CD8 PerCP Cy5.5, CD3 APC CY7 (BioLegend).

Cells were counted and resuspended at a density of 1×10^6 cells/mL in PBS. 2 μ M of final concentration of Cell-Trace Violet Dye (CTV) (Life Technologies) were added to the cells and incubated for 20 minutes at 37 °C under an atmosphere of 5% CO₂. Cells were resuspended at five times volume in fresh pre-warmed RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), antibiotics (penicillin, 10,000 units/mL; streptomycin, 10,000 μ g/mL; Thermo Life Technologies), and 10% FBS (Hyclone) for 5 minutes at room temperature. Cells were washed 2 times at 1000 rpm for 10 minutes at counted and resuspended at a density of 2×10^6 cells/mL in RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), antibiotics (penicillin, 10,000 units/mL; streptomycin, 10,000 μ g/mL; Thermo Life Technologies), and 10% FBS (Hyclone). Cells (0.1 mL) were transferred in a 96-well flat-bottom plate, and subjected to either: no treatment (media), or stimulated with 18t15-12s (250 nM) for a short-term exposure of either 180 minutes (kick) or 12-16 hours (overnight kick) at 37 °C under an atmosphere of 5% CO₂. Then, all conditions of NK cells were washed and resuspended in complete RPMI 1640 medium supplemented with 7t15-21s (200 nM) plus the anti-tissue factor antibody (α TF Ab, 100 nM) and incubated for 7 days (long-term). Cells were replenished with 7t15-21s (200 nM) plus α TF Ab (100 nM) to keep the cell density at approximately 2×10^6 cells/mL every 48 to 72 hrs. To assess cell proliferation, NK cells were harvested and surface stained for CD56 for 30 minutes. After surface staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma)). After two washes, cells were analyzed by Flow Cytometry (Celesta-BD Bioscience).

Analysis of the cell proliferation of NK cells stimulated (kick) for 180 minutes or 12-16 hours (Overnight) with 18t15-12s and supported for 7 days in culture by 7t15-21s (200 nM) plus α TF Ab (100 nM) increased proliferative capacity compared to unstimulated cells supported with 7t15-21s (200 nM) plus α TF Ab (100 nM). As shown in Figure 37A, compared to the control, which are unstimulated cells supported with 7t15-21s (200 nM) plus α TF Ab (100 nM), the treatment group stimulated with cytokines (kick and overnight kick) shows increased proliferation of NK cells (number of divisions). The control group shows dividing cells in different stages of divisions compared to few terminal divisions in NK cells stimulated for 180 minutes

(kick) or 12-16 hours (overnight kick) with 18t15-12s and supported for 7 days in culture by 7t15-21s (200 nM) plus α TF Ab (100 nM). Figure 37B represents the percentage of cell proliferation.

5 Taken together, this result suggests that NK cells derived from cord blood, stimulated 18t15-12s and supported by 7t15-21s plus α TF Ab has increased proliferative capacity.

OTHER EMBODIMENTS

10 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of promoting the activation and proliferation of a natural killer cell, the method comprising:

(a) contacting a natural killer cell in a liquid culture medium comprising an effective amount of a first multi-chain chimeric polypeptide for a first period of time under conditions that allow for the differentiation of the natural killer cell; and

(b) contacting the natural killer cell in a liquid culture medium comprising an effective amount of (i) a second multi-chain chimeric polypeptide, and (ii) an IgG1 antibody construct, for a second period of time under conditions that allow for the activation and proliferation of the natural killer cell,

wherein the first multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the first multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the first multi-chain chimeric polypeptide, and the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12; and

wherein the second multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains, and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the second multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the second multi-chain chimeric polypeptide; the first target-

binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide each independently bind specifically to a receptor for IL-7 or a receptor for IL-21, and the IgG1 antibody construct comprises at least one antigen-binding domain that binds specifically to the linker domain.

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

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

4. The method of any one of claims 1-3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide.

5. The method of any one of claims 1-3, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

6. The method of any one of claims 1-5, wherein the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide.

7. The method of any one of claims 1-5, wherein the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

8. The method of any one of claims 1-7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

9. The method of claim 8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

10. The method of claim 9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

11. The method of claim 10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

12. The method of claim 11, wherein the soluble human tissue factor domain comprises SEQ ID NO: 5.

13. The method of claim 8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9.

14. The method of claim 13, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9.

15. The method of claim 14, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9.

16. The method of claim 15, wherein the soluble human tissue factor domain comprises SEQ ID NO: 9.

17. The method of claim 8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10.

18. The method of claim 17, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10.

19. The method of claim 18, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 10.

20. The method of claim 19, wherein the soluble human tissue factor domain comprises SEQ ID NO: 10.

21. The method of any one of claims 8-11, 13-15, and 17-19, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

22. The method of claim 21, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

23. The method of any one of claims 1-22, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

24. The method of any one of claims 1-23, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

25. The method of any one of claims 1-24, wherein the first multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

26. The method of any one of claims 1-25, wherein the first chimeric polypeptide and/or the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end.

27. The method of claim 26, wherein the signal sequence comprises SEQ ID NO: 28.

28. The method of claim 27, wherein the signal sequence is SEQ ID NO: 28.

29. The method of any one of claims 1-28, wherein the pair of affinity domains in the first multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15.

30. The method of claim 29, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

31. The method of claim 29, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26.

32. The method of claim 31, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26.

33. The method of claim 32, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26.

34. The method of claim 33, wherein the soluble IL-15 comprises SEQ ID NO: 26.

35. The method of any one of claims 29-34, wherein the sushi domain of IL15R α comprises a sushi domain from human IL15R α .

36. The method of claim 35, wherein the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24.

37. The methods of claim 36, wherein the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24.

38. The method of claim 37, wherein the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24.

39. The method of claim 38, wherein the sushi domain from human IL15R α comprises SEQ ID NO: 24.

40. The method of claim 35, wherein the sushi domain from human IL15R α is a mature full-length IL15R α .

41. The method of any one of claims 1-28, wherein the pair of affinity domains in the first multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag

modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

42. The method of any one of claims 1-41, wherein the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide are each independently a soluble IL-15 or a soluble IL-18.

43. The method of any one of claims 1-42, wherein the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18.

44. The method of any one of claims 1-42, wherein the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12.

45. The method of claim 53, wherein the first target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-18.

46. The method of claim 45, wherein the soluble IL-18 is a soluble human IL-18.

47. The method of claim 46, wherein the soluble human IL-18 comprises a sequence at least 80% identical to SEQ ID NO: 20.

48. The method of claim 47, wherein the soluble human IL-18 comprises a sequence at least 90% identical to SEQ ID NO: 20.

49. The method of claim 48, wherein the soluble human IL-18 comprises a sequence at least 95% identical to SEQ ID NO: 20.

50. The method of claim 49, wherein the soluble human IL-18 comprises a sequence of SEQ ID NO: 20.

51. The method of any one of claims 44-50, wherein the second target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-12.

52. The method of claim 51, wherein the soluble IL-12 is a soluble human IL-12.

53. The method of claim 52, wherein the soluble human IL-12 comprises a sequence of soluble human IL-12 β (p40) and a sequence of soluble human IL-12 α (p35).

54. The method of claim 53, wherein the soluble human IL-15 further comprises a linker sequence between the sequence of soluble IL-12 β (p40) and the sequence of soluble human IL-12 α (p35).

55. The method of claim 54, wherein the linker sequence comprises SEQ ID NO: 11.

56. The method of any one of claims 53-55, wherein the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 80% identical to SEQ ID NO: 14.

57. The method of claim 56, wherein the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 90% identical to SEQ ID NO: 14.

58. The method of claim 57, wherein the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 95% identical to SEQ ID NO: 14.

59. The method of claim 58, wherein the sequence of soluble human IL-12 β (p40) comprises SEQ ID NO: 14.

60. The method of any one of claims 53-59, wherein the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 80% identical to SEQ ID NO: 16.

61. The method of claim 60, wherein the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 90% identical to SEQ ID NO: 16.

62. The method claim 61, wherein the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 95% identical to SEQ ID NO: 16.

63. The method of claim 62, wherein the sequence of soluble human IL-12 α (p35) comprises SEQ ID NO: 16.

64. The method of claim 1, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKD
SQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGH
DNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDESGTTN
TVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTEC
DLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQ
PTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSS
GKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQKEG
EFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPCKVTAMKCFLELQV
ISLESGDASIHTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 63).

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

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

67. The method of claim 66, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 63.

68. The method of claim 67, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises

MKWVTFISLLFLFSSAYSYFGKLESKLSVIRNLNDQVLFDQGNRPLFEDMTD
SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPD
NIKDTKSDIIFQRSVPGHDNKMQFESSYEGYFLACEKERDLFKLILKKEDEL
GDRSIMFTVQNEDESGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQIS
TKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGE
PLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLR
DVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFVSQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESD
VHPCKVTAMKCFLELQVISLES GDASIHTVENLIILANNSLSSNGNVTESG
CKECEEELEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 64).

69. The method of any one of claims 1 and 64-68, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK
TLTIQVKEFGDAGQYTCHKGGEVLSSHLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAE
RVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIR
DIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKR
EKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCSGGGGSGG
GGSGGGGSRNLPVATPDPGMFPC LHHSQNLLRAVSNMLQKARQTLEFYPCTS
EEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALN
FNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPM

SVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTP
SLKCIR (SEQ ID NO: 65).

70. The method of claim 69, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 65.

71. The method of claim 70, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 65.

72. The method of claim 71, wherein the second chimeric polypeptide comprises SEQ ID NO: 65.

73. The method of claim 72, wherein the second chimeric polypeptide comprises

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEED
GITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHLLLLHKKE
DGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRG
SSDPQGVTCGAATLSAERVGRDNKEYEYSVEQCEDSACPAAEESLPIEVMVD
AVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPH
SYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSS
WSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC LHHSQNLLRAV
SNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRET
SFITNGSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFL
DQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNASITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLT
ECVLNKATNVAHWTPSLKCIR (SEQ ID NO: 66).

74. The method of any one of claims 1-73, wherein the first target-binding domain and the linker domain in the first chimeric polypeptide in the second multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide.

75. The method of any one of claims 1-73, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker

sequence between the first target-binding domain and the linker domain in the first chimeric polypeptide.

76. The method of any one of claims 1-75, wherein the linker domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide in the second multi-chain chimeric polypeptide.

77. The method of any one of claims 1-75, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the linker domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

78. The method of any one of claims 1-77, wherein the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide of the second multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide.

79. The method of any one of claims 1-77, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

80. The method of any one of claims 1-79, wherein the linker domain is a soluble tissue factor domain.

81. The method of claim 80, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

82. The method of claim 81, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

83. The method of claim 82, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

84. The method of claim 83, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

85. The method of claim 84, wherein the soluble human tissue factor domain comprises SEQ ID NO: 5.

86. The method of claim 81, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9.

87. The method of claim 86, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9.

88. The method of claim 87, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9.

89. The method of claim 88, wherein the soluble human tissue factor domain comprises SEQ ID NO: 9.

90. The method of claim 81, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10.

91. The method of claim 90, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10.

92. The method of claim 91, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 10.

93. The method of claim 92, wherein the soluble human tissue factor domain comprises SEQ ID NO: 10.

94. The method of any one of claims 82-84, 86-88, and 90-92, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

95. The method of claim 94, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

96. The method of any one of claims 81-95, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

97. The method of any one of claims 81-96, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

98. The method of any one of claims 81-97, wherein the second multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

99. The method of any one of claims 1-98, wherein the first chimeric polypeptide and/or the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end.

100. The method of claim 99, wherein the signal sequence comprises SEQ ID NO: 28.

101. The method of claim 100, wherein the signal sequence is SEQ ID NO: 28.

102. The method of any one of claims 1-101, wherein the pair of affinity domains in the second multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15.

103. The method of claim 102, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

104. The method of claim 102, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26.

105. The method of claim 104, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26.

106. The method of claim 105, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26.

107. The method of claim 106, wherein the soluble IL-15 comprises SEQ ID NO: 26.

108. The method of any one of claims 102-107, wherein the sushi domain of IL15R α comprises a sushi domain from human IL15R α .

109. The method of claim 108, wherein the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24.

110. The methods of claim 109, wherein the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24.

111. The method of claim 110, wherein the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24.

112. The method of claim 111, wherein the sushi domain from human IL15R α comprises SEQ ID NO: 24.

113. The method of claim 108, wherein the sushi domain from human IL15R α is a mature full-length IL15R α .

114. The method of any one of claims 1-101, wherein the pair of affinity domains in the second multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

115. The method of any one of claims 1-114, wherein the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide are each independently a soluble IL-7 or a soluble IL-21.

116. The method of any one of claims 1-115, wherein the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7.

117. The method of any one of claims 1-115, wherein the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21.

118. The method of claim 117, wherein the first target-binding domain in the second multi-chain chimeric polypeptide comprises a soluble IL-21.

119. The method of claim 118, wherein the soluble IL-21 is a soluble human IL-21.

120. The method of claim 119, wherein the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 22.

121. The method of claim 120, wherein the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 22.

122. The method of claim 121, wherein the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 22.

123. The method of claim 122, wherein the soluble human IL-21 comprises a sequence of SEQ ID NO: 22.

124. The method of any one of claims 117-123, wherein the second target-binding domain comprises a soluble IL-7.

125. The method of claim 124, wherein the soluble IL-7 is a soluble human IL-7.

126. The method of claim 125, wherein the soluble human IL-7 comprises a sequence at least 80% identical to SEQ ID NO: 23.

127. The method of claim 126, wherein the soluble human IL-7 comprises a sequence at least 90% identical to SEQ ID NO: 23.

128. The method of claim 127, wherein the soluble human IL-7 comprises a sequence at least 95% identical to SEQ ID NO: 23.

129. The method of claim 128, wherein the soluble human IL-7 comprises a sequence of SEQ ID NO: 23.

130. The method of claim 1, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKE
GMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLEIKTCWNKILMGTKEHSGTT
NTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTE
CDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSS
SGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRVTNRKSTDSPVECMGQEK
GEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ
VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 67).

131. The method of claim 130, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 67.

132. The method of claim 131, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 67.

133. The method of claim 132, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 67.

134. The method of claim 133, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises
MKWVTFISLLFLFSSAYSDCDIEGKDQKQYESVLMVSIQQLDSMKEIGSNCL
NNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTT
ILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKT
CWNKILMGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQIST
KSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEP
LYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRD
VFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTV
NRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDV
HPCKVTAMKCFLELQVISLESGDASIHDVTENLIILANNSLSSNGNVTESGC
KECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 68).

135. The method of any one of claims 1 and 130-134, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to
QGQDRHMRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
AQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRER
YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 69).

136. The method of claim 135, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 69.

137. The method of claim 136, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 69.

138. The method of claim 137, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 69.

139. The method of claim 138, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises

MKWVTFISLLFLFSSAYSQGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAP
EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQK
HRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPMSV
EHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATNV AHWTPSL
KCIR (SEQ ID NO: 70).

140. The method of claim 1, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

QGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
AQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNL TWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFS
YPAGNVESTGSAGEPLYENSPEFTPLYETNLGQPTIQSFEQVGTKVNVTVDE
RTLVRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKG
ENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLI
QSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:
71).

141. The method of claim 140, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 71.

142. The method of claim 141, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 71.

143. The method of claim 142, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 71.

144. The method of claim 143, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises
 MGVKVLFA LICI A V A E A Q G Q D R H M I R M R Q L I D I V D Q L K N Y V N D L V P E F L P A P
 E D V E T N C E W S A F S C F Q K A Q L K S A N T G N N E R I I N V S I K K L K R K P P S T N A G R R Q K
 H R L T C P S C D S Y E K K P P K E F L E R F K S L L Q K M I H Q H L S S R T H G S E D S S G T T N T V A
 A Y N L T W K S T N F K T I L E W E P K P V N Q V Y T V Q I S T K S G D W K S K C F Y T T D T E C D L T
 D E I V K D V K Q T Y L A R V F S Y P A G N V E S T G S A G E P L Y E N S P E F T P Y L E T N L G Q P T I
 Q S F E Q V G T K V N V T V E D E R T L V R R N N T F L S L R D V F G K D L I Y T L Y Y W K S S S S G K
 K T A K T N T N E F L I D V D K G E N Y C F S V Q A V I P S R T V N R K S T D S P V E C M G Q E K G E F
 R E N W V N V I S D L K K I E D L I Q S M H I D A T L Y T E S D V H P S C K V T A M K C F L L E L Q V I S
 L E S G D A S I H D T V E N L I L A N N S L S S N G N V T E S G C K E C E E E L E E K N I K E F L Q S F V H I
 V Q M F I N T S (SEQ ID NO: 72).

145. The method of any one of claims 1 and 140-144, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to

DCDIEGKD G K Q Y E S V L M V S I D Q L L D S M K E I G S N C L N N E F N F F K R H I C D A N K E
 G M F L F R A A R K L R Q F L K M N S T G D F D L H L L K V S E G T T I L L N C T G Q V K G R K P A A L
 G E A Q P T K S L E E N K S L K E Q K K L N D L C F L K R L L Q E I K T C W N K I L M G T K E H I T C P P
 P M S V E H A D I W V K S Y S L Y S R E R Y I C N S G F K R K A G T S S L T E C V L N K A T N V A H W T
 T P S L K C I R (SEQ ID NO: 73).

146. The method of claim 145, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 73.

147. The method of claim 146, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 73.

148. The method of claim 147, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 73.

149. The method of claim 148, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises
MGVKVLFALICIAVAEADCDIEGKDQKQYESVLMVSIQLLDSMKEIGSNCL
NNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTT
ILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKT
CWNKILMGTKEHITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSS
LTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 74).

150. The method of any one of claims 1-149, wherein the IgG1 antibody construct is a monoclonal IgG1 antibody, where both antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain.

151. The method of any one of claims 1-149, wherein the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

152. The method of any one of claims 1-151, wherein the first period of time is about 15 minutes to about 4 hours.

153. The method of claim 152, wherein the first period of time is about 30 minutes to about 3.5 hours.

154. The method of claim 153, wherein the first period of time is about 1 hour to about 3.5 hours.

155. The method of claim 154, wherein the first period of time is about 2 hours to about 3.5 hours.

156. The method of any one of claims 1-155, wherein the liquid culture medium in step (a) and/or step (b) is a serum-containing liquid culture medium.

157. The method of any one of claims 1-156, wherein the NK cells are in the liquid culture medium in step (a) at a density of about 4.0×10^7 cells/mL to about 1.5×10^8 cells/mL.

158. The method of claim 157, wherein the NK cells are in the liquid culture medium in step (a) at a density of about 5.0×10^7 cells/mL to about 2.0×10^8 cells/mL.

159. The method of any one of claims 1-158, wherein the second period of time is about 1 day to about 30 days.

160. The method of claim 159, wherein the second period of time is about 7 days to about 30 days.

161. The method of claim 160, wherein the second period of time is about 14 days to about 21 days.

162. The method of any one of claims 1-161, wherein the liquid culture medium in step (b) comprises the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.5:1 to about 2:1.

163. The method of claim 162, wherein the liquid culture medium in step (b) comprises the second multi-chain chimeric polypeptide and the IgG1 antibody construct at a molar ratio of about 0.8:1 to about 1.2:1.

164. The method of any one of claims 1-163, wherein the NK cells are in the liquid culture medium in step (b) are maintained at a density of about 0.5×10^6 cells/mL to about 2.0×10^6 cells/mL.

165. The method of claim 164, wherein the NK cells are in the liquid culture medium in step (b) are maintained at a density of about 1.0×10^6 cells/mL to about 2.0×10^6 cells/mL.

166. The method of any one of claims 1-165, wherein the NK cell was previously obtained from a subject.

167. The method of any one of claims 1-165, wherein the NK cell was previously obtained from umbilical cord blood.

168. The method of any one of claims 1-165, wherein the NK cell was previously obtained from an induced pluripotent stem cell.

169. The method of claim 166 or 167, wherein the method further comprises obtaining the NK cell from the subject prior to step (a).

170. The method of any one of claims 1-169, wherein the NK cell has previously been genetically modified to express a chimeric antigen receptor or a recombinant T-cell receptor.

171. The method of any one of claims 1-169, wherein the method further comprises, between step (a) and step (b), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

172. The method of any one of claims 1-169, wherein the method further comprises, prior to step (a), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

173. The method of any one of claims 1-169, wherein the method further comprises, during step (b), introducing into the NK cell a nucleic acid encoding a chimeric antigen-receptor or a recombinant T-cell receptor.

174. The method of any one of claims 1-173, wherein the method further comprises, after step (b), isolating the NK cell.

175. The method of any one of claims 1-174, wherein after step (b), the NK cell has an increased level of expression or secretion of one or more proteins selected

from the group consisting of: TNF- α , IFN- γ , granzyme A, granzyme B, perforin, 2B4, CD8, CD11a, CD16, CD25, CD27, CD48, CD49d, CD54, CD56, CD58, CD62L, CD69, CD70, CD94, CD137, CD158a, CD158b, CD158e, CD178, CD226, CD253, NKG2C, NKG2D, LIR-1, LILR-B1, KIR2DL1, KIR3DL1, KIR2DL2, KIR2DL3, CXCR3, NKp30, NKp44, NKp46, NKG2D, DNAM-1, TRAIL, FasL, CXCR3, CXCR4, LTB, MX1, BAX, TNF- α , and IFN- γ as compared to the level of expression or secretion of the one or more proteins prior to step (a).

176. The method of any one of claims 1-177, wherein the method further comprises, after the contacting step, administering the NK cell to a subject in need thereof.

177. The method of claim 176, wherein the subject has been identified or diagnosed as having an age-related disease or condition.

178. The method of claim 177, wherein the age-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

179. The method of claim 176, wherein the subject has been identified or diagnosed as having a cancer.

180. The method of claim 179, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma,

glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

181. The method of claim 176, wherein the subject has been diagnosed or identified as having an infectious disease.

182. The method of claim 181, 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.

183. An activated NK cell produced by the method of any one of claims 1-182.

184. A pharmaceutical composition comprising the activated NK cell of claim 183.

185. A kit comprising a pharmaceutical composition comprising the activated NK cell of claim 183.

186. 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 activated NK cell of claim 183 or the pharmaceutical composition of claim 184.

187. The method of claim 186, wherein the subject has been identified or diagnosed as having a cancer.

188. The method of claim 187, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

189. The method of claim 186, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

190. The method of claim 189, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

191. The method of claim 186, wherein the subject has been diagnosed or identified as having an infectious disease.

192. The method of claim 191, 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.

193. A method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the activated NK cell or the activated T cell of claim 183 or the pharmaceutical composition of claim 184.

194. The method of claim 193, wherein the subject has been identified or diagnosed as having a cancer.

195. The method of claim 194, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

196. The method of claim 193, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

197. The method of claim 196, wherein the aging-related disease or condition is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, and renal dysfunction.

198. The method of claim 197, wherein the subject has been diagnosed or identified as having an infectious disease.

199. The method of claim 198, 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.

200. A kit comprising:

(a) a first multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the first multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the first multi-chain chimeric polypeptide, and the first target-binding domain and the second target-binding domain in the first

multi-chain chimeric polypeptide each independently bind specifically to a receptor of IL-18 or a receptor of IL-12;

(b) a second multi-chain chimeric polypeptide comprises:

a first chimeric polypeptide comprising a first target-binding domain, a linker domain, a first domain of a pair of affinity domains, and

a second chimeric polypeptide comprising a second domain of a pair of affinity domains, and a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide in the second multi-chain chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains in the second multi-chain chimeric polypeptide; and the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide each independently bind specifically to a receptor for IL-7 or a receptor for IL-21; and

(c) an IgG1 antibody that comprises at least one antigen-binding domain that binds specifically to the linker domain.

201. The kit of claim 200, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide in the first multi-chain chimeric polypeptide.

202. The kit of claim 200, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

203. The kit of any one of claims 200-202, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide.

204. The kit of any one of claims 200-202, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker

sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

205. The kit of any one of claims 200-204, wherein the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide in the first multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide.

206. The kit of any one of claims 200-204, wherein the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

207. The kit of any one of claims 200-206, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

208. The kit of claim 207, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

209. The kit of claim 208, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

210. The kit of claim 209, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

211. The kit of claim 210, wherein the soluble human tissue factor domain comprises SEQ ID NO: 5.

212. The kit of claim 207, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9.

213. The kit of claim 212, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9.

214. The kit of claim 213, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9.

215. The kit of claim 214, wherein the soluble human tissue factor domain comprises SEQ ID NO: 9.

216. The kit of claim 207, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10.

217. The kit of claim 216, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10.

218. The kit of claim 217, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 10.

219. The kit of claim 218, wherein the soluble human tissue factor domain comprises SEQ ID NO: 10.

220. The kit of any one of claims 207-210, 212-214, and 216-218, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

221. The kit of claim 220, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

222. The kit of any one of claims 200-221, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

223. The kit of any one of claims 200-222, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

224. The kit of any one of claims 200-223, wherein the first multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

225. The kit of any one of claims 200-224, wherein the first chimeric polypeptide and/or the second chimeric polypeptide in the first multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end.

226. The kit of claim 225, wherein the signal sequence comprises SEQ ID NO: 28.

227. The kit of claim 226, wherein the signal sequence is SEQ ID NO: 28.

228. The kit of any one of claims 200-227, wherein the pair of affinity domains in the first multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15.

229. The kit of claim 228, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

230. The kit of claim 228, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26.

231. The kit of claim 230, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26.

232. The kit of claim 231, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26.

233. The kit of claim 232, wherein the soluble IL-15 comprises SEQ ID NO: 26.

234. The kit of any one of claims 228-233, wherein the sushi domain of IL15R α comprises a sushi domain from human IL15R α .

235. The kit of claim 234, wherein the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24.

236. The kit of claim 235, wherein the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24.

237. The kit of claim 236, wherein the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24.

238. The kit of claim 237, wherein the sushi domain from human IL15R α comprises SEQ ID NO: 24.

239. The kit of claim 234, wherein the sushi domain from human IL15R α is a mature full-length IL15R α .

240. The kit of any one of claims 200-227, wherein the pair of affinity domains in the first multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

241. The kit of any one of claims 200-240, wherein the first target-binding domain and the second target-binding domain in the first multi-chain chimeric polypeptide are each independently a soluble IL-15 or a soluble IL-18.

242. The kit of any one of claims 200-241, wherein the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18.

243. The kit of any one of claims 200-241, wherein the first target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-18, and the second target-binding domain in the first multi-chain chimeric polypeptide binds specifically to a receptor for IL-12.

244. The kit of claim 252, wherein the first target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-18.

245. The kit of claim 244, wherein the soluble IL-18 is a soluble human IL-18.

246. The kit of claim 245, wherein the soluble human IL-18 comprises a sequence at least 80% identical to SEQ ID NO: 20.

247. The kit of claim 246, wherein the soluble human IL-18 comprises a sequence at least 90% identical to SEQ ID NO: 20.

248. The kit of claim 247, wherein the soluble human IL-18 comprises a sequence at least 95% identical to SEQ ID NO: 20.

249. The kit of claim 248, wherein the soluble human IL-18 comprises a sequence of SEQ ID NO: 20.

250. The kit of any one of claims 243-249, wherein the second target-binding domain in the first multi-chain chimeric polypeptide comprises a soluble IL-12.

251. The kit of claim 250, wherein the soluble IL-12 is a soluble human IL-12.

252. The kit of claim 251, wherein the soluble human IL-12 comprises a sequence of soluble human IL-12 β (p40) and a sequence of soluble human IL-12 α (p35).

253. The kit of claim 252, wherein the soluble human IL-15 further comprises a linker sequence between the sequence of soluble IL-12 β (p40) and the sequence of soluble human IL-12 α (p35).

254. The kit of claim 253, wherein the linker sequence comprises SEQ ID NO: 11.

255. The kit of any one of claims 252-254, wherein the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 80% identical to SEQ ID NO: 14.

256. The kit of claim 255, wherein the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 90% identical to SEQ ID NO: 14.

257. The kit of claim 256, wherein the sequence of soluble human IL-12 β (p40) comprises a sequence that is at least 95% identical to SEQ ID NO: 14.

258. The kit of claim 257, wherein the sequence of soluble human IL-12 β (p40) comprises SEQ ID NO: 14.

259. The kit of any one of claims 252-258, wherein the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 80% identical to SEQ ID NO: 16.

260. The kit of claim 259, wherein the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 90% identical to SEQ ID NO: 16.

261. The kit claim 260, wherein the sequence of soluble human IL-12 α (p35) comprises a sequence that is at least 95% identical to SEQ ID NO: 16.

262. The kit of claim 261, wherein the sequence of soluble human IL-12 α (p35) comprises SEQ ID NO: 16.

263. The kit of claim 200, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 63.

264. The kit of claim 263, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 63.

265. The kit of claim 264, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 63.

266. The kit of claim 265, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 63.

267. The kit of claim 266, wherein the first chimeric polypeptide in the first multi-chain chimeric polypeptide comprises SEQ ID NO: 64.

268. The kit of any one of claims 200 and 263-267, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 65.

269. The kit of claim 268, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 65.

270. The kit of claim 269, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 65.

271. The kit of claim 270, wherein the second chimeric polypeptide comprises SEQ ID NO: 65.

272. The kit of claim 271, wherein the second chimeric polypeptide comprises SEQ ID NO: 66.

273. The kit of any one of claims 200-272, wherein the first target-binding domain and the linker domain in the first chimeric polypeptide in the second multi-chain chimeric polypeptide directly abut each other in the first chimeric polypeptide.

274. The kit of any one of claims 200-272, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the linker domain in the first chimeric polypeptide.

275. The kit of any one of claims 200-274, wherein the linker domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide in the second multi-chain chimeric polypeptide.

276. The kit of any one of claims 200-274, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the linker domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

277. The kit of any one of claims 200-276, wherein the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide of the second multi-chain chimeric polypeptide directly abut each other in the second chimeric polypeptide.

278. The kit of any one of claims 200-276, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

279. The kit of any one of claims 200-278, wherein the linker domain is a soluble tissue factor domain.

280. The kit of claim 279, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

281. The kit of claim 280, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

282. The kit of claim 281, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

283. The kit of claim 282, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 5.

284. The kit of claim 283, wherein the soluble human tissue factor domain comprises SEQ ID NO: 5.

285. The kit of claim 280, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 9.

286. The kit of claim 285, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 9.

287. The kit of claim 284, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 9.

288. The kit of claim 285, wherein the soluble human tissue factor domain comprises SEQ ID NO: 9.

289. The kit of claim 278, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10.

290. The kit of claim 289, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10.

291. The kit of claim 290, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 3.

292. The kit of claim 291, wherein the soluble human tissue factor domain comprises SEQ ID NO: 10.

293. The kit of any one of claims 279-281, 283-285, and 289-291, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

294. The kit of claim 293, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

295. The kit of any one of claims 278-294, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

296. The kit of any one of claims 278-295, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

297. The kit of any one of claims 278-296, wherein the second multi-chain chimeric polypeptide does not stimulate coagulation in a mammal.

298. The kit of any one of claims 198-297, wherein the first chimeric polypeptide and/or the second chimeric polypeptide in the second multi-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end.

299. The kit of claim 298, wherein the signal sequence comprises SEQ ID NO: 28.

300. The kit of claim 299, wherein the signal sequence is SEQ ID NO: 28.

301. The kit of any one of claims 198-298, wherein the pair of affinity domains in the second multi-chain chimeric polypeptide is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15.

302. The kit of claim 301, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

303. The kit of claim 301, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 26.

304. The kit of claim 303, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 26.

305. The kit of claim 304, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 26.

306. The kit of claim 305, wherein the soluble IL-15 comprises SEQ ID NO: 26.

307. The kit of any one of claims 301-306, wherein the sushi domain of IL15R α comprises a sushi domain from human IL15R α .

308. The kit of claim 307, wherein the sushi domain from human IL15R α comprises a sequence that is 80% identical to SEQ ID NO: 24.

309. The kit of claim 308, wherein the sushi domain from human IL15R α comprises a sequence that is 90% identical to SEQ ID NO: 24.

310. The kit of claim 309, wherein the sushi domain from human IL15R α comprises a sequence that is 95% identical to SEQ ID NO: 24.

311. The kit of claim 310, wherein the sushi domain from human IL15R α comprises SEQ ID NO: 24.

312. The kit of claim 307, wherein the sushi domain from human IL15R α is a mature full-length IL15R α .

313. The kit of any one of claims 200-300, wherein the pair of affinity domains in the second multi-chain chimeric polypeptide is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

314. The kit of any one of claims 200-313, wherein the first target-binding domain and the second target-binding domain in the second multi-chain chimeric polypeptide are each independently a soluble IL-7 or a soluble IL-21.

315. The kit of any one of claims 200-314, wherein the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-7.

316. The kit of any one of claims 200-314, wherein the first target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a

receptor for IL-7, and the second target-binding domain in the second multi-chain chimeric polypeptide binds specifically to a receptor for IL-21.

317. The kit of claim 316, wherein the first target-binding domain in the second multi-chain chimeric polypeptide comprises a soluble IL-21.

318. The kit of claim 317, wherein the soluble IL-21 is a soluble human IL-21.

319. The kit of claim 318, wherein the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 22.

320. The kit of claim 319, wherein the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 22.

321. The kit of claim 320, wherein the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 22.

322. The kit of claim 321, wherein the soluble human IL-21 comprises a sequence of SEQ ID NO: 22.

323. The kit of any one of claims 316-322, wherein the second target-binding domain comprises a soluble IL-7.

324. The kit of claim 323, wherein the soluble IL-7 is a soluble human IL-7.

325. The kit of claim 324, wherein the soluble human IL-7 comprises a sequence at least 80% identical to SEQ ID NO: 23.

326. The kit of claim 325, wherein the soluble human IL-7 comprises a sequence at least 90% identical to SEQ ID NO: 23.

327. The kit of claim 326, wherein the soluble human IL-7 comprises a sequence at least 95% identical to SEQ ID NO: 23.

328. The kit of claim 327, wherein the soluble human IL-7 comprises a sequence of SEQ ID NO: 23.

329. The kit of claim 200, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 67.

330. The kit of claim 329, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 67.

331. The kit of claim 330, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 67.

332. The kit of claim 331, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 67.

333. The kit of claim 332, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 68.

334. The kit of any one of claims 200 and 329-333, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 69.

335. The kit of claim 334, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 69.

336. The kit of claim 335, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 69.

337. The kit of claim 336, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 69.

338. The kit of claim 337, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 70.

339. The kit of claim 200, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 71.

340. The kit of claim 339, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 71.

341. The kit of claim 340, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 71.

342. The kit of claim 341, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 71.

343. The kit of claim 342, wherein the first chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 72.

344. The kit of any one of claims 200 and 339-343, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 73.

345. The kit of claim 344, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 73.

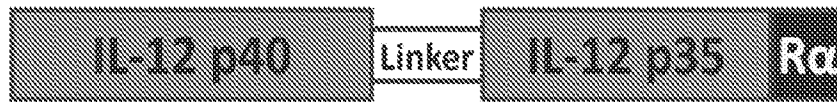
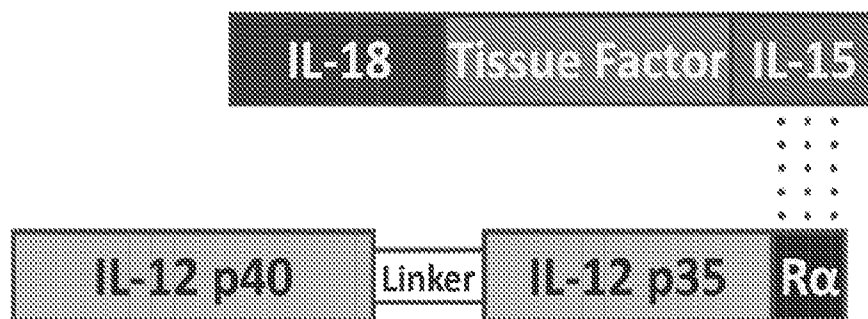
346. The kit of claim 345, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 73.

347. The kit of claim 346, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 73.

348. The kit of claim 347, wherein the second chimeric polypeptide in the second multi-chain chimeric polypeptide comprises SEQ ID NO: 74.

349. The kit of any one of claims 200-348, wherein the IgG1 antibody construct is a monoclonal IgG1 antibody, where both antigen-binding domains in the monoclonal IgG1 antibody bind specifically to the linker domain.

350. The kit of any one of claims 200-348, wherein the IgG1 antibody construct is a bispecific IgG1 antibody, where one of the two antigen-binding domains in the bispecific IgG1 antibody binds specifically to the linker domain.

**Figure 1****Figure 2****Figure 3**

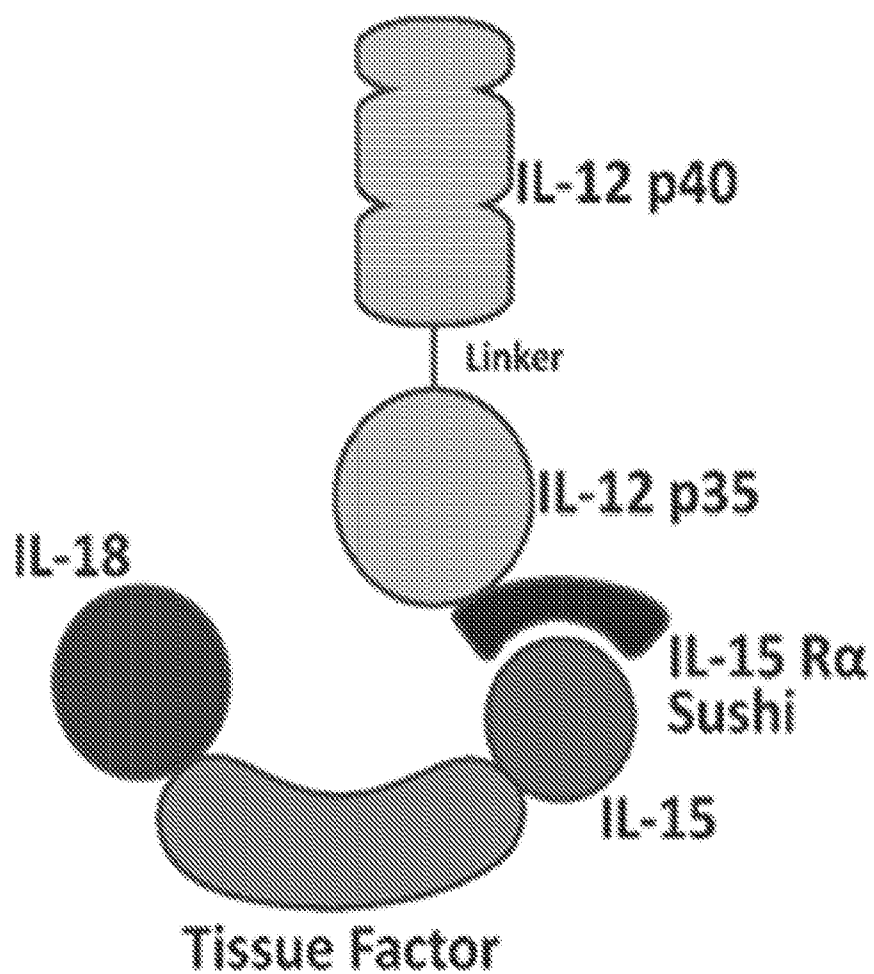
**Figure 4**



Figure 5

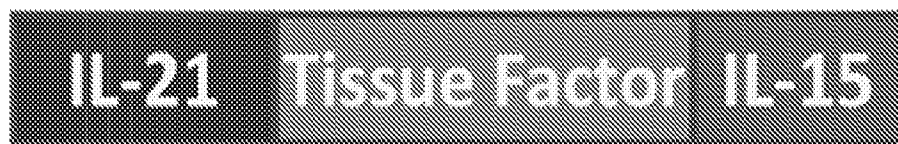


Figure 6

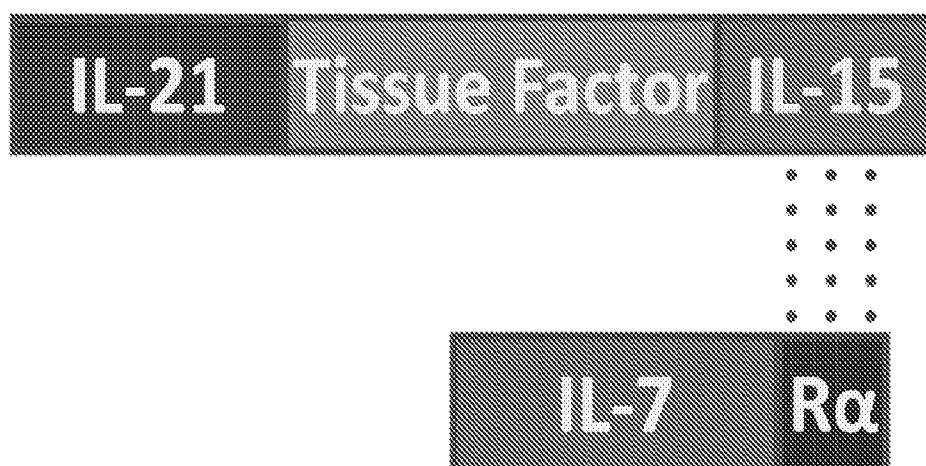


Figure 7

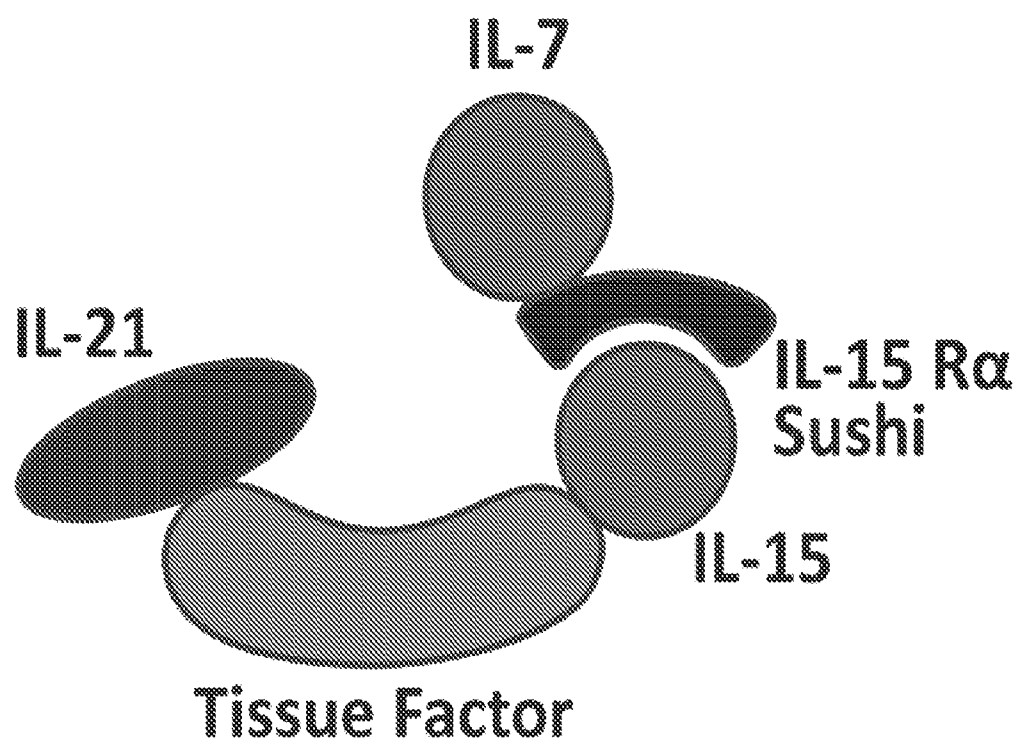
**Figure 8**



Figure 9

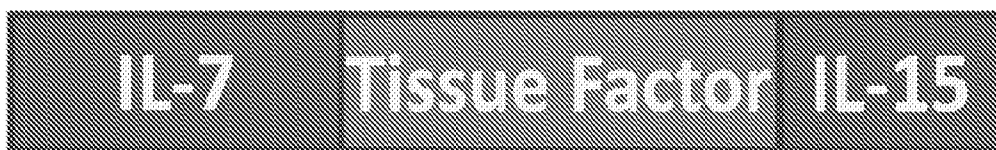


Figure 10

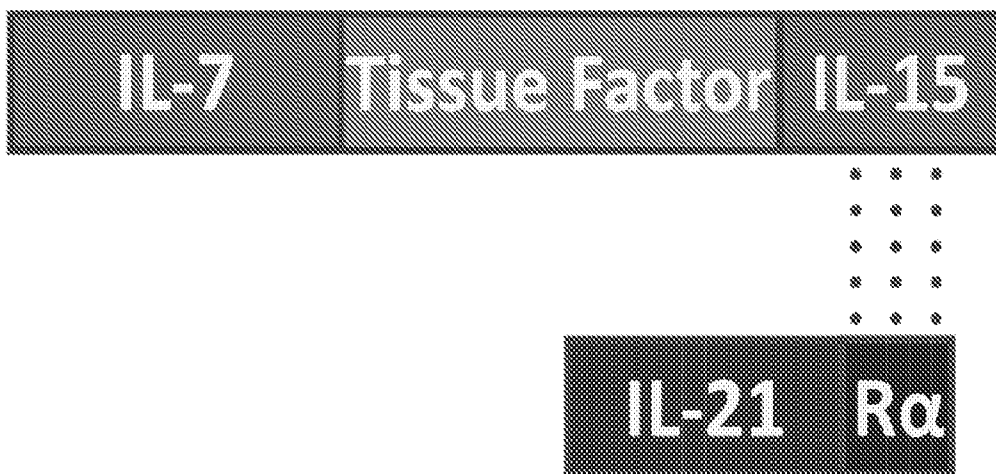


Figure 11

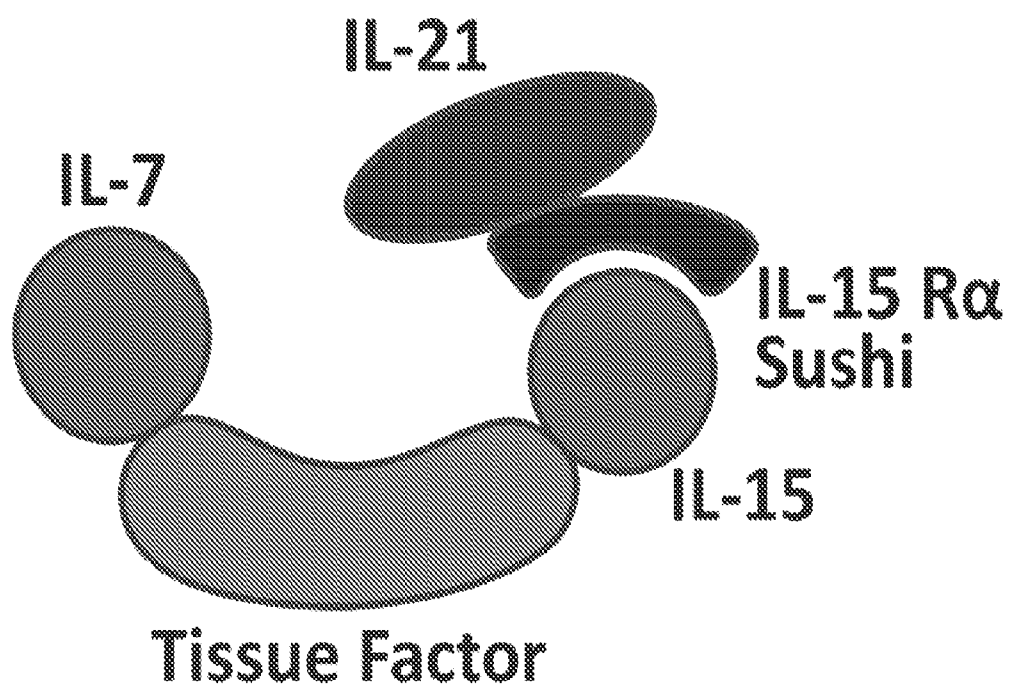


Figure 12

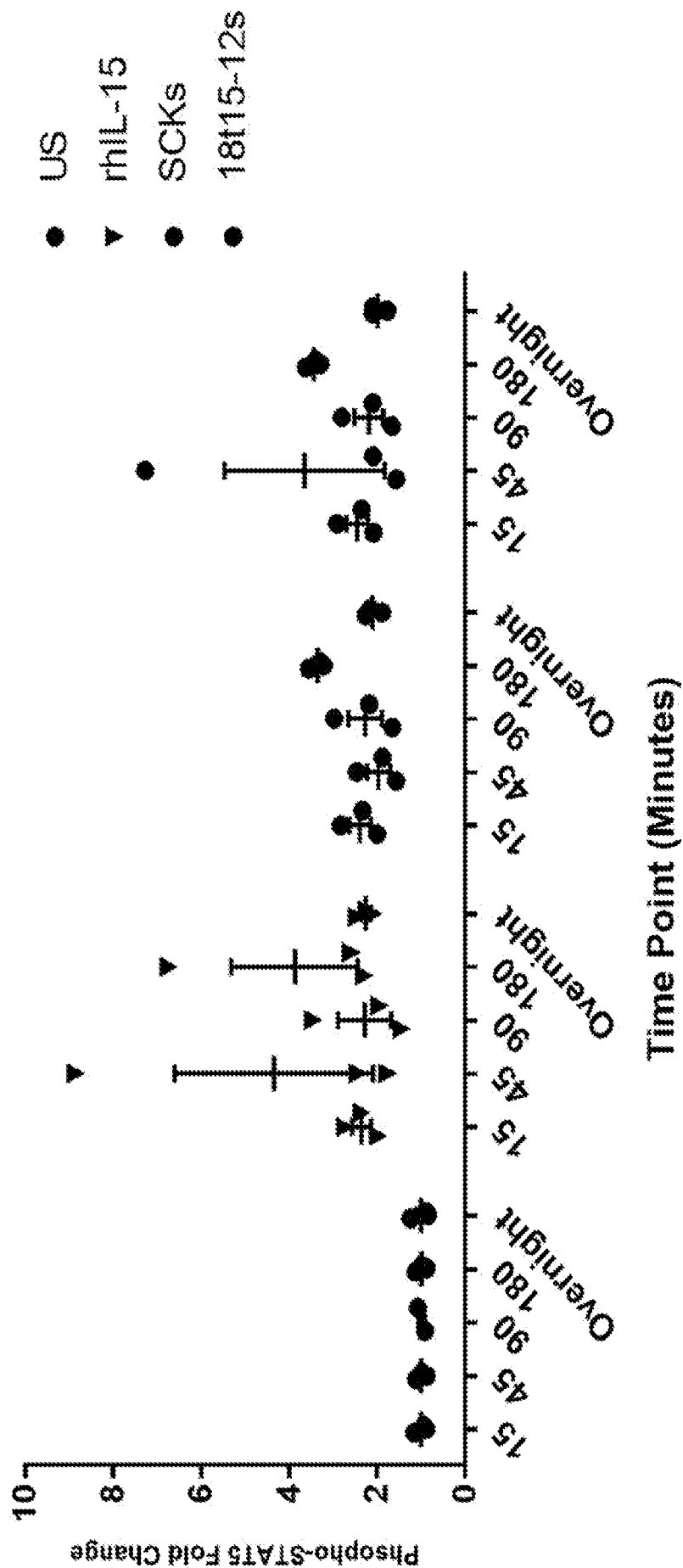


Figure 13A

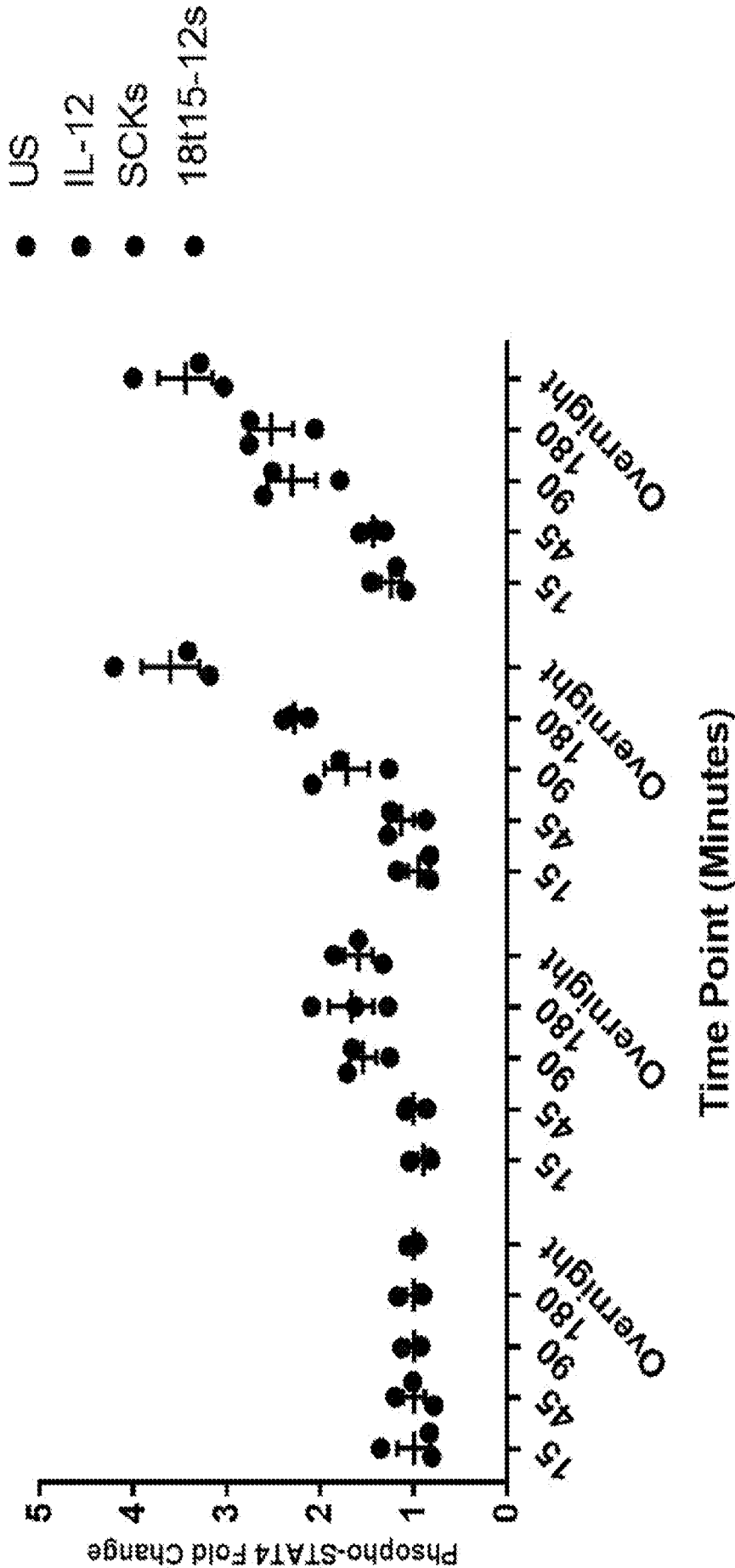
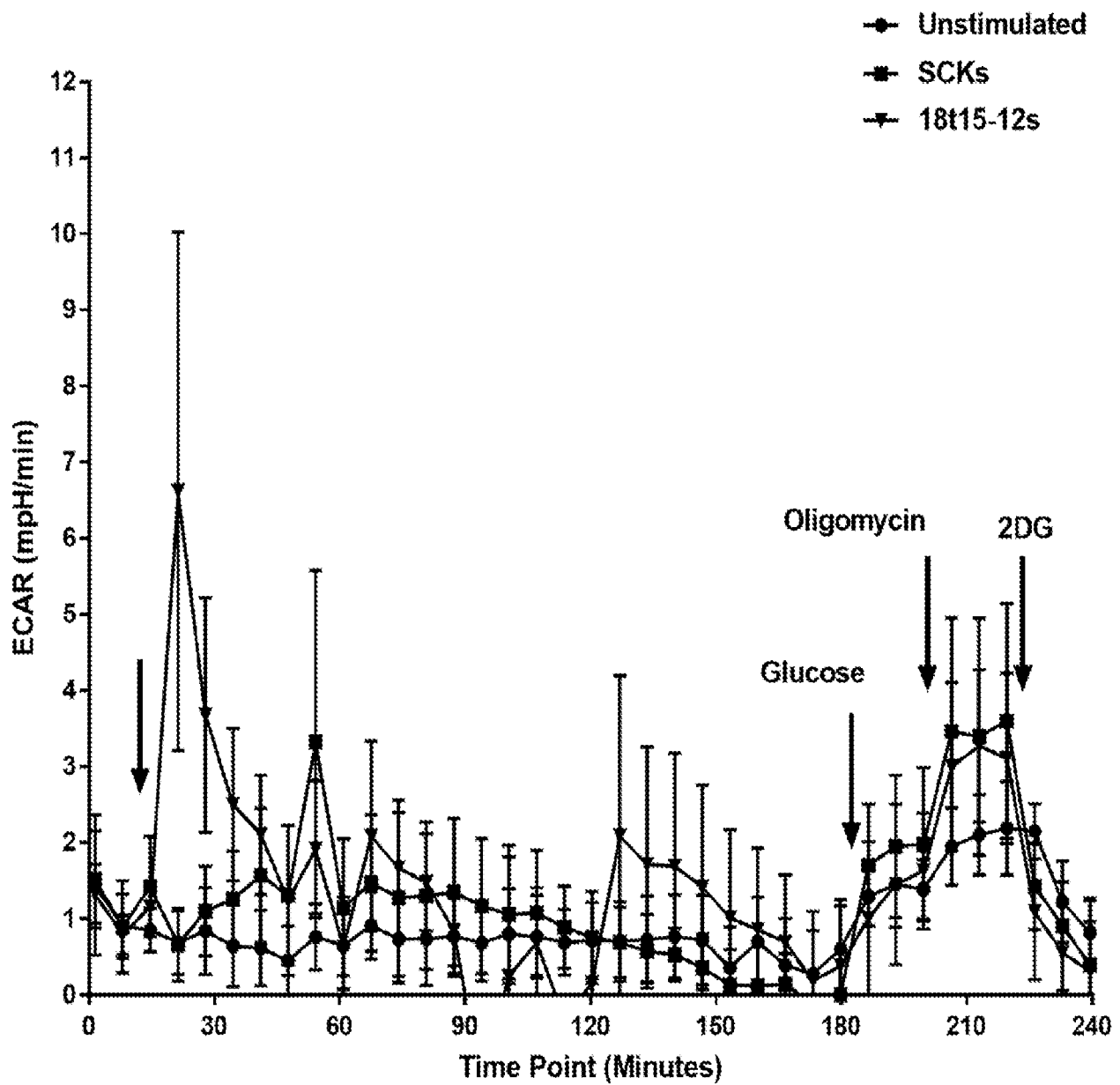
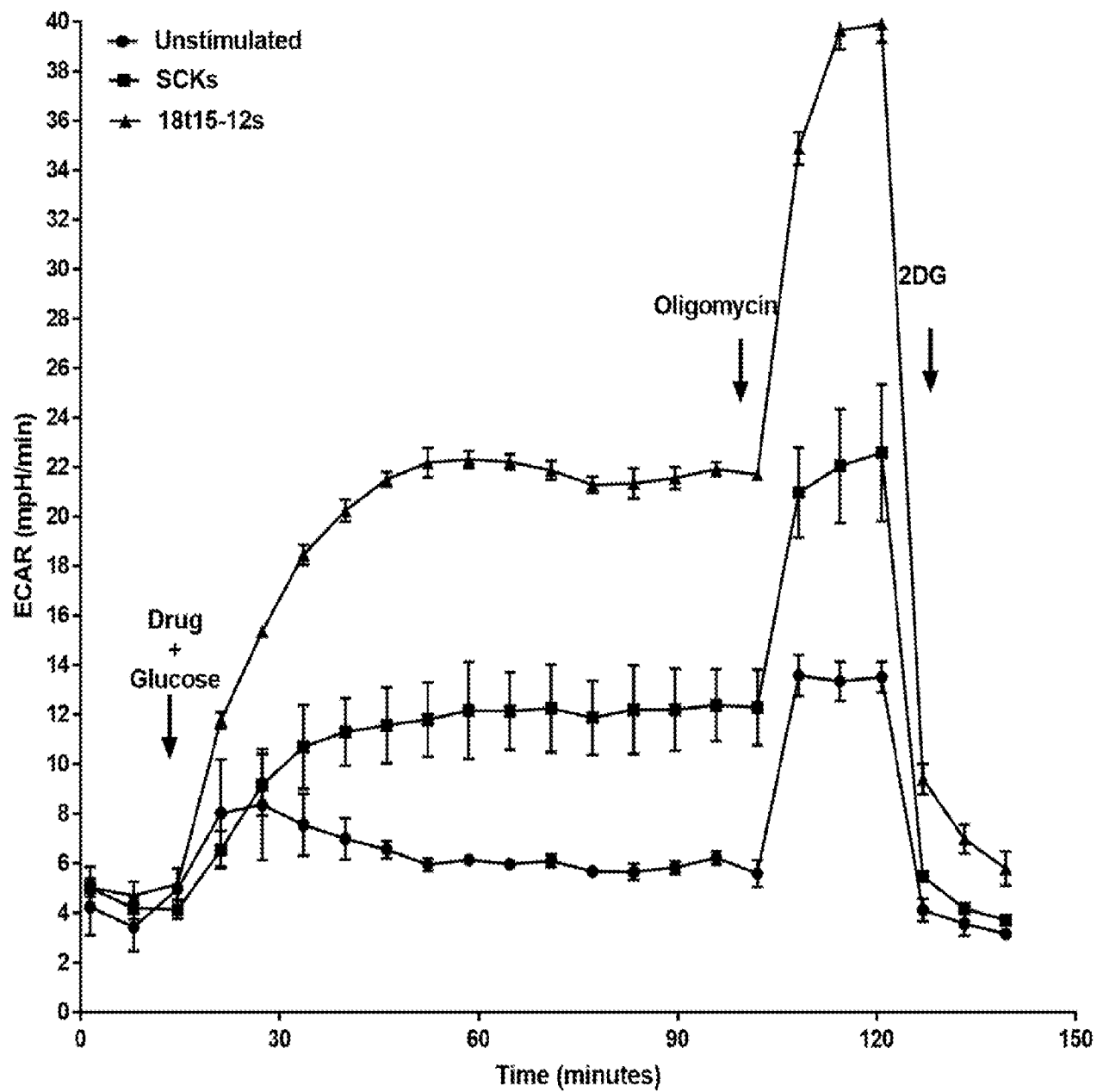


Figure 13B

**Figure 14**

**Figure 15**

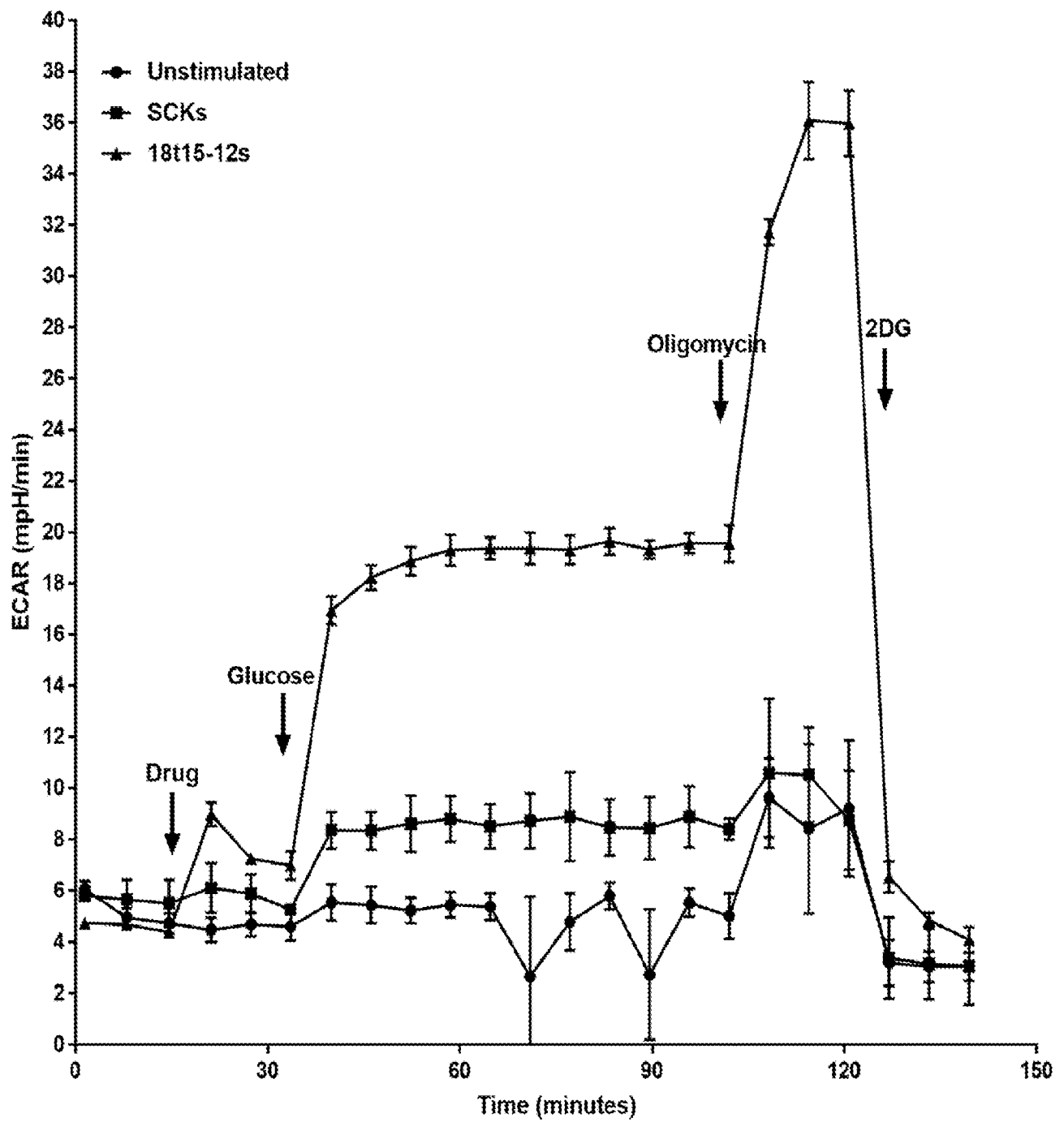
**Figure 16**

Figure 17A

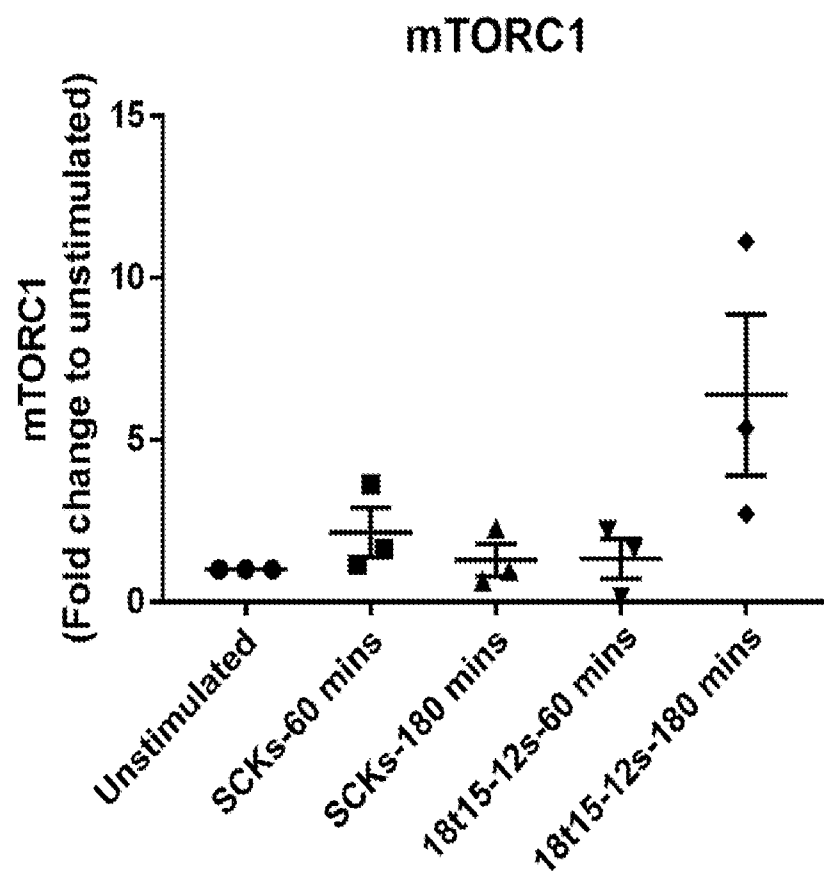
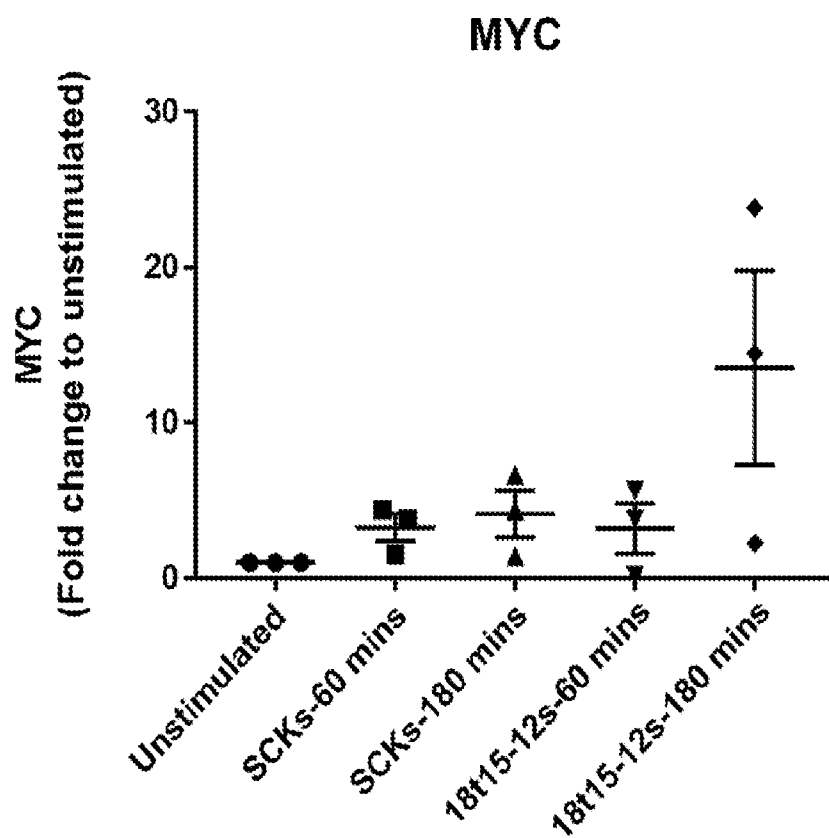


Figure 17B



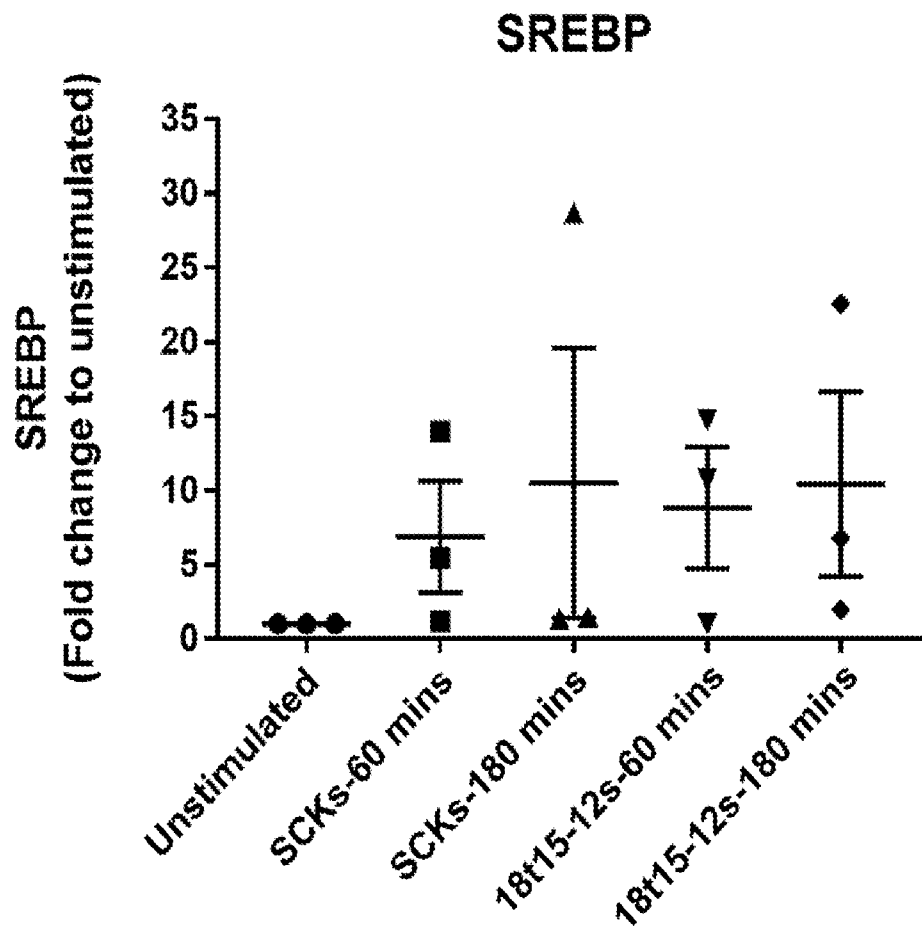


Figure 17C

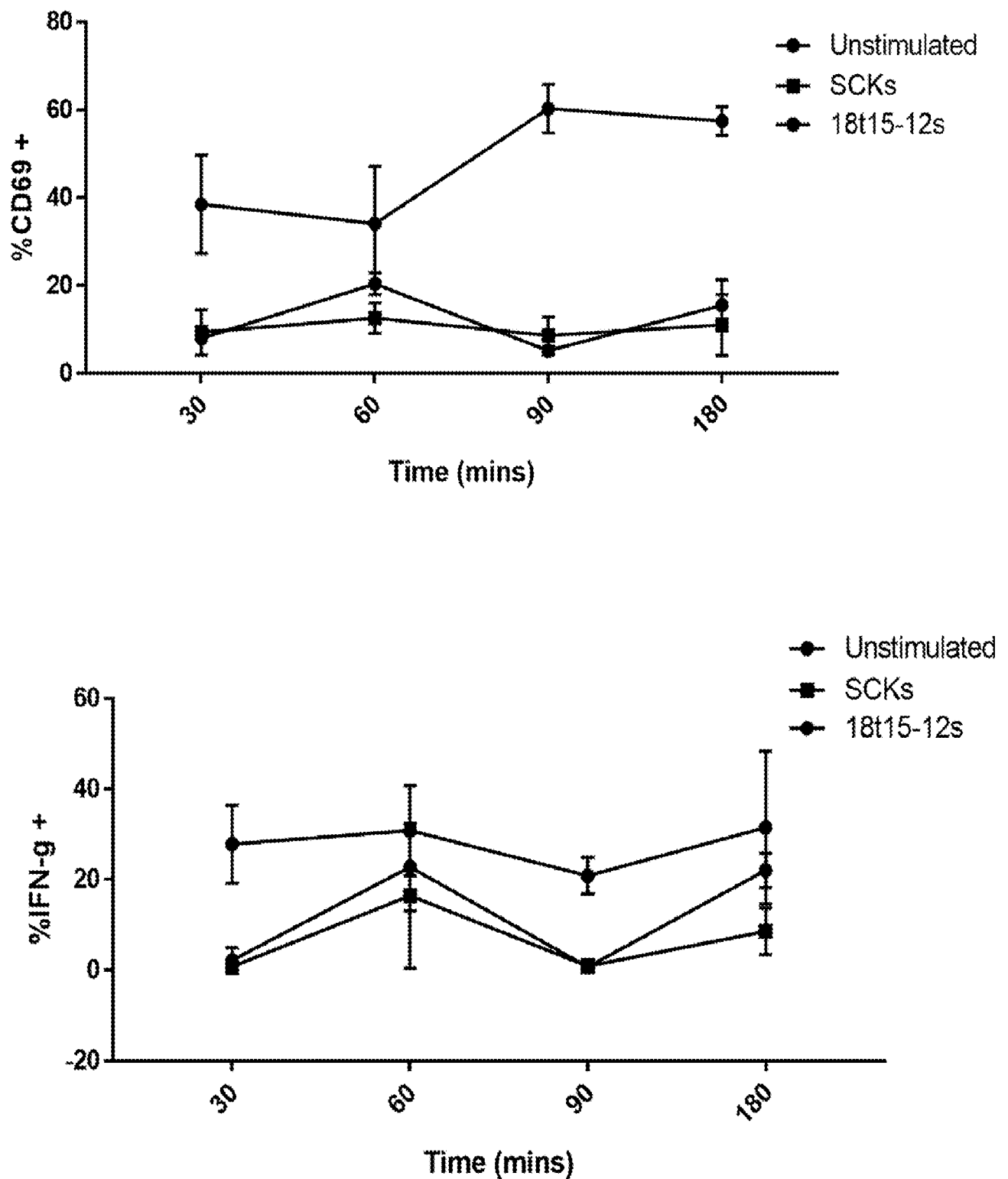
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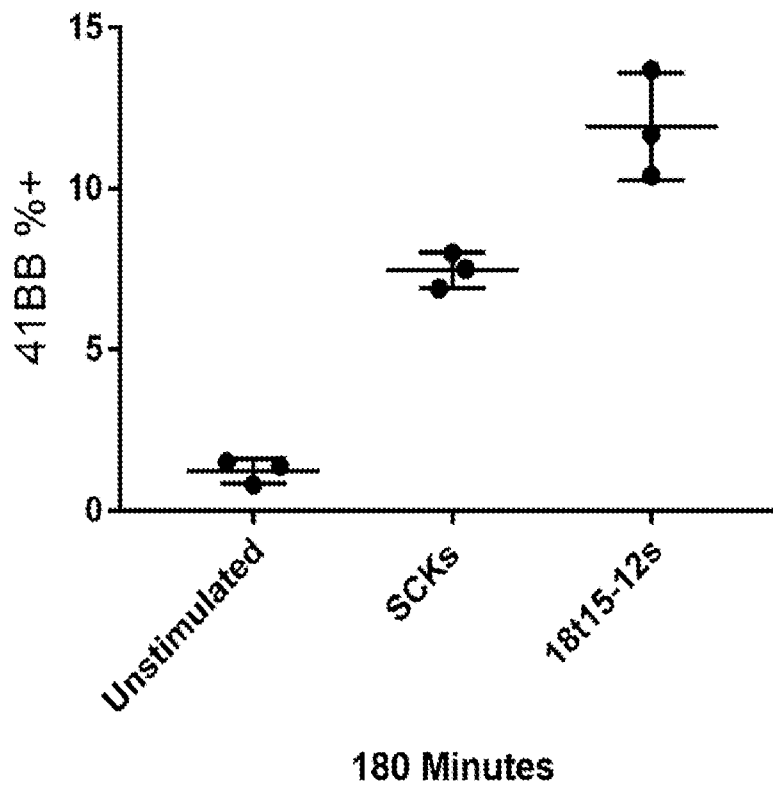
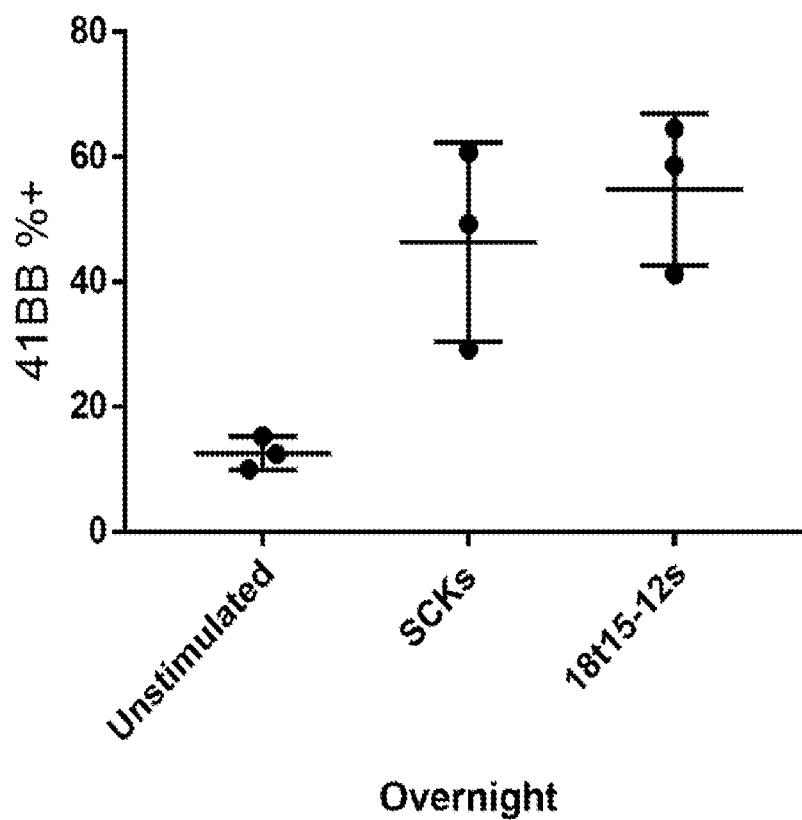
Figure 19A**Figure 19B**

Figure 19C

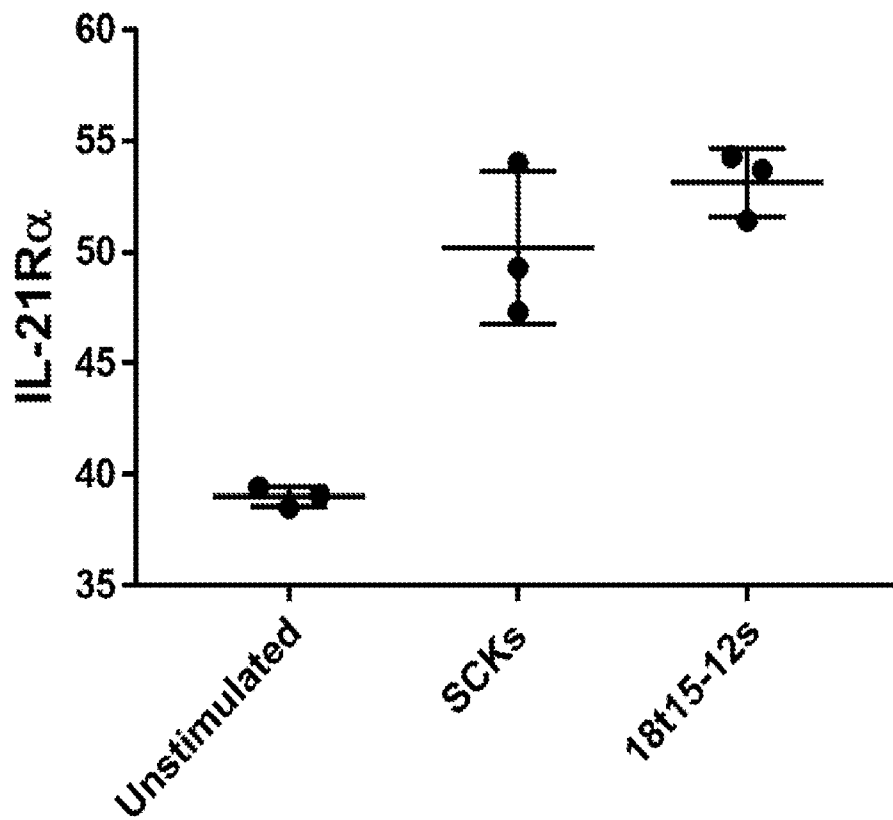
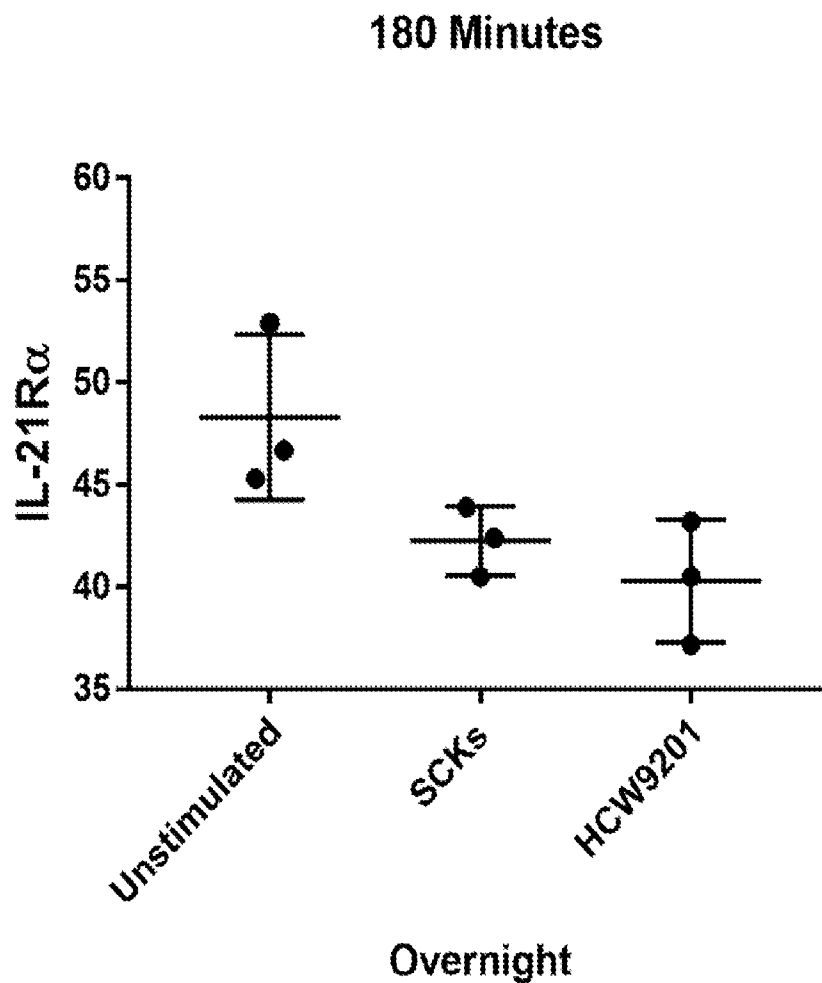
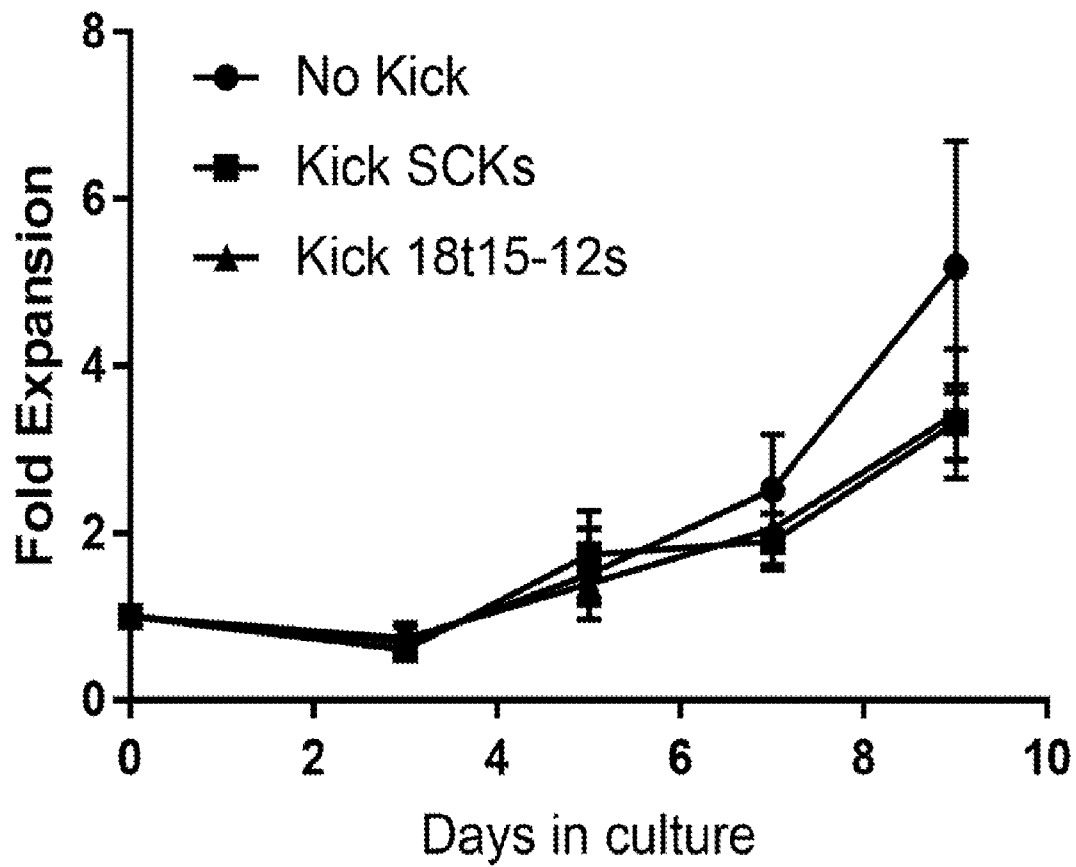
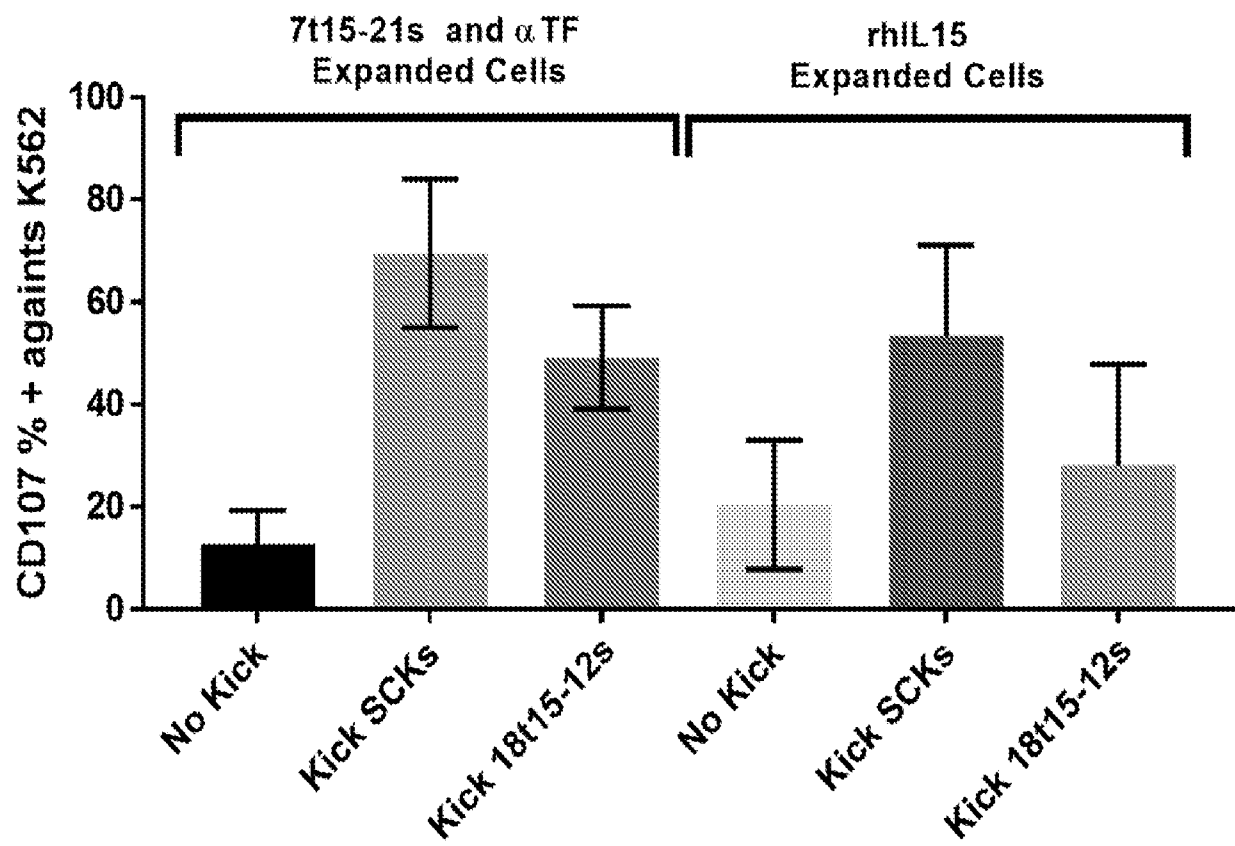
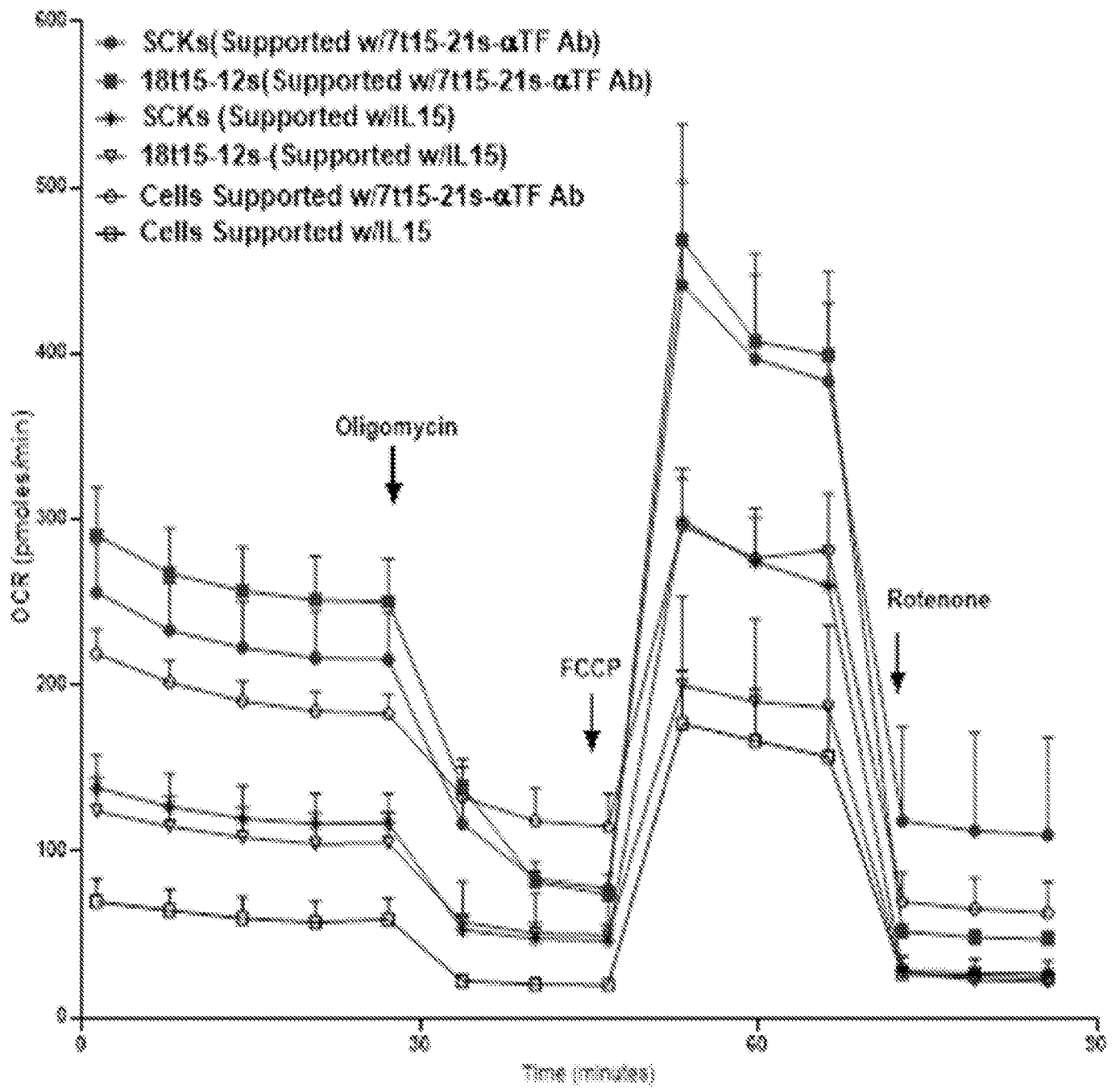


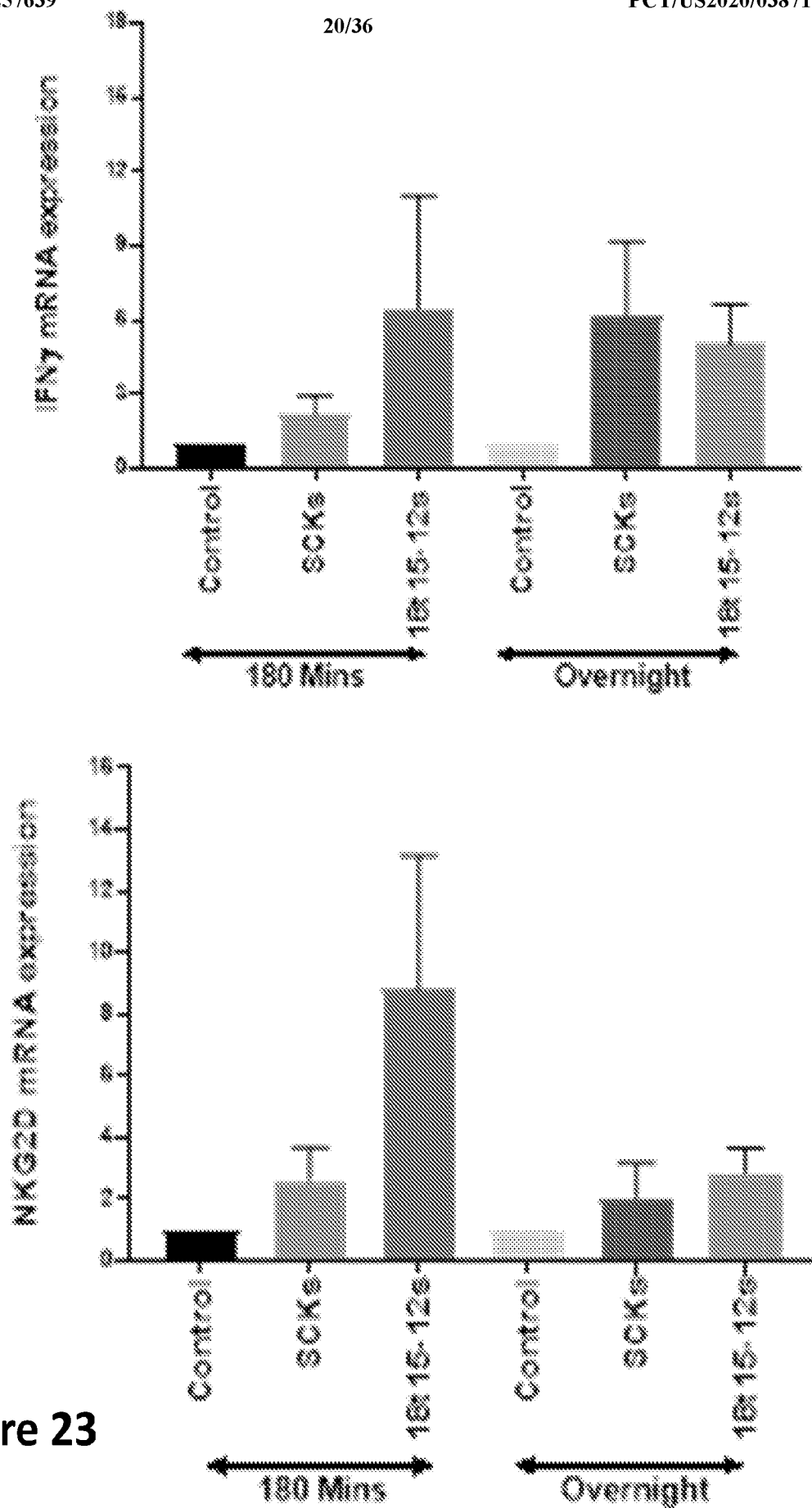
Figure 19D

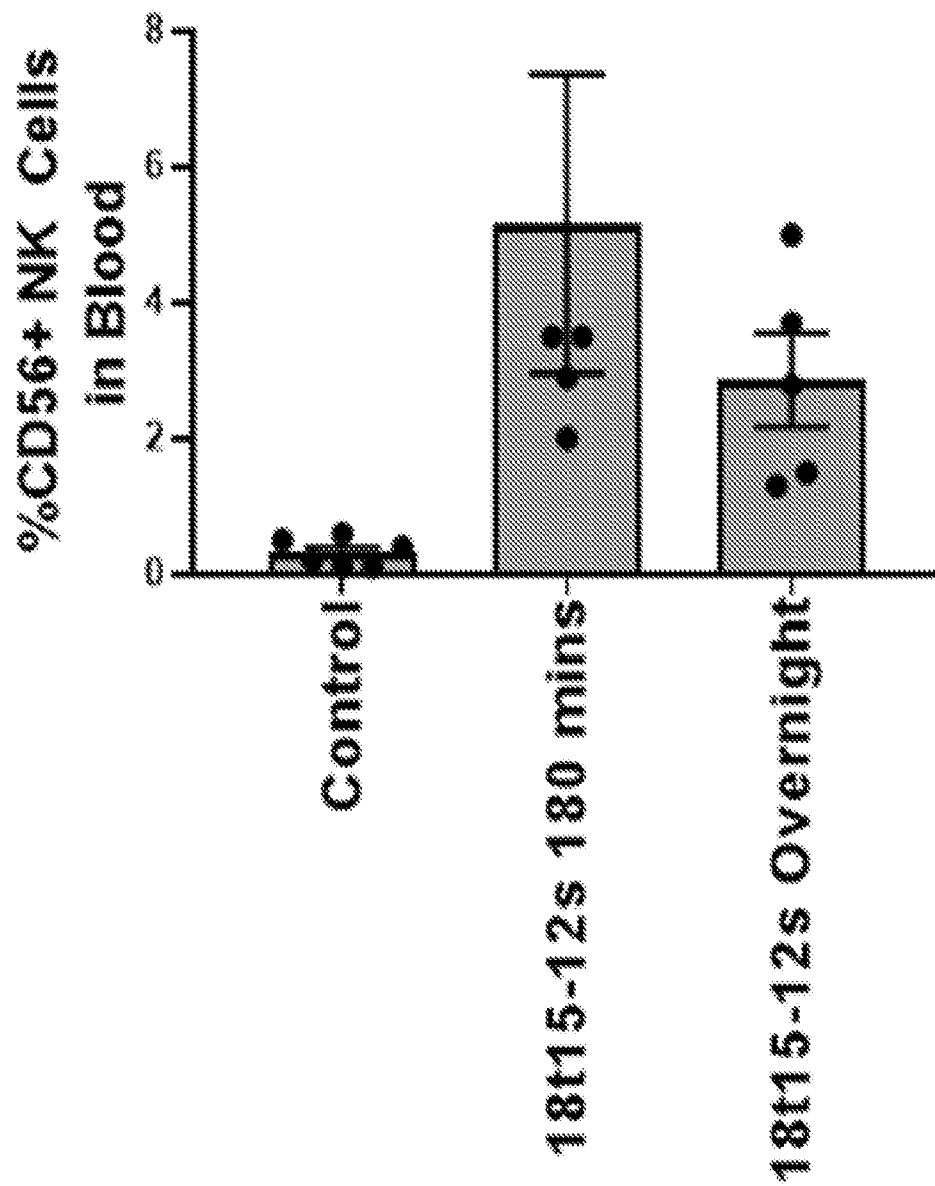


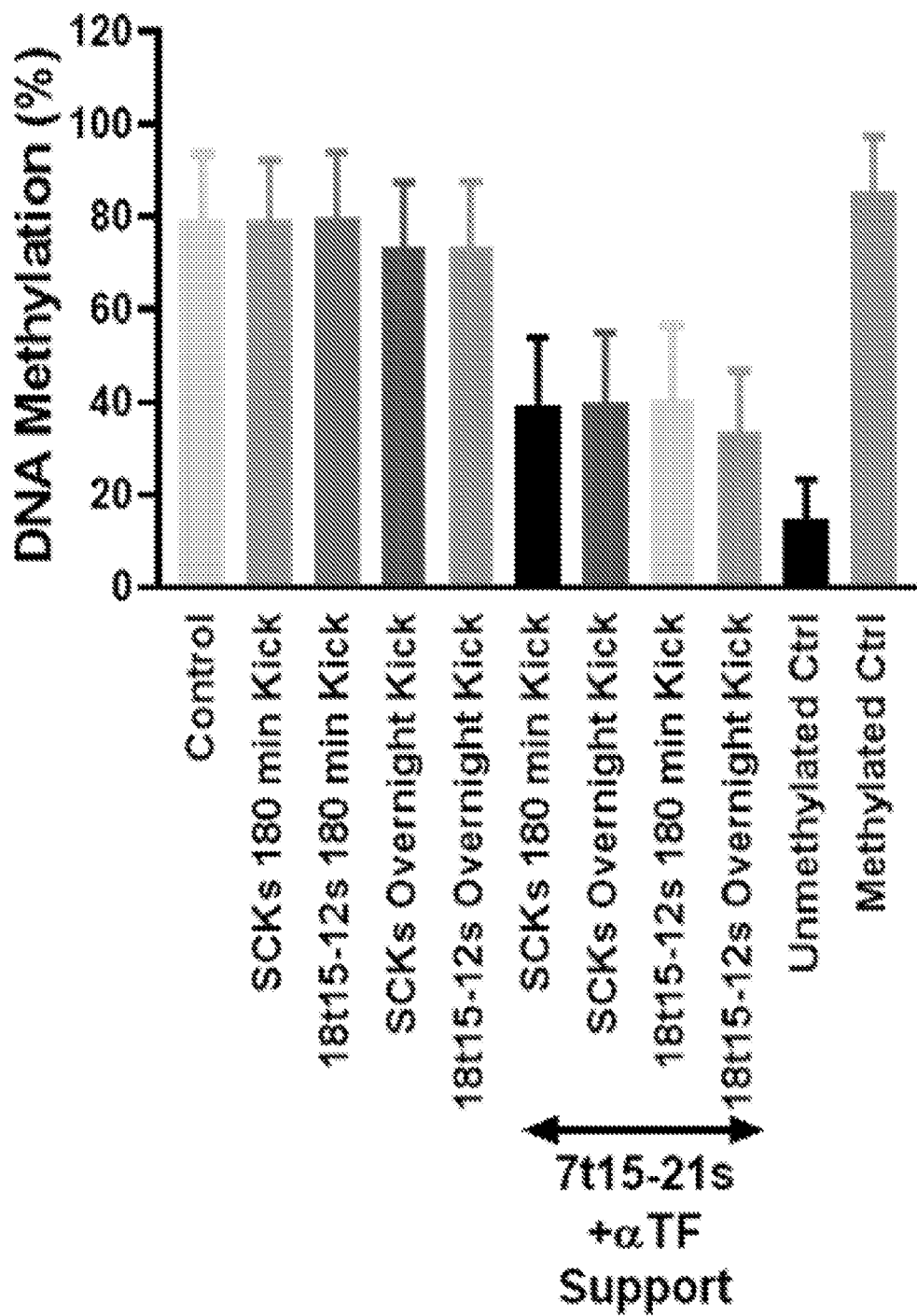
**Figure 20**

**Figure 21**

**Figure 22**

**Figure 23**

**Figure 24**

**Figure 25**

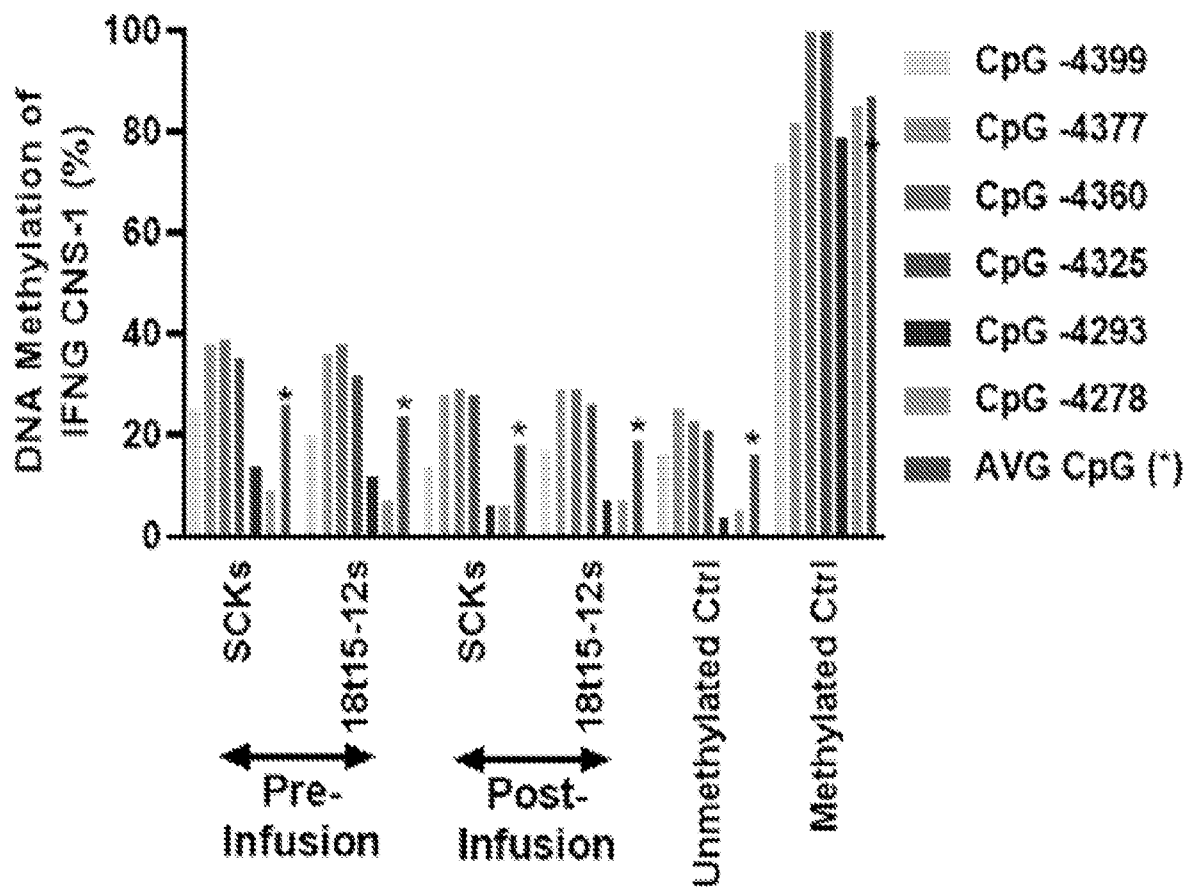


Figure 26

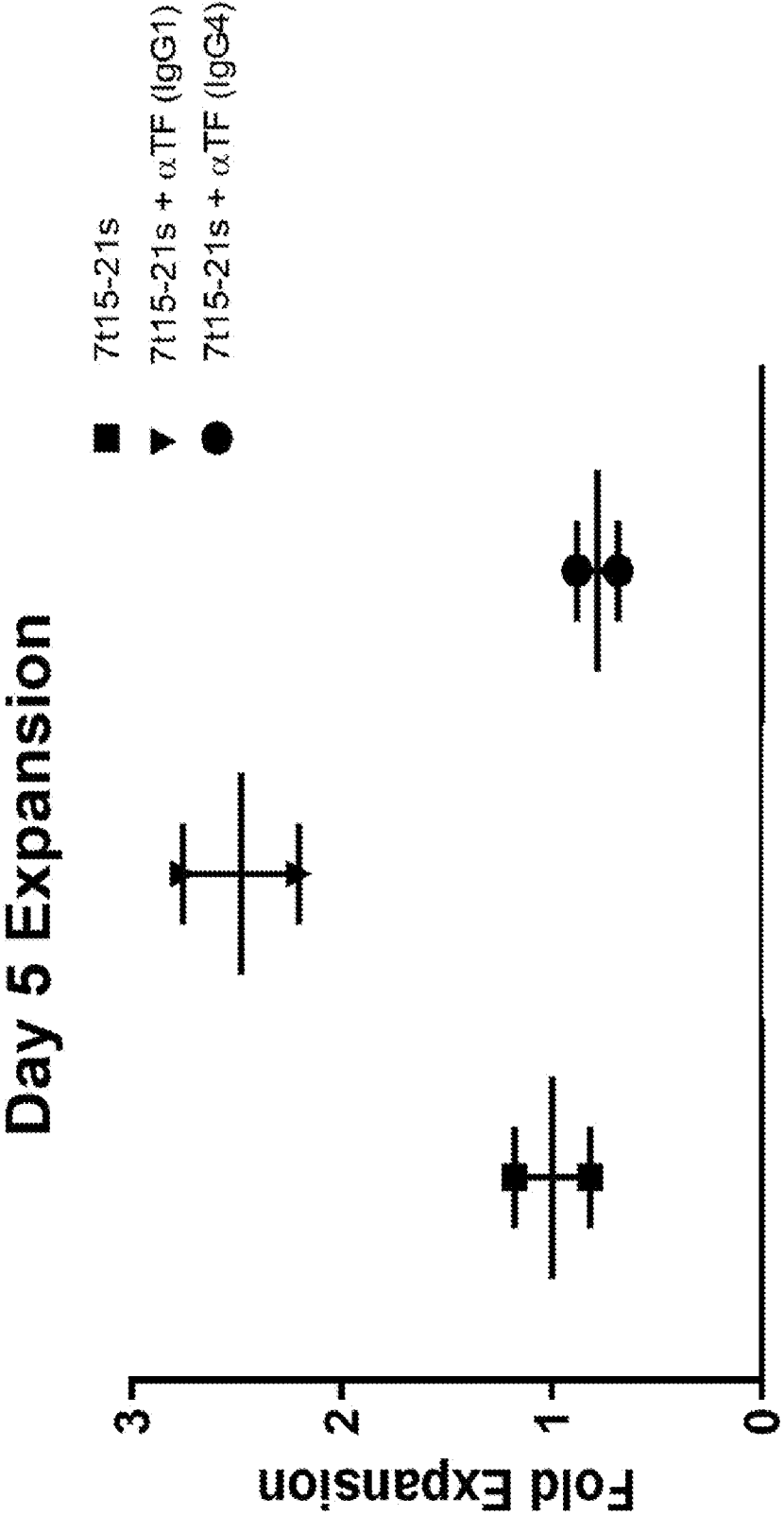


Figure 27

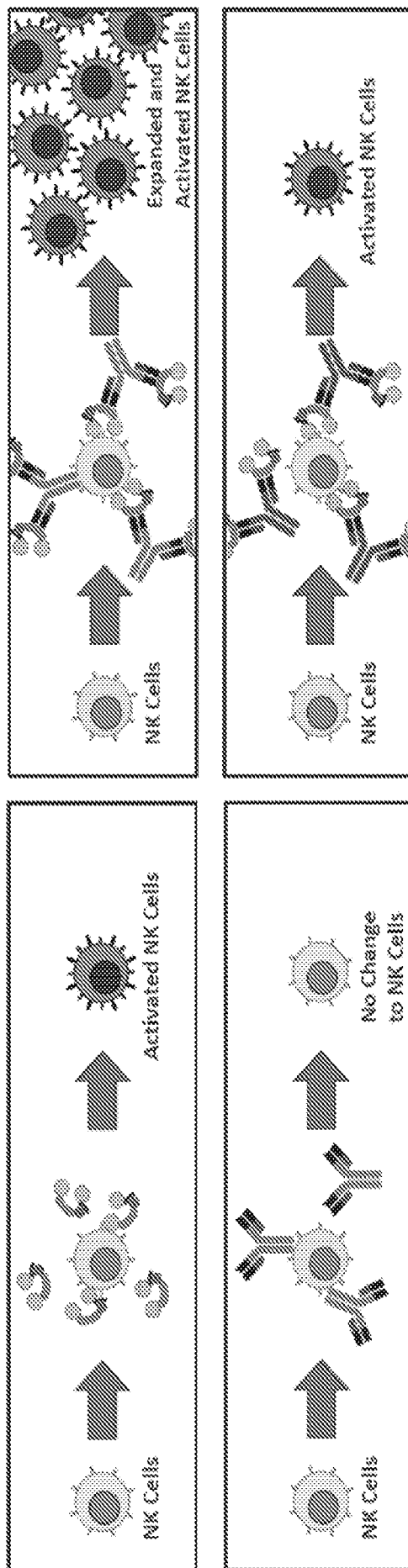
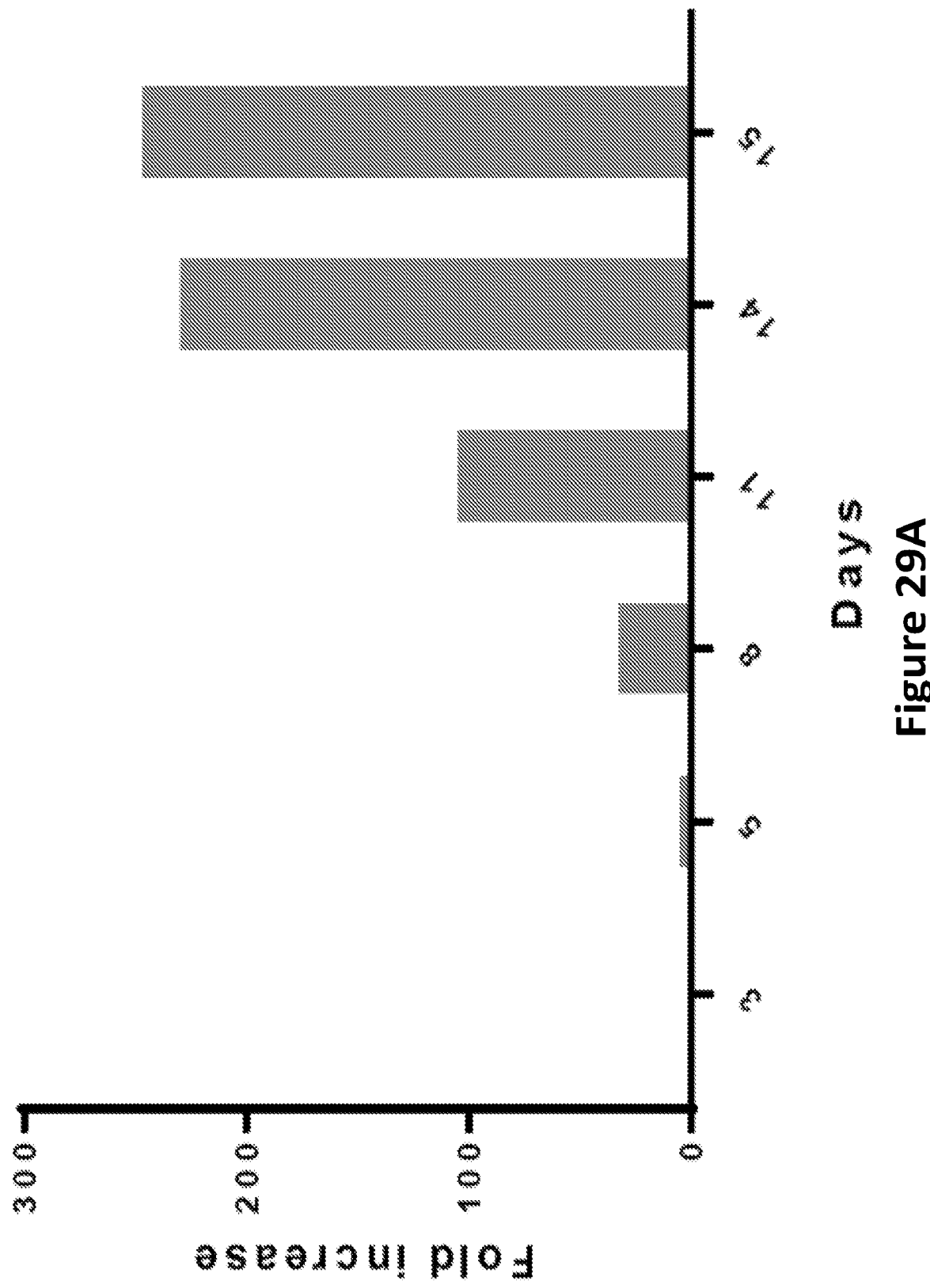


Figure 28



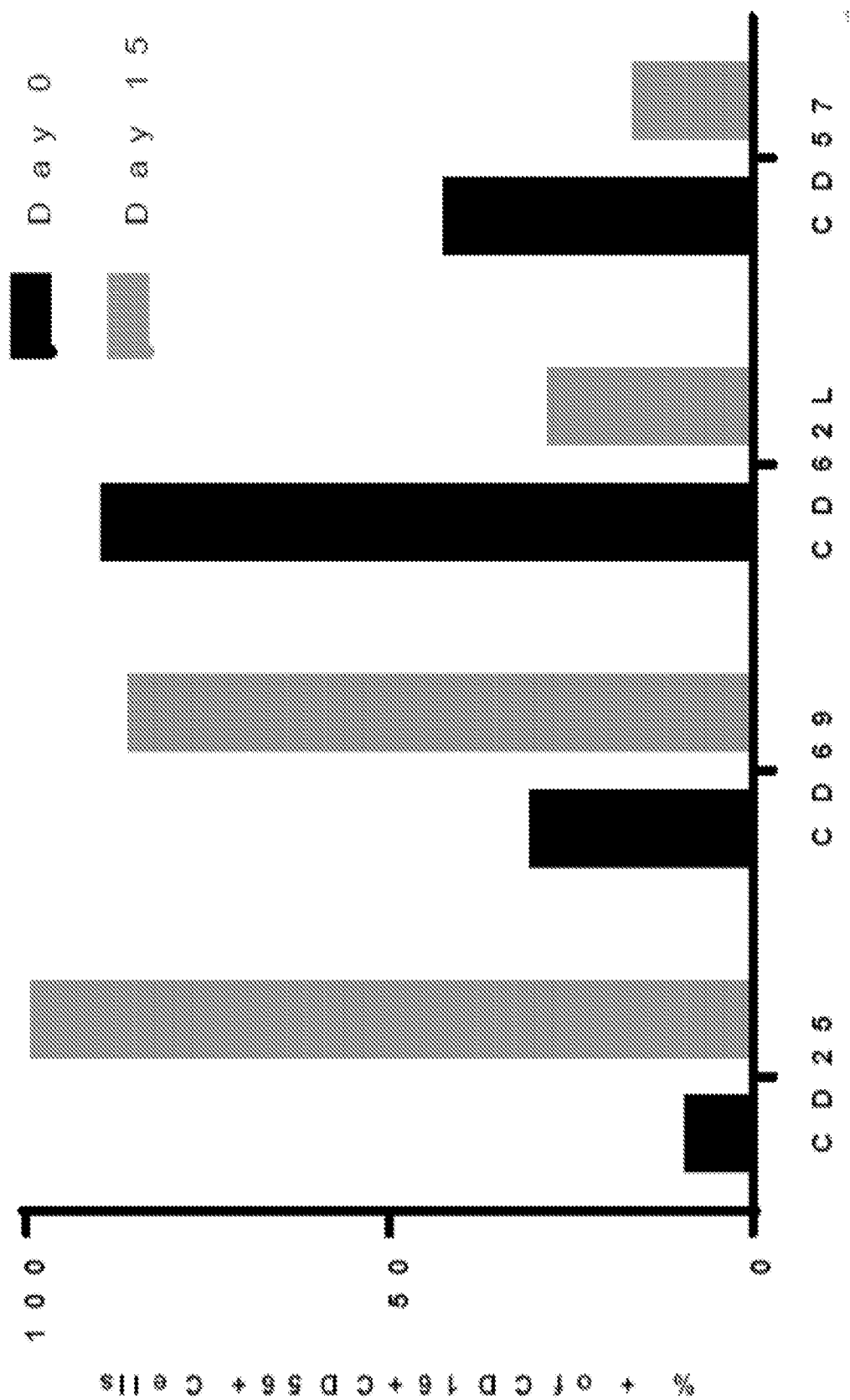
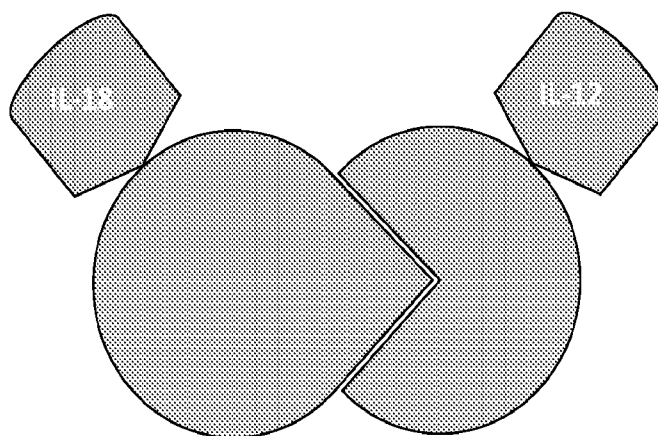


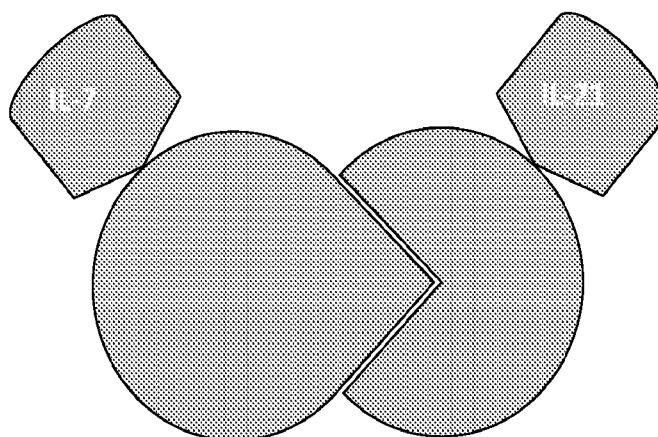
Figure 29B

28/36
Kick



18t15-12s for
Cytokine Induced Memory Like (CIML) NK Cells

Expand



7t15-21s with α TF for
Expansion of NK Cells

Figure 30

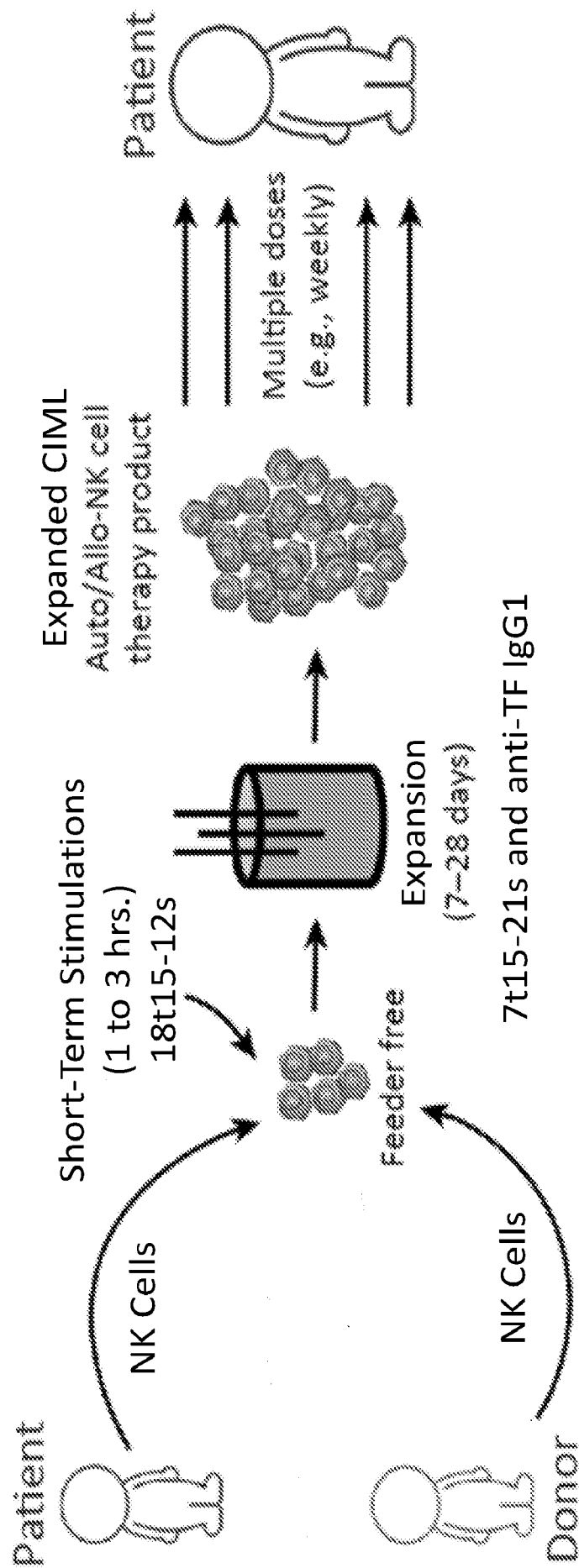


Figure 31

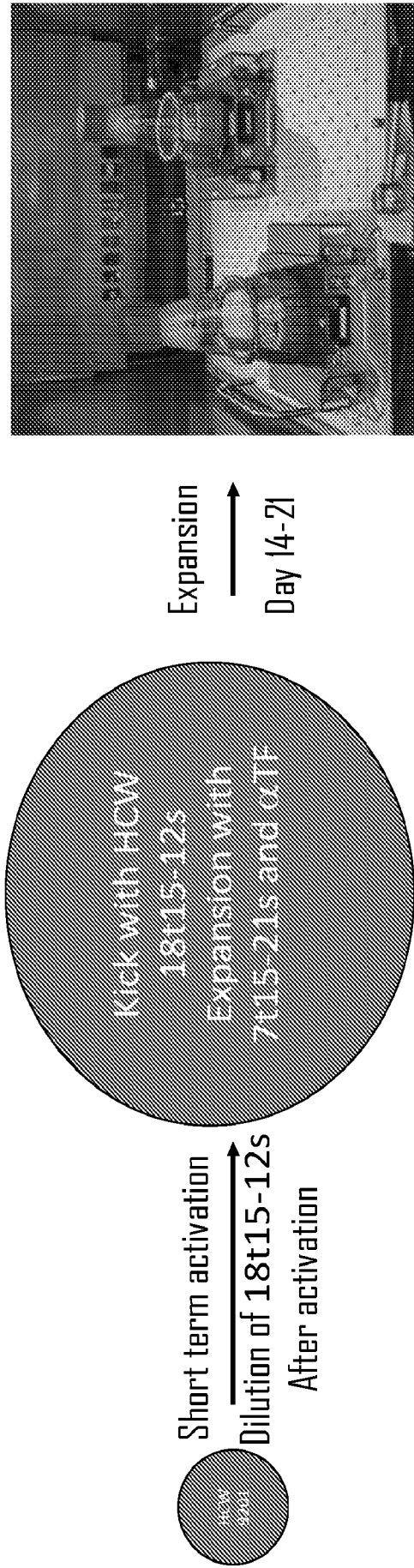


Figure 32

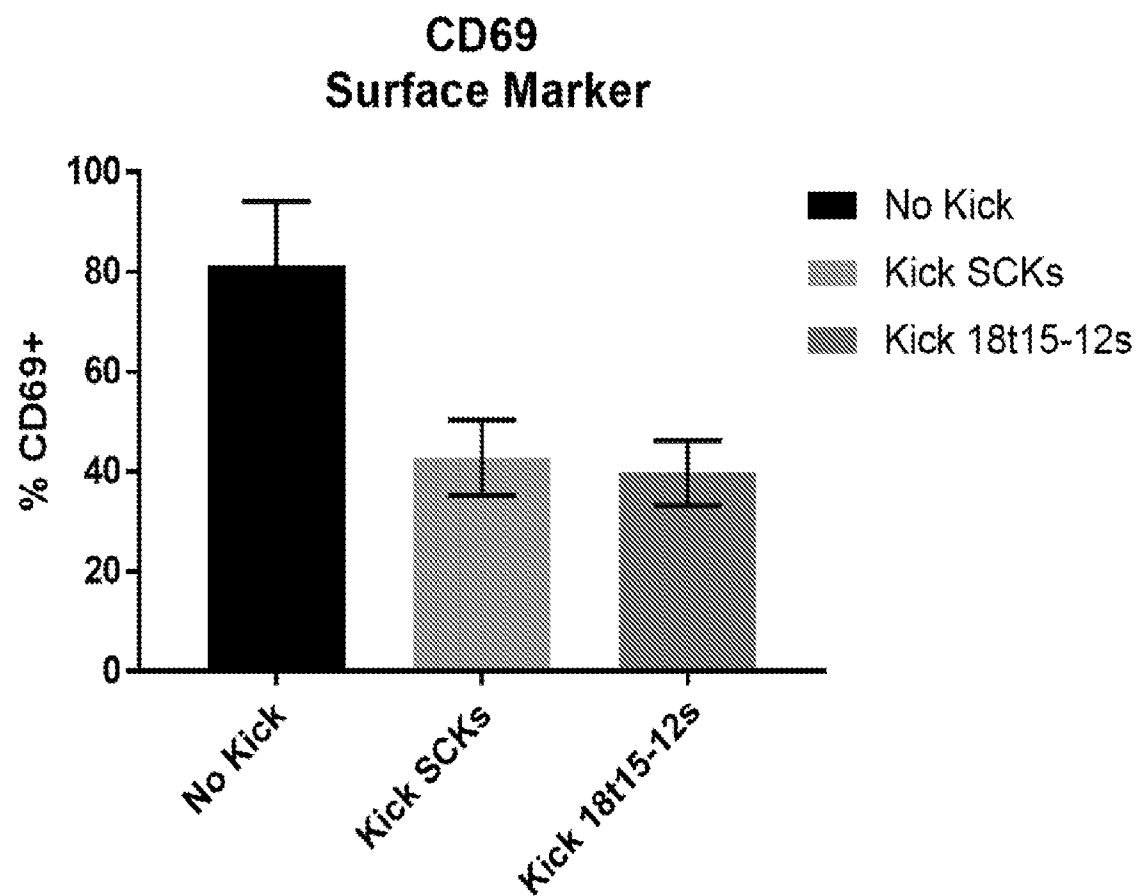
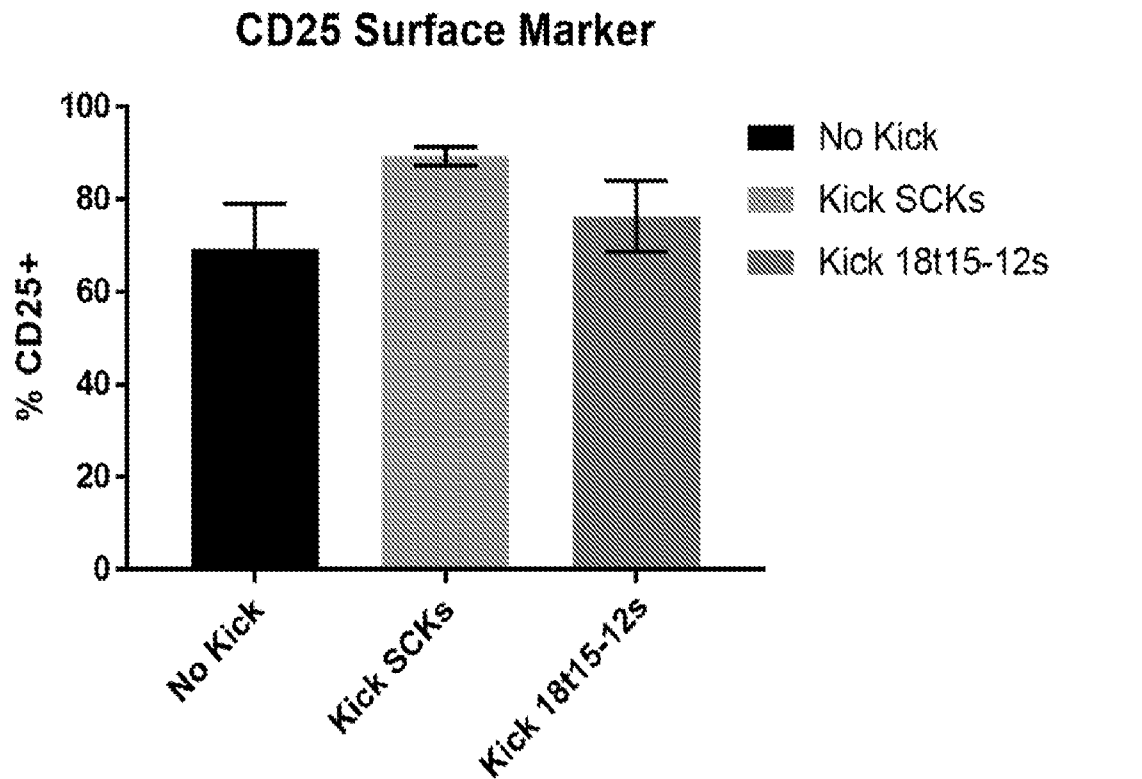
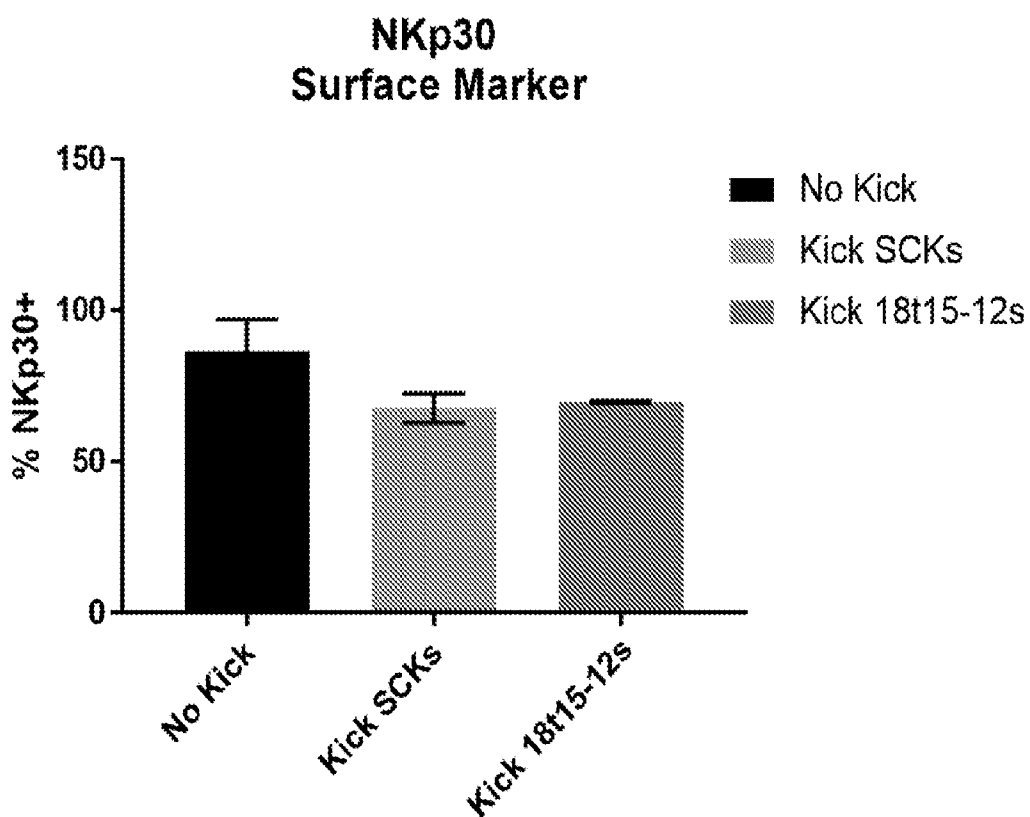
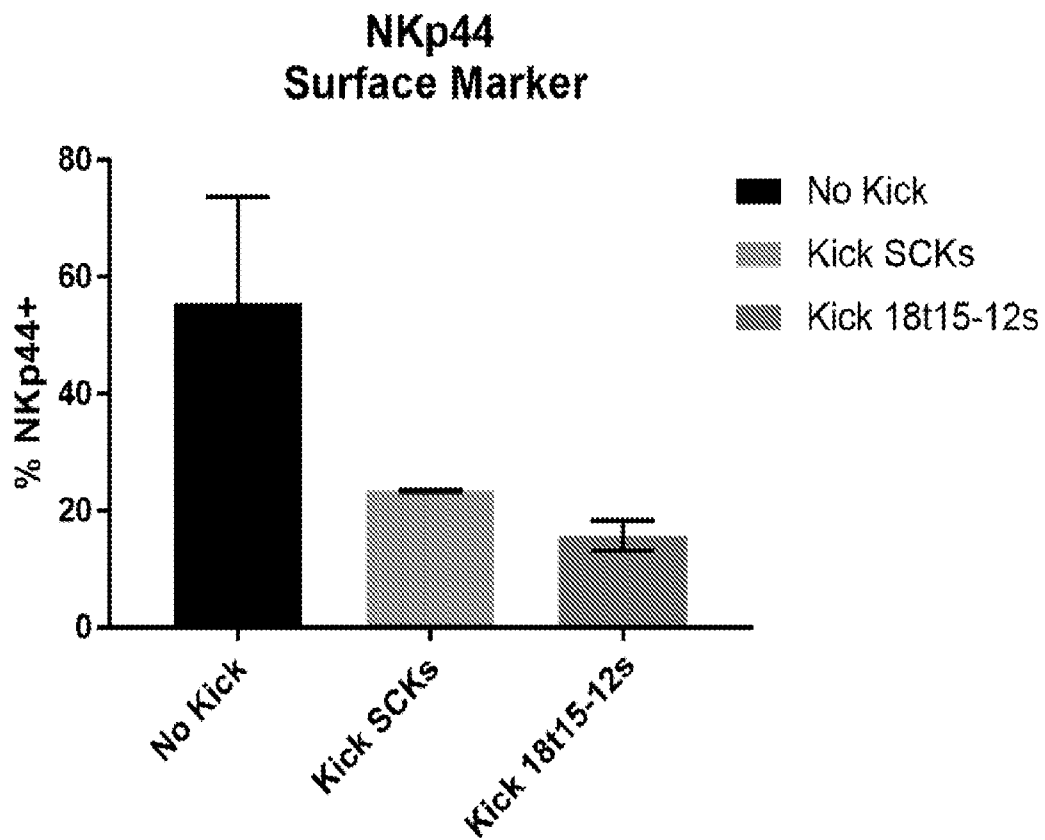
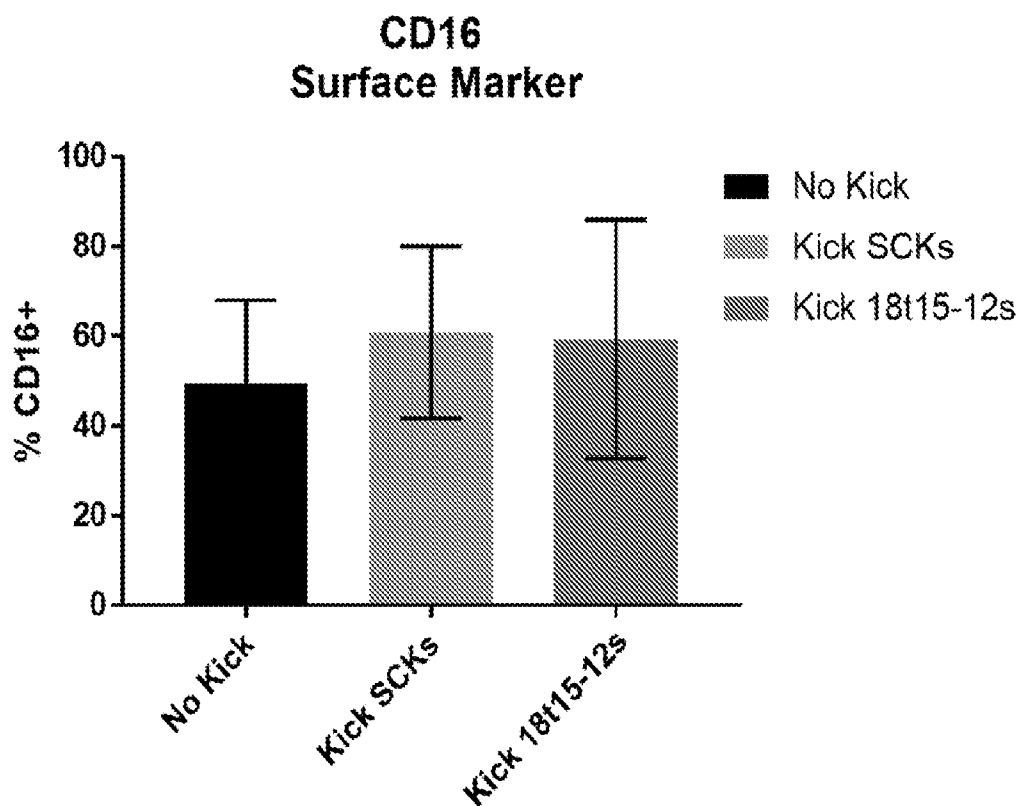
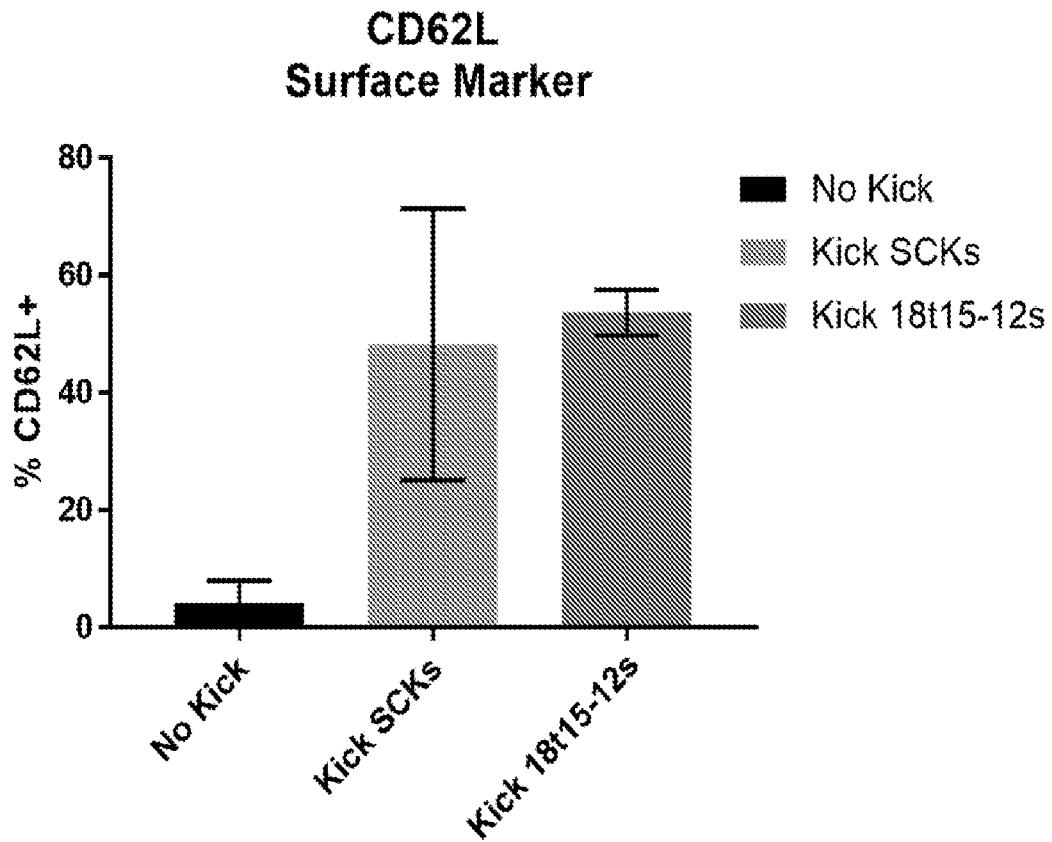
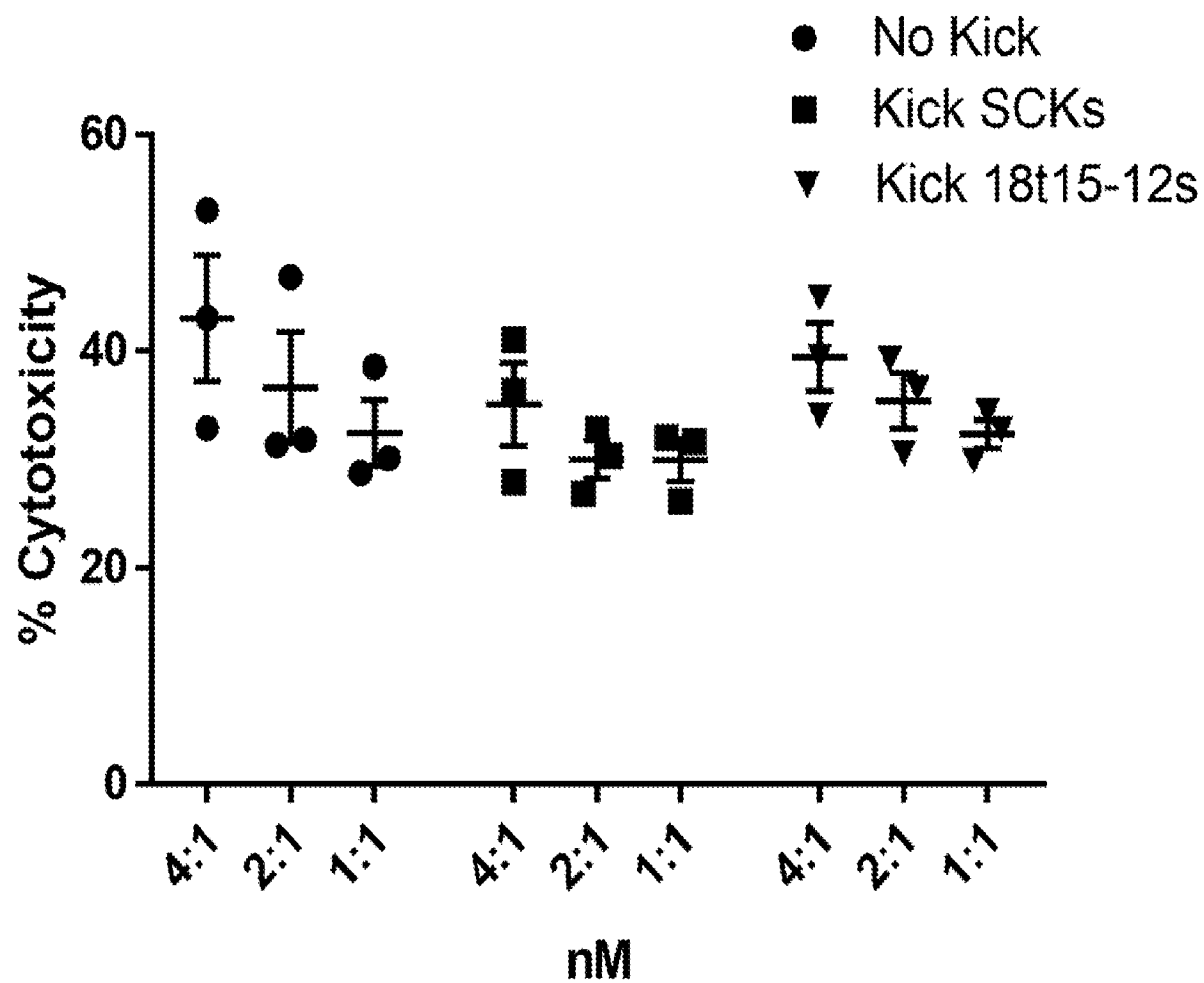
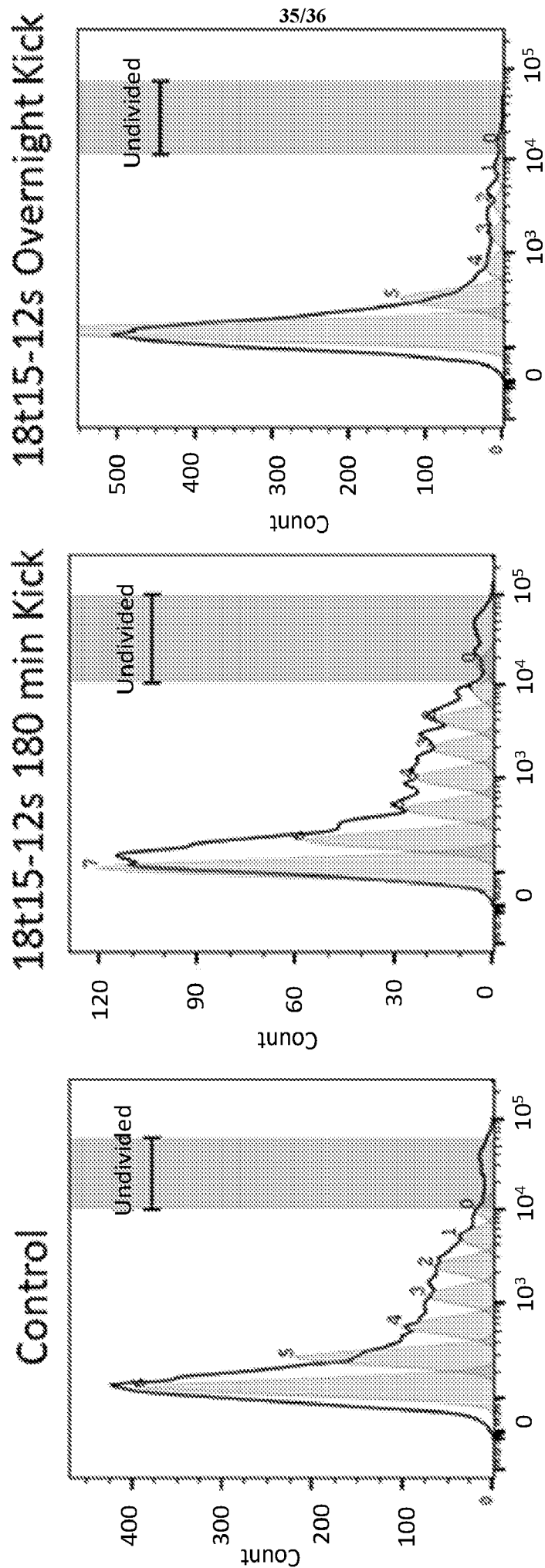


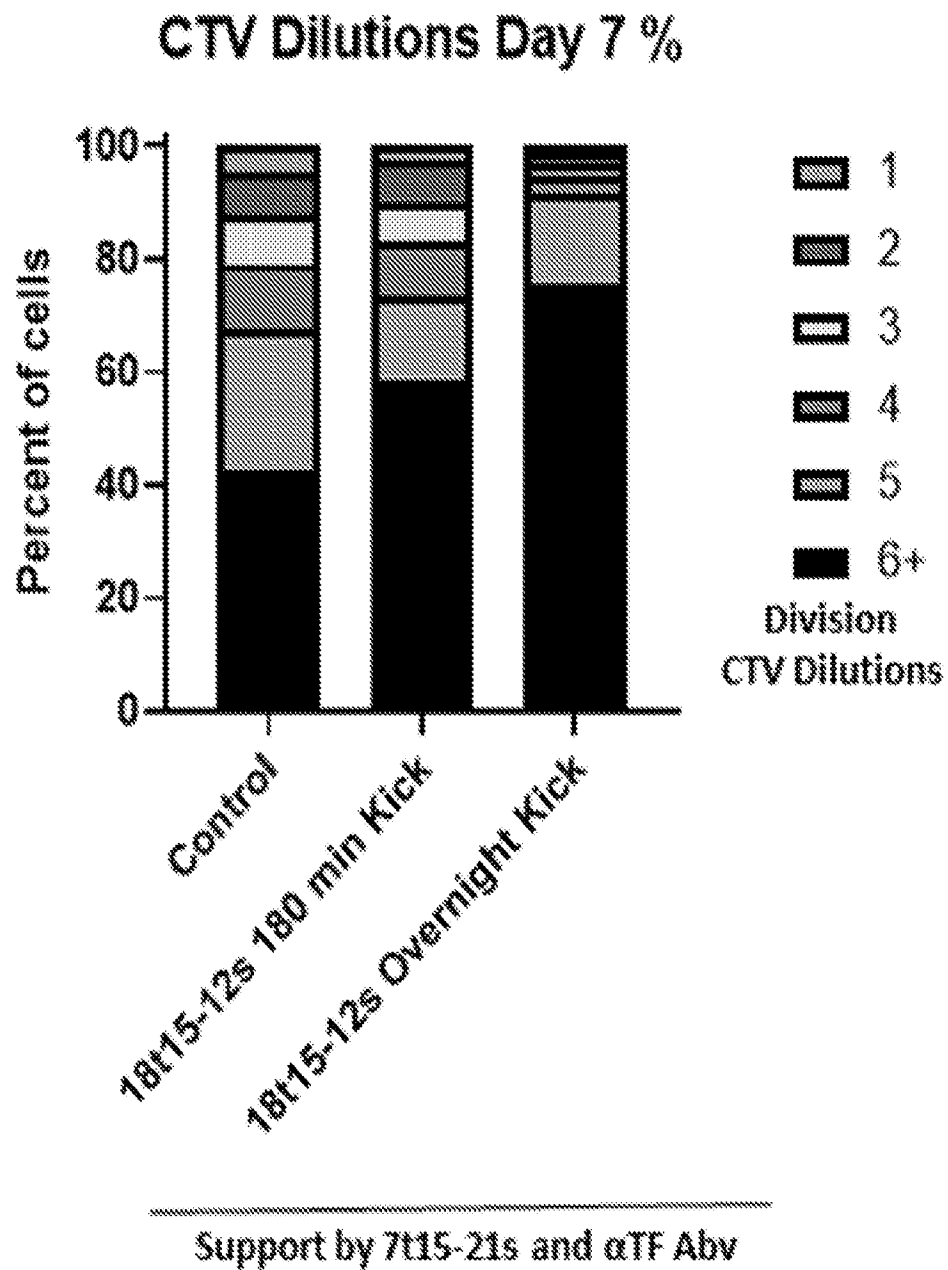
Figure 33

**Figure 34**

**Figure 35**

**Figure 36**



**Figure 37B**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/038717

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0783 A61P35/00 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61P A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018/165208 A1 (ALTOR BIOSCIENCE CORP [US]) 13 September 2018 (2018-09-13) page 5 - page 7; claims 1-40; sequence 1 -----	1-350
A	WO 2018/158350 A1 (AFFIMED GMBH [DE]) 7 September 2018 (2018-09-07) page 30; claims 1-16; examples 1-6 -----	1-350
A	GAURAV NAYYAR ET AL: "Overcoming Resistance to Natural Killer Cell Based Immunotherapies for Solid Tumors", FRONTIERS IN ONCOLOGY, vol. 9, 11 February 2019 (2019-02-11), XP055700879, DOI: 10.3389/fonc.2019.00051 the whole document ----- -/--	1-350

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

8 October 2020

Date of mailing of the international search report

16/10/2020

Name and mailing address of the ISA/

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Authorized officer

Schmidt-Yodlee, H

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/038717

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/096158 A2 (DOMANTIS LTD [GB]; TOMLINSON IAN [GB] ET AL.) 14 August 2008 (2008-08-14) Annex 2; page 257, line 6 - line 11; claims 1-79 -----	1-350
X,P	WO 2020/047299 A1 (HCW BIOLOGICS INC [US]) 5 March 2020 (2020-03-05) claims 1-45; figures 1-144; sequences 72,73, 76,77 -----	1-350

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/038717

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