

CHIMERIC TRANSMEMBRANE PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 62/688,810, filed on June 22, 2018; the entire contents of which are herein incorporated by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 21, 2019, is named CDL-700_PF-43159-0072WO1_SL.txt and is 176,317 bytes in size.

TECHNICAL FIELD

The present disclosure relates to the field of biotechnology, and more specifically, to chimeric transmembrane proteins and uses thereof.

BACKGROUND

5 Interleukin-7 (IL-7) receptor is expressed on the cell surface (either temporary or permanent) of various cell types, including naïve and memory T-cells. Upon binding to IL-7, the IL-7 receptor transduces a signal into the cell via activation of the Janus kinases—JAK1 and JAK3 (Palmer et al., *Cell. Mol. Immunol.* 5(2):79-89, 2008).

10 While many improvements have been made to generate a secondary proliferative signal in second generation chimeric antigen receptor (CAR) T-cells (through the inclusion of co-stimulatory signaling domains such as CD28 and 4-1BB in chimeric antigen receptors), this signal remains antigen-dependent often does not have sufficient co-stimulatory activity in solid tumors that have low tumor associated
15 antigen levels.

In another attempt to provide sufficient co-stimulatory activity in CAR T-cells, co-administration of cytokines belonging to the common gamma (γ c) chain receptor family have been used. However, prolonged usage of recombinant, pro-

proliferative cytokines, such as IL-2, is often associated with severe side effects, limiting the duration and dosage of administration. In view of the above, alternative, improved methods of providing a co-stimulatory signal to CAR T-cells are desired.

SUMMARY

5 The present disclosure is based, at least in part, on the discovery that a protein that includes a transmembrane domain of an alpha chain of interleukin-7 receptor that includes the sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more (e.g., two, three, or four) of the following modifications: (i) alanine-leucine-leucine at amino acids positions 15 through 17 in SEQ ID NO: 1 has been
10 replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine; (ii) a cysteine-proline-threonine has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) a proline-proline-cysteine-leucine has been inserted between amino acid positions 4 and 5 in SEQ ID NO: 1, demonstrates increased downstream signaling as compared to a corresponding protein that includes a wildtype
15 transmembrane domain of an alpha chain of interleukin-7 receptor or a corresponding protein that includes the sequence of SEQ ID NO: 1. Representative modified transmembrane domains have a sequence of SEQ ID NO: 2, 4, 6 or 8.

 The present disclosure is also based, at least in part, on the discovery that a chimeric transmembrane protein that includes an extracellular interleukin-15 domain;
20 an extracellular sushi domain from an alpha chain of interleukin-15 receptor; and a transmembrane domain from an alpha chain of interleukin-7 receptor provide for antigen-independent co-stimulation of a chimeric antigen receptor (CAR) T-cell.

 In view of this discovery, provided herein are chimeric transmembrane proteins that include an extracellular IL-15 domain, an extracellular sushi domain
25 from an alpha chain of interleukin-15 receptor, and a transmembrane domain of an alpha chain of interleukin-7 receptor. Also provided are proteins that include a transmembrane domain of an alpha chain of interleukin-7 receptor that includes the sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more (e.g., two, three, or four) of the following modifications: (i) alanine-leucine-leucine at
30 amino acids positions 15 through 17 in SEQ ID NO: 1 has been replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine (ii) a cysteine-proline-threonine has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) a proline-proline-cysteine-leucine has been inserted between amino acid

positions 4 and 5 in SEQ ID NO: 1. Representative modified transmembrane domains have a sequence of SEQ ID NO: 2, 4, 6 or 8.

Also provided are nucleic acids encoding these chimeric transmembrane proteins or these proteins, vectors including any of these nucleic acids, and mammalian cells that include any of these nucleic acids or vectors. Also provided herein are methods of treating a cancer, methods of inducing cell death in a cancer cell (e.g., apoptosis and/or necrosis), and methods of decreasing the risk of developing a metastasis or an additional metastasis in a subject in need thereof that include administering any of the mammalian cells described herein to the subject.

In some embodiments, provided herein is a protein that includes a transmembrane domain of an alpha chain of interleukin-7 receptor, wherein the transmembrane domain includes the sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more of the following modifications: (i) alanine-leucine-leucine at amino acids positions 15 through 17 in SEQ ID NO: 1 has been replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine; (ii) a cysteine-proline-threonine has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) a proline-proline-cysteine-leucine has been inserted between amino acid positions 4 and 5 in SEQ ID NO: 1. Representative modified transmembrane domains have a sequence of SEQ ID NO: 2, 4, 6 or 8.

In some embodiments, the protein further includes an intracellular domain of an alpha chain of interleukin-7 receptor (e.g., a wildtype alpha chain of interleukin-7 receptor such as, without limitation, a wildtype human alpha chain of interleukin-7 receptor). In some embodiments, the protein further includes an intracellular domain that includes a sequence of SEQ ID NO: 45 or a sequence that is at least 80% identical to the sequence of an intracellular domain of a wildtype alpha chain of interleukin-7 receptor (e.g., a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45). In some embodiments, the intracellular domain is at least 90% or 95% identical to SEQ ID NO: 45. In some embodiments, the protein further includes an intracellular domain of a wildtype alpha chain of interleukin-7 receptor having one or both of: (i) one to ten amino acids deleted from the N-terminus of the sequence of the intracellular domain of the wildtype alpha chain of interleukin-7 receptor (e.g., wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45) and (ii) one to ten amino acids deleted from the C-terminus of the sequence

of the intracellular domain of the wildtype alpha chain of interleukin-7 receptor (e.g., wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45).

In some embodiments, the protein further includes an extracellular domain of an alpha chain of interleukin-7 receptor (e.g., a wildtype alpha chain of interleukin-7 receptor such as, without limitation, a wildtype human alpha chain of interleukin-7 receptor). In some embodiments, the protein further includes an extracellular domain that includes a sequence of SEQ ID NO: 11 or a sequence that is at least 80% identical to the sequence of an extracellular domain of a wildtype alpha chain of interleukin-7 receptor (e.g., a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 11). In some embodiments, the extracellular domain is at least 90% or 95% identical to SEQ ID NO: 11. In some embodiments, the protein further includes an extracellular domain of a wildtype alpha chain of interleukin-7 receptor having one or both of: (i) one to ten amino acids deleted from the N-terminus of the sequence of the extracellular domain of the wildtype alpha chain of interleukin-7 receptor (e.g., wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 11) and (ii) one to ten amino acids deleted from the C-terminus of the sequence of the extracellular domain of the wildtype alpha chain of interleukin-7 receptor (e.g., wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 11).

Also provided herein are nucleic acids encoding any of the variety of proteins described herein. Also provided herein are vectors that include any of the nucleic acids encoding any of the variety of proteins described herein. In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of proteins described herein include a promoter operably linked to the nucleic acid, and optionally, an enhancer sequence operably linked to the nucleic acid. In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of proteins described herein include a poly(A) sequence operably linked to the nucleic acid. In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of proteins described herein are lentiviral or adenoviral vectors.

In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of proteins described herein include a sequence encoding a chimeric antigen receptor. In some embodiments, the chimeric antigen receptor binds specifically to a tumor antigen (e.g., a tumor antigen is selected from the group consisting of: glypican-3, BCMA, MAGE, MUC16, CD19, WT-1, CD22, LI-CAM,

ROR-1, CEA, 4-1BB, ETA, 5T4, adenocarcinoma antigen, alpha-fetoprotein (AFP), BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD20, CD125 CD200, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, 5 CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, 10 PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, and vimentin). In some embodiments, the chimeric antigen receptor comprises one or more co-stimulatory signaling domains selected from the group consisting of: 4-1BB, 15 CD27, OX40, CD40, CD28, GITR, CD2, CD5, ICAM-1, CD11a, Lck, TNFR-I, TNFR-II, FasR, CD30, ICOS, LIGHT, NKG2C, B7-H3, DAP-10, and DAP-12.

Also provided herein are mammalian cells that include any of the variety of nucleic acids encoding any of the variety of proteins described herein or vectors that include such nucleic acids described herein. In some embodiments, the mammalian 20 cell is an immune cell. In some embodiments, the immune cell is selected from the group consisting of: a CD4⁺ T cell, a CD8⁺ T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, a macrophage, a regulatory T cell, and a helper T cell. In some embodiments, the mammalian cell was previously obtained from a subject or is a daughter cell of a mammalian cell that was previously obtained from the subject. In 25 some embodiments, the mammalian cell is a human cell.

Also provided herein are pharmaceutical compositions that include any of the variety of vectors that include any of the nucleic acids encoding any of the variety of proteins described herein and a pharmaceutically acceptable carrier. Also provided 30 herein are pharmaceutical compositions that include any of the variety of mammalian cells that include vectors that include any of the nucleic acids encoding any of the variety of proteins described herein.

Also provided herein are sets of vectors that include a first vector that includes any of the nucleic acids encoding any of the variety of proteins described herein, and

a second vector that includes a sequence encoding a chimeric antigen receptor. In some embodiments of such sets of vectors, the chimeric antigen receptor binds specifically to a tumor antigen (e.g., a tumor antigen is selected from the group consisting of: glypican-3, BCMA, MAGE, MUC16, CD19, WT-1, CD22, LI-CAM, ROR-1, CEA, 4-1BB, ETA, 5T4, adenocarcinoma antigen, alpha-fetoprotein (AFP), BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD20, CD125 CD200, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, and vimentin). In some embodiments of such sets of vectors, the chimeric antigen receptor comprises one or more co-stimulatory signaling domains selected from the group consisting of: 4-1BB, CD27, OX40, CD40, CD28, GITR, CD2, CD5, ICAM-1, CD11a, Lck, TNFR-I, TNFR-II, FasR, CD30, ICOS, LIGHT, NKG2C, B7-H3, DAP-10, and DAP-12. In some embodiments of such sets of vectors, both of the first vector and the second vector is a lentiviral or adenoviral vector. In some embodiments of sets of vectors provided herein, the second vector further includes a promoter operably linked to the sequence encoding the chimeric antigen receptor, and optionally, an enhancer operably linked to the sequence encoding the chimeric antigen receptor. In some embodiments of such sets of vectors, the second vector further comprises a poly(A) sequence operably linked to the sequence encoding the chimeric antigen receptor.

Also provided herein are mammalian cells that include any of the variety of sets of vectors provided herein that include a first vector that includes any of the nucleic acids encoding any of the variety of proteins described herein, and a second vector that includes a sequence encoding a chimeric antigen receptor. In some embodiments, the mammalian cell is an immune cell. In some embodiments, the

immune cell is selected from the group consisting of: a CD4+ T cell, a CD8+ T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, a macrophage, a regulatory T cell, and a helper T cell. In some embodiments, the mammalian cell was previously obtained from a subject or is a daughter cell of a mammalian cell that was previously
5 obtained from the subject. In some embodiments, the mammalian cell is a human cell.

Also provided herein are pharmaceutical compositions that include any of the variety of sets of vectors provided herein (e.g., sets of vectors that include a first vector that includes any of the nucleic acids encoding any of the variety of proteins
10 described herein, and a second vector that includes a sequence encoding a chimeric antigen receptor) and a pharmaceutically acceptable carrier. Also provided herein are pharmaceutical compositions that include any of the variety of mammalian cells that include such sets of vectors.

Also provided herein are chimeric transmembrane proteins that include: an
15 extracellular interleukin-15 domain; an extracellular sushi domain from an alpha chain of interleukin-15 receptor; and a transmembrane domain from an alpha chain of interleukin-7 receptor. In some embodiments, the extracellular interleukin-15 domain includes a sequence of a wildtype interleukin-15 protein. In some embodiments, a wildtype interleukin-15 protein is a wildtype human interleukin-15 protein (e.g., a
20 wildtype human interleukin-15 protein having a sequence of SEQ ID NO: 22 or SEQ ID NO: 24). In some embodiments, the extracellular interleukin-15 domain comprises a sequence that is at least 80% identical to the sequence of a wildtype interleukin-15 protein (e.g., a wildtype human interleukin-15 protein such as, without limitation, a wildtype human interleukin-15 protein having a sequence of SEQ ID
25 NO: 22 or SEQ ID NO: 24). In some embodiments, the extracellular interleukin-15 domain comprises a sequence that is at least 95% identical to the sequence of a wildtype interleukin-15 protein (e.g., a wildtype human interleukin-15 protein such as, without limitation, a wildtype human interleukin-15 protein having a sequence of
30 SEQ ID NO: 22 or SEQ ID NO: 24). In some embodiments, the extracellular interleukin-15 domain is a sequence of a wildtype interleukin-15 protein having one or both of (i) one to ten amino acids removed from the N-terminus of the sequence of the wildtype interleukin-15 protein (e.g., a wildtype human interleukin-15 protein such as, without limitation, a wildtype human interleukin-15 protein having a

sequence of SEQ ID NO: 22 or SEQ ID NO: 24), and (ii) one to ten amino acids removed from the C-terminus of the sequence of the wildtype interleukin-15 protein (e.g., a wildtype human interleukin-15 protein such as, without limitation, a wildtype human interleukin-15 protein having a sequence of SEQ ID NO: 22 or SEQ ID NO: 24).

In some embodiments, the chimeric transmembrane protein further includes a linker sequence positioned between the extracellular interleukin-15 domain and the extracellular sushi domain. In some embodiments, the linker sequence is about 2 amino acids to about 50 amino acids. In some embodiments, the linker sequence is about 4 amino acids to about 40 amino acids. In some embodiments, the linker is a naturally-occurring amino acid sequence. In some embodiments, the linker sequence is not a naturally-occurring amino acid sequence. In some embodiments, the linker sequence includes a sequence of SEQ ID NO: 92. In some embodiments, the linker sequence consists of a sequence of SEQ ID NO: 92.

In some embodiments, the chimeric transmembrane protein further includes an additional linker sequence between the extracellular sushi domain and the transmembrane domain. In some embodiments, the additional linker sequence is about 2 amino acids to about 50 amino acids. In some embodiments, the additional linker sequence is about 4 amino acids to about 40 amino acids. In some embodiments, the additional linker is a naturally-occurring amino acid sequence. In some embodiments, the additional linker sequence is not a naturally-occurring amino acid sequence.

In some embodiments of chimeric transmembrane proteins having an extracellular sushi domain, the extracellular sushi domain includes a sushi domain from a wildtype alpha chain of interleukin-15 receptor. In some embodiments, the wildtype alpha chain of interleukin-15 receptor is wildtype human alpha chain of interleukin-15 receptor. In some embodiments, the extracellular sushi domain of the wildtype human alpha chain of interleukin-15 receptor includes SEQ ID NO: 36 or SEQ ID NO: 37. In some embodiments, the extracellular sushi domain is at least 80% identical to a sequence of an extracellular sushi domain of a wildtype alpha chain of interleukin-15 receptor (e.g., a wildtype human alpha chain of interleukin-15 receptor such as, without limitation, a wildtype human alpha chain of interleukin-15 receptor having a sequence of SEQ ID NO: 36 or SEQ ID NO: 37). In some embodiments, the

extracellular sushi domain is at least 95% identical to a sequence of an extracellular sushi domain of a wildtype alpha chain of interleukin-15 receptor (e.g., a wildtype human alpha chain of interleukin-15 receptor such as, without limitation, a wildtype human alpha chain of interleukin-15 receptor having a sequence of SEQ ID NO: 36 or
5 SEQ ID NO: 37). In some embodiments, the extracellular sushi domain is a sequence of an extracellular sushi domain of a wildtype alpha chain of interleukin-15 receptor having one or both of (i) one to five amino acids removed from the N-terminus of the sequence of the extracellular sushi domain of the wildtype alpha chain of interleukin-15 receptor (e.g., a wildtype human alpha chain of interleukin-15 receptor such as,
10 without limitation, a wildtype human alpha chain of interleukin-15 receptor having a sequence of SEQ ID NO: 36 or SEQ ID NO: 37), and (ii) one to five amino acids removed from the C-terminus of the sequence of the extracellular sushi domain of the wildtype alpha chain of interleukin-15 receptor (e.g., a wildtype human alpha chain of interleukin-15 receptor such as, without limitation, a wildtype human alpha chain of
15 interleukin-15 receptor having a sequence of SEQ ID NO: 36 or SEQ ID NO: 37).

In some embodiments of chimeric transmembrane proteins having a transmembrane domain from an alpha chain of interleukin-7 receptor, the transmembrane domain includes a transmembrane domain from a wildtype alpha chain of interleukin-7 receptor. In some embodiments, the wildtype alpha chain of the
20 interleukin-7 receptor is a wildtype alpha chain of a human interleukin-7 receptor. In some embodiments, the transmembrane domain of the wildtype alpha chain of a human interleukin-7 receptor includes a sequence of
PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1). In some embodiments, the transmembrane domain of the wildtype alpha chain of a human interleukin-7 receptor
25 includes a sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more of the following modifications: (i) alanine-leucine-leucine at amino acid positions 15 through 17 in SEQ ID NO: 1 has been replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine; (ii) a cysteine-proline-threonine has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) a
30 proline-proline-cysteine-leucine has been inserted between amino acid positions 4 and 5 in SEQ ID NO: 1. Representative modified transmembrane domains have a sequence of SEQ ID NO: 2, 4, 6 or 8. In some embodiments, the transmembrane domain is at least 80% identical to a sequence of a transmembrane domain of a

wildtype alpha chain of interleukin-7 receptor (e.g., a transmembrane domain of a wildtype alpha chain interleukin-7 receptor such as, without limitation, a transmembrane domain of a wildtype alpha chain of a human interleukin-7 receptor having a sequence of SEQ ID NO: 1). In some embodiments, the transmembrane domain is at least 95% identical to a sequence of a transmembrane domain of a wildtype alpha chain of interleukin-7 receptor (e.g., a transmembrane domain of a wildtype alpha chain interleukin-7 receptor such as, without limitation, a transmembrane domain of a wildtype alpha chain of a human interleukin-7 receptor having a sequence of SEQ ID NO: 1). In some embodiments, the transmembrane domain is a sequence of a transmembrane domain of a wildtype alpha chain of interleukin-7 receptor having one or both of (i) one to three amino acids removed from the N-terminus of the sequence of the transmembrane domain of the wildtype alpha chain of interleukin-2 receptor (e.g., a transmembrane domain of a wildtype alpha chain interleukin-7 receptor such as, without limitation, a transmembrane domain of a wildtype alpha chain of a human interleukin-7 receptor having a sequence of SEQ ID NO: 1), and (ii) one to three amino acids removed from the C-terminus of the sequence of the transmembrane domain of the wildtype alpha chain of interleukin-7 receptor (e.g., a transmembrane domain of a wildtype alpha chain interleukin-7 receptor such as, without limitation, a transmembrane domain of a wildtype alpha chain of a human interleukin-7 receptor having a sequence of SEQ ID NO: 1).

In some embodiments of chimeric transmembrane proteins provided herein, the chimeric transmembrane protein further includes an intracellular domain of an alpha chain of interleukin-7 receptor. In some embodiments, the alpha chain of interleukin-7 receptor is a wildtype alpha chain of interleukin-7 receptor. In some embodiments, the wildtype alpha chain of interleukin-7 receptor is a wildtype human alpha chain of interleukin-7 receptor. In some embodiments, the intracellular domain includes a sequence of SEQ ID NO: 45. In some embodiments, the intracellular domain includes a sequence that is at least 80% identical to the sequence of an intracellular domain of a wildtype alpha chain of interleukin-7 receptor (e.g., a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45). In some embodiments, the intracellular domain is at least 90% or 95% identical to SEQ ID NO: 45. In some embodiments, the intracellular domain is a sequence of an

intracellular domain of a wildtype alpha chain of interleukin-7 receptor having one or both of: (i) one to ten amino acids deleted from the N-terminus of the sequence of the intracellular domain of the wildtype alpha chain of interleukin-7 receptor (e.g., a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45) and (ii)
 5 one to ten amino acids deleted from the C-terminus of the sequence of the intracellular domain of the wildtype alpha chain of interleukin-7 receptor (e.g., a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45).

Also provided herein are nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein. Also provided herein are vectors that
 10 include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein. In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein include a promoter operably linked to the nucleic acid, and optionally, an enhancer sequence operably linked to the nucleic acid. In some
 15 embodiments, vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein include a poly(A) sequence operably linked to the nucleic acid. In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein are lentiviral or adenoviral vectors.

In some embodiments, vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein include a sequence encoding a chimeric antigen receptor. In some embodiments, the chimeric antigen receptor binds specifically to a tumor antigen (e.g., a tumor antigen is selected from the group consisting of: glypican-3, BCMA, MAGE, MUC16, CD19,
 25 WT-1, CD22, LI-CAM, ROR-1, CEA, 4-1BB, ETA, 5T4, adenocarcinoma antigen, alpha-fetoprotein (AFP), BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA- IX), C-MET, CCR4, CD152, CD20, CD125 CD200, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3,
 30 FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-

glycolylneuraminic acid, NPC-1C, PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, and vimentin). In some embodiments, the chimeric antigen
5 receptor comprises one or more co-stimulatory signaling domains selected from the group consisting of: 4-1BB, CD27, OX40, CD40, CD28, GITR, CD2, CD5, ICAM-1, CD11a, Lck, TNFR-I, TNFR-II, FasR, CD30, ICOS, LIGHT, NKG2C, B7-H3, DAP-10, and DAP-12.

Also provided herein are mammalian cells that include any of the variety of
10 nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein or vectors that include such nucleic acids described herein. In some embodiments, the mammalian cell is an immune cell. In some embodiments, the immune cell is selected from the group consisting of: a CD4⁺ T cell, a CD8⁺ T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, a macrophage, a regulatory T
15 cell, and a helper T cell. In some embodiments, the mammalian cell was previously obtained from a subject or is a daughter cell of a mammalian cell that was previously obtained from the subject. In some embodiments, the mammalian cell is a human cell.

Also provided herein are pharmaceutical compositions that include any of the
20 variety of vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein and a pharmaceutically acceptable carrier. Also provided herein are pharmaceutical compositions that include any of the variety of mammalian cells that include vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein.

Also provided herein are sets of vectors that include a first vector that is any of
25 the vectors that include any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein, and a second vector that includes a sequence encoding a chimeric antigen receptor. In some embodiments of sets of such vectors, the chimeric antigen receptor binds specifically to a tumor antigen (e.g.,
30 a tumor antigen is selected from the group consisting of: glypican-3, BCMA, MAGE, MUC16, CD19, WT-1, CD22, LI-CAM, ROR-1, CEA, 4-1BB, ETA, 5T4, adenocarcinoma antigen, alpha-fetoprotein (AFP), BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA- IX), C-MET, CCR4, CD152, CD20,

CD125 CD200, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha \beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, and vimentin). In some embodiments of such sets of vectors, the chimeric antigen receptor comprises one or more co-stimulatory signaling domains selected from the group consisting of: 4-1BB, CD27, OX40, CD40, CD28, GITR, CD2, CD5, ICAM-1, CD11a, Lck, TNFR-I, TNFR-II, FasR, CD30, ICOS, LIGHT, NKG2C, B7-H3, DAP-10, and DAP-12. In some embodiments of such sets of vectors, both of the first vector and the second vector is a lentiviral or adenoviral vector. In some embodiments of such sets of vectors, the second vector further includes a promoter operably linked to the sequence encoding the chimeric antigen receptor, and optionally, an enhancer operably linked to the sequence encoding the chimeric antigen receptor. In some embodiments of such sets of vectors, the second vector further comprises a poly(A) sequence operably linked to the sequence encoding the chimeric antigen receptor.

Also provided herein are mammalian cells that include any of the variety of sets of vectors described herein that include a first vector that includes any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein, and a second vector that includes a sequence encoding a chimeric antigen receptor. In some embodiments, the mammalian cell is an immune cell. In some embodiments, the immune cell is selected from the group consisting of: a CD4⁺ T cell, a CD8⁺ T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, a macrophage, a regulatory T cell, and a helper T cell. In some embodiments, the mammalian cell was previously obtained from a subject or is a daughter cell of a mammalian cell that was previously obtained from the subject. In some embodiments, the mammalian cell is a human cell.

Also provided herein are pharmaceutical compositions that include any of the variety of sets of vectors described herein (e.g., sets of vectors that include a first vector that includes any of the nucleic acids encoding any of the variety of chimeric transmembrane proteins described herein, and a second vector that includes a sequence encoding a chimeric antigen receptor) and a pharmaceutically acceptable carrier. Also provided herein are pharmaceutical compositions that include any of the variety of mammalian cells that include such sets of vectors.

Also provided herein are kits that include any of the variety of pharmaceutical compositions described herein (e.g., pharmaceutical compositions that include any of the variety of vectors or sets of vectors described herein).

Also provided herein are methods of treating a cancer in a subject in need thereof, the methods including: administering a therapeutically effective amount of any of the variety of mammalian cells described herein (e.g., mammalian cells that include any of the variety of nucleic acids, vectors, or sets of vectors described herein). In some embodiments, the subject is a human and the mammalian cell is human. In some embodiments, the mammalian cell was previously obtained from the subject or is a daughter cell of a cell previously obtained from the subject.

Also provided herein are methods of reducing the volume of a solid tumor in a subject in need thereof, the methods including: administering a therapeutically effective amount of any of the variety of mammalian cells described herein (e.g., mammalian cells that include any of the variety of nucleic acids, vectors, or sets of vectors described herein). In some embodiments, the subject is a human and the mammalian cell is human. In some embodiments, the mammalian cell was previously obtained from the subject or is a daughter cell of a cell previously obtained from the subject.

Also provided herein are methods of inducing cell death in a cancer cell in a subject in need thereof, the methods including: administering a therapeutically effective amount of any of the variety of mammalian cells described herein (e.g., mammalian cells that include any of the variety of nucleic acids, vectors, or sets of vectors described herein). In some embodiments, the subject is a human and the mammalian cell is human. In some embodiments, the mammalian cell was previously obtained from the subject or is a daughter cell of a cell previously obtained from the subject.

Also provided herein are methods of decreasing the risk of developing a metastasis or an additional metastasis in a subject having a cancer, the methods including: administering a therapeutically effective amount of any of the variety of mammalian cells described herein (e.g., mammalian cells that include any of the variety of nucleic acids, vectors, or sets of vectors described herein). In some 5 embodiments, the subject is a human and the mammalian cell is human. In some embodiments, the mammalian cell was previously obtained from the subject or is a daughter cell of a cell previously obtained from the subject.

Also provided herein are methods of activating STAT5 signaling in an immune 10 cell, the methods including introducing into the immune cell any of the variety of nucleic acids described herein (e.g., nucleic acids encoding any of the variety of proteins or chimeric transmembrane proteins described herein). In some embodiments, the immune cell is obtained from the subject. In some embodiments, the subject has been identified as having a cancer. In some embodiments, the immune 15 cell is selected from the group consisting of: a CD4⁺ T cell, a CD8⁺ T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, a macrophage, a regulatory T cell, and a helper T cell.

The use of the term “a” before a noun is meant “one or more” of the particular noun. For example, the phrase “a mammalian cell” means “one or more mammalian 20 cell.” In some examples, the term “a” can mean a single unit of the particular noun.

The terms “chimeric antigen receptor” and “CAR” are used interchangeably herein, and refer to artificial multi-module molecules capable of triggering or inhibiting the activation of an immune cell which generally but not exclusively comprise an extracellular domain (e.g., a ligand/antigen binding domain), a 25 transmembrane domain and one or more intracellular signaling domains. CAR molecules and derivatives thereof (e.g., CAR variants) are described, e.g., in PCT Application No. US2014/016527; Fedorov et al., *Sci Transl Med* (2013) 5(215):215ra172; Glienke et al., *Front Pharmacol* (2015) 6:21; Kakarla & Gottschalk, *Cancer J* (2014) 20(2):151-5; Riddell et al., *Cancer J* (2014) 20(2):141-4; 30 Pegram et al., *Cancer J* (2014) 20(2):127-33; Cheadle et al., *Immunol Rev* (2014) 257(1):91-106; Barrett et al., *Annu Rev Med* (2014) 65:333-47; Sadelain et al., *Cancer Discov* (2013) 3(4):388-98; Cartellieri et al., *J Biomed Biotechnol* (2010) 956304; the disclosures of which are incorporated herein by reference in their entirety. A CAR

can be a single-chain chimeric antigen receptor or a multi-chain chimeric antigen receptor.

5 The term “transmembrane domain” means a domain of a polypeptide that includes at least one contiguous amino acid sequence that traverses a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell. For example, a transmembrane domain can include one, two, three, four, five, six, seven, eight, nine, or ten contiguous amino acid sequences that each traverse a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell. As is known in the art, a transmembrane domain can, e.g., include at least one (e.g., two, three, four, five, six, seven, eight, 10 nine, or ten) contiguous amino acid sequence (that traverses a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell) that has α -helical secondary structure in the lipid bilayer. In some embodiments, a transmembrane domain can include two or more contiguous amino acid sequences (that each traverse a lipid bilayer when present in the corresponding 15 endogenous polypeptide when expressed in a mammalian cell) that form a β -barrel secondary structure in the lipid bilayer. Non-limiting examples of transmembrane domains are described herein. Additional examples of transmembrane domains are known in the art.

20 The term “antigen-binding domain” means a domain that binds specifically to a target antigen. In some examples, an antigen-binding domain can be formed from the amino acids present within a single-chain polypeptide. In other examples, an antigen-binding domain can be formed from amino acids present within a first single-chain polypeptide and the amino acids present in one or more additional single-chain polypeptides (e.g., a second single-chain polypeptide). Non-limiting examples of 25 antigen-binding domains are described herein, including, without limitation, scFvs, or ligand-binding domains (LBDs) of growth factors. Additional examples of antigen-binding domains are known in the art.

30 As used herein, the term “antigen” refers generally to a binding partner specifically recognized by an antigen-binding domain described herein. Exemplary antigens include different classes of molecules, such as, but not limited to, polypeptides and peptide fragments thereof, small molecules, lipids, carbohydrates, and nucleic acids. Non-limiting examples of antigen or antigens that can be

specifically bound by any of the antigen-binding domains are described herein. Additional examples of antigen or antigens that can be specifically bound by any of the antigen-binding domains are known in the art.

5 The term “intracellular signaling domain” means an intracellular signaling domain from an endogenous signaling transmembrane polypeptide expressed in an immune cell (e.g., a T lymphocyte) that promotes downstream immune cell signaling (e.g., T-cell receptor signaling) and/or immune cell activation (e.g., T cell activation). Non-limiting examples of intracellular signaling domains are described herein. Additional examples of intracellular signaling domains are known in the art. See,
10 e.g., Chen et al., *Nature Reviews Immunol.* 13:227-242, 2013.

The term “immunoreceptor tyrosine-based activation motif” or “ITAM” means an amino acid motif that includes a four amino-acid consensus sequence of a tyrosine separated from a leucine or an isoleucine by two other amino acids (YxxL/I). The tyrosine residue in the four-amino acid consensus sequence becomes phosphorylated
15 following interaction of a signaling pathway kinase (e.g., a lymphocyte signaling pathway kinase). Non-limiting examples of ITAMs are described herein. Additional examples of ITAMs are known in the art.

The phrase “treatment of cancer” means a reduction in the number, frequency, or severity of one or more (e.g., two, three, four, or five) symptoms of a cancer in a
20 subject having a cancer, a reduction in the number of cancer cells and/or tumors present in a subject, and/or a reduction in the size of one or more solid tumors present in a subject.

As used herein, “extracellular domain” describes a portion of a polypeptide (e.g., a domain) that is present in the extracellular space when the polypeptide is
25 expressed in a mammalian cell (e.g., a human cell).

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
30 be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their

entirety. In case of conflict, the present specification, including definitions, will control.

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

5

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a schematic diagram of IL7R α . ECD = extracellular domain. TMD = transmembrane domain. ICD = intracellular domain. Relevant regions, motifs, and amino acid modifications are indicated.

Figure 2 is a schematic diagram of IL7R α , showing relevant motifs, and mechanisms of signaling activation.

Figure 3 is a set of flow cytometric data showing the expression of modified IL7R α proteins on the surface of primary T cells. The data shown are from day 6 post-activation.

Figure 4 is a schematic diagram of an exemplary chimeric transmembrane protein. The relevant motifs are indicated, along with the mechanism of signaling activation. The IL15R α sushi domain is indicated by the arrow.

Figure 5 is a set of flow cytometric data showing the expression of chimeric transmembrane proteins on the surface of primary T cells. The data shown are from day 6 post-activation.

Figure 6A is a graph showing the percentage of different types of CD8⁺ T cells when primary human T cells were transduced with lentiviral vectors to express one of the specific IL7R α proteins or one of the specific chimeric membrane proteins shown.

Figure 6B is a set of two graphs of flow cytometry data showing the expression of PD-1 (left graph) or CD25 (right graph) in primary human T cells expressing different IL7R α mutant proteins and chimeric transmembrane proteins in primary human T cells at day 15 post-activation.

Figure 7A is an immunoblot showing the level of phosphorylated STAT5 in primary human T cells left untransduced ("UT") or transduced with lentiviral encoding one of the specific IL7R α proteins or one of the specific chimeric membrane proteins shown.

Figure 7B is a set of two bar graphs showing the expression of various IL7R α mutant proteins and chimeric transmembrane proteins in primary human T cells at day 15 post-activation.

Figure 8A shows schematic diagrams of IL7R α mutant expression constructs and a 1928z chimeric antigen receptor expression construct that were used to co-infect primary T-cells.

Figure 8B shows diagrammatically the 1928z chimeric antigen receptor being co-expressed at the T-cell surface with EKV or Ins_CPT IL7R α mutant proteins, the mbIL15-17R α Ins_PPCL chimeric transmembrane protein, or the mbIL15 chimeric transmembrane protein.

Figure 8C is a set of flow cytometric data showing the expression of the 1928z chimeric antigen receptor, EKV or Ins_CPT IL7R α mutant proteins, the mbIL15-17R α Ins_PPCL chimeric transmembrane protein, and the mbIL15 protein on the surface of primary T cells. The data shown are from day 6 post-activation.

Figure 8D is a set of two graphs showing that the number of cells expressing mbIL15-IL7R α _Ins_PPCL and mbIL15 decreased from day 6 to day 14 in culture, while the number of cell expressing IL7R α _EKV, IL7R α _Ins_CPT, and the 1928z chimeric antigen receptor remained relatively constant over the same period.

Figure 9 is a set of two graphs showing that CAR T-cells expressing various IL7R α mutants and chimeric transmembrane proteins maintain less differentiated memory phenotype at day 14 post activation.

Figure 10 is a set of immunoblots showing the levels of phosphorylated STAT5 and STAT3 and total protein level of BCL-XL in primary human T cells left untransduced or transduced with lentiviral vectors encoding one of the specific IL7R α proteins or one of the specific chimeric transmembrane proteins with and without CAR (1928z).

Figure 11A is a line graph showing the expansion of cells that express the various IL7R α mutants and Figure 11B is a bar graph showing fold expansion of CAR-T cells over 21 days of serial encounter with Nalm6 target cells; together Figures 11A and 11B show that CAR T-cells expressing IL7R α mutants demonstrate superior expansion compared to control CAR upon serial exposure to antigen at a 1:1 ratio effector to target (E:T) ratio.

Figure 12A is a line graph showing percent lysis of CD19+ Nalm6 B-cells in a luciferase-based overnight killing assay.

Figure 12B is a line graph showing percent lysis of CD19- K562 cells in a luciferase-based overnight killing assay.

Figure 13 is a set of bar graphs showing that CAR T-cells expressing IL7R α mutants maintained a less differentiated memory phenotype at day 14 following serial exposure to antigen.

Figure 14A is a line graph showing the expansion of cells expressing the various IL7R α mutants and Figure 14B is a bar graph showing fold expansion of CAR-T cells over 21 days following single encounter with antigen at a 1:3 E:T ratio; together Figures 14A and 14B show that CAR T-cells expressing IL7R α mutants demonstrate superior expansion to control CAR upon a single exposure to target.

Figure 15 is a set of bar graphs showing that CAR T-cells expressing IL7R α mutants maintained a less differentiated memory phenotype at day 14 following single exposure to antigen.

Figure 16 is the measured flux (photons/second) over time in NOD-SCID IL2Rgamma^{null} mice administered 0.5×10^6 Nalm6_luc cells on day 1, followed by administration of 0.1×10^6 cells non-transduced T cells, or T cells carrying CD19 CAR alone, CD19 CAR + IL7RaCPT, CD19 CAR + IL7RaMCP, or CD19 CAR + IL7RaPPCL. The data shown are the mean $\pm$ standard deviation.

Figure 17 is the change in body weight over time in NOD-SCID IL2Rgamma^{null} mice administered 0.5×10^6 Nalm6_luc cells on day 1, followed by administration of 0.1×10^6 cells non-transduced T cells, or T cells carrying CD19 CAR alone, CD19 CAR + IL7RaCPT, CD19 CAR + IL7RaMCP, or CD19 CAR + IL7RaPPCL. The data shown are the mean $\pm$ standard deviation.

Figure 18 is the relative mean fluorescence intensity (MFI) of pSTAT5a,b and pSTAT3 in non-transduced T cells, or T cells carrying CD19 CAR alone, CD19 CAR + IL7RaCPT, CD19 CAR + IL7RaMCP, or CD19 CAR + IL7RaPPCL. The mean fluorescence intensity data are compared to the mean fluorescence intensity measured in non-transduced T cells.

Figure 19 is a graph showing the fold-expansion of GPC3 CAR positive T cells and GPC3 CAR + IL7Ra CPT positive T-cells co-cultured with Hep3B target cells from American Type Culture Company (ATCC, Manassas, VA) (0.5×10^6 CAR T cells co-cultured with 0.5×10^6 Hep3B target cells) over time (as described in Example 9).

DETAILED DESCRIPTION

Provided herein are proteins that include a transmembrane domain of an alpha chain of interleukin-7 receptor, where the transmembrane domain includes the sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more
5 (e.g., two, three, or four) of the following modifications: (i) alanine-leucine-leucine at amino acids positions 15 through 17 in SEQ ID NO: 1 has been replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine; (ii) a cysteine-proline-threonine has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) a proline-proline-cysteine-leucine has been inserted between amino acid
10 positions 4 and 5 in SEQ ID NO: 1. Representative modified transmembrane domains have a sequence of SEQ ID NO: 2, 4, 6 or 8.

Also provided herein are chimeric transmembrane proteins that include an extracellular IL-15 domain, an extracellular sushi domain from an alpha chain of interleukin-15 receptor, and a transmembrane domain of an alpha chain of interleukin-
15 7 receptor.

Also provided are nucleic acids encoding any of these chimeric transmembrane proteins and proteins, vectors including any of these nucleic acids, and mammalian cells that include any of these nucleic acids or vectors. Also provided herein are methods of treating a cancer, methods of inducing cell death in a cancer cell (e.g., apoptosis and/or necrosis), and methods of decreasing the risk of
20 developing a metastasis or an additional metastasis in a subject in need thereof that include administering any of the mammalian cells described herein to the subject.

The chimeric transmembrane proteins and proteins provided herein can be used to maintain CAR T-cells in the absence of exogenous cytokine support or antigen stimulation, thus providing for the T-cell stimulatory effects without the dose-limiting toxicities associated with prolonged administration of a cytokine, e.g.,
25 soluble, recombinant IL-2.

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

Proteins Including a Transmembrane Domain of an Alpha Chain of Interleukin-7 Receptor

Provided herein are proteins that include a transmembrane domain of an alpha chain of interleukin-7 receptor, where the transmembrane domain includes the sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more (e.g., one, two, three, or four) of the following modifications: (i) alanine-leucine-leucine at amino acids positions 15 through 17 in SEQ ID NO: 1 has been replaced with a different three-amino acid sequence (e.g., has been replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine); (ii) one to three (e.g., one, two, or three) amino acids (e.g., cysteine-proline-threonine) has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) one to four (e.g., one, two, three, or four) amino acids (e.g., proline-proline-cysteine-leucine) has been inserted between amino acid positions 4 and 5 in SEQ ID NO: 1. Representative modified transmembrane domains have a sequence of SEQ ID NO: 2, 4, 6 or 8.

In some embodiments, the transmembrane domain includes the sequence of SEQ ID NO: 1 with the alanine-leucine-leucine at amino acid positions 15 through 17 in SEQ ID NO: 1 replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine. In some embodiments, the transmembrane domain includes the sequence of SEQ ID NO: 1 with cysteine-proline-threonine inserted between amino acid positions 5 and 6 in SEQ ID NO: 1. In some embodiments, the transmembrane domain includes the sequence of SEQ ID NO: 1 with a proline-proline-cysteine-leucine inserted between amino acid positions 4 and 5 in SEQ ID NO: 1.

In some embodiments, the proteins provided herein (e.g., mature or precursor protein) can be, e.g., about 40 amino acids to about 800 amino acids, about 40 amino acids to about 750 amino acids, about 40 amino acids to about 700 amino acids, about 40 amino acids to about 650 amino acids, about 40 amino acids to about 600 amino acids, about 40 amino acids to about 550 amino acids, about 40 amino acids to about 500 amino acids, about 40 amino acids to about 450 amino acids, about 40 amino acids to about 400 amino acids, about 40 amino acids to about 350 amino acids, about 40 amino acids to about 300 amino acids, about 40 amino acids to about 250 amino acids, about 40 amino acids to about 200 amino acids, about 40 amino acids to about 180 amino acids, about 40 amino acids to about 160 amino acids, about 40 amino acids to about 140 amino acids, about 40 amino acids to about 120 amino acids, about

40 amino acids to about 100 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 60 amino acids, about 60 amino acids to about 800 amino acids, about 60 amino acids to about 750 amino acids, about 60 amino acids to about 700 amino acids, about 60 amino acids to about 650 amino acids, about 5
60 amino acids to about 600 amino acids, about 60 amino acids to about 550 amino acids, about 60 amino acids to about 500 amino acids, about 60 amino acids to about 450 amino acids, about 60 amino acids to about 400 amino acids, about 60 amino acids to about 350 amino acids, about 60 amino acids to about 300 amino acids, about 60 amino acids to about 250 amino acids, about 60 amino acids to about 200 amino
10 acids, about 60 amino acids to about 180 amino acids, about 60 amino acids to about 160 amino acids, about 60 amino acids to about 140 amino acids, about 60 amino acids to about 120 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 80 amino acids, about 80 amino acids to about 800 amino acids, about 80 amino acids to about 750 amino acids, about 80 amino acids to about
15 700 amino acids, about 80 amino acids to about 650 amino acids, about 80 amino acids to about 600 amino acids, about 80 amino acids to about 550 amino acids, about 80 amino acids to about 500 amino acids, about 80 amino acids to about 450 amino acids, about 80 amino acids to about 400 amino acids, about 80 amino acids to about 350 amino acids, about 80 amino acids to about 300 amino acids, about 80 amino
20 acids to about 250 amino acids, about 80 amino acids to about 200 amino acids, about 80 amino acids to about 180 amino acids, about 80 amino acids to about 160 amino acids, about 80 amino acids to about 140 amino acids, about 80 amino acids to about 120 amino acids, about 80 amino acids to about 100 amino acids, about 100 amino acids to about 800 amino acids, about 100 amino acids to about 750 amino acids,
25 about 100 amino acids to about 700 amino acids, about 100 amino acids to about 650 amino acids, about 100 amino acids to about 600 amino acids, about 100 amino acids to about 550 amino acids, about 100 amino acids to about 500 amino acids, about 100 amino acids to about 450 amino acids, about 100 amino acids to about 400 amino acids, about 100 amino acids to about 350 amino acids, about 100 amino acids to about 300 amino acids, about 100 amino acids to about 250 amino acids, about 100
30 amino acids to about 200 amino acids, about 100 amino acids to about 180 amino acids, about 100 amino acids to about 160 amino acids, about 100 amino acids to about 140 amino acids, about 100 amino acids to about 120 amino acids, about 120

[illegible]

[illegible]

amino acids to about 700 amino acids, about 450 amino acids to about 650 amino acids, about 450 amino acids to about 600 amino acids, about 450 amino acids to about 550 amino acids, about 450 amino acids to about 500 amino acids, about 500 amino acids to about 800 amino acids, about 500 amino acids to about 750 amino acids, about 500 amino acids to about 700 amino acids, about 500 amino acids to about 650 amino acids, about 500 amino acids to about 600 amino acids, about 500 amino acids to about 550 amino acids, about 550 amino acids to about 800 amino acids, about 550 amino acids to about 750 amino acids, about 550 amino acids to about 700 amino acids, about 550 amino acids to about 650 amino acids, about 550 amino acids to about 600 amino acids, about 600 amino acids to about 800 amino acids, about 600 amino acids to about 750 amino acids, about 600 amino acids to about 700 amino acids, about 600 amino acids to about 650 amino acids, about 650 amino acids to about 800 amino acids, about 650 amino acids to about 750 amino acids, about 650 amino acids to about 700 amino acids, about 700 amino acids to about 800 amino acids, about 700 amino acids to about 750 amino acids, or about 750 amino acids to about 800 amino acids.

In some embodiments, the protein comprises or is SEQ ID NO: 2 (shown below).

Exemplary Protein (SEQ ID NO: 2) (IL7RA EKA Protein, EKA sequence is underlined)

PILLTISILSFFSVEKAVILACVLW

Nucleic Acid Encoding IL7RA EKA (SEQ ID NO: 3, nucleic acid sequence encoding the EKA sequence is underlined)

cccatcctgctgaccatcagcatcctgagcttcttcagcgtggagaggtggtgatcctggcctgcgtgctgtgg

In some embodiments, the protein comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to

SEQ ID NO: 2. In some embodiments, a protein includes a sequence that differs from SEQ ID NO: 2 by one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids). In some embodiments, any of the proteins provided herein can further include one or more additional amino acids (e.g., 1 amino acid to 300 amino acids, 1 amino acid to about 250 amino acids, 1 amino acid to about 200 amino acids, 1 amino acid to about 150 amino acids, 1 amino acid to about 100 amino acids, 1 amino acid to about 50 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 250 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 50 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, or about 10 amino acids to about 50 amino acids), e.g., in addition to SEQ ID NO: 2. Additionally or alternatively, a protein can lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the N-terminus of SEQ ID NO: 2 and/or lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the C-terminus of SEQ ID NO: 2.

In some embodiments, a nucleic acid encoding the protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 3. In some embodiments, a nucleic acid encoding the protein includes a sequence that differs from SEQ ID NO: 3 by one to sixty nucleotides (e.g., 1 to 60 nucleotides, 1 to 55 nucleotides, 1 to 50 nucleotides, 1 to 45 nucleotides, 1 to 40 nucleotides, 1 to 35 nucleotides, 1 to 30 nucleotides, 1 to 25 nucleotides, 1 to 20 nucleotides, 1 to 15 nucleotides, 1 to 10 nucleotides, or 1 to 5 nucleotides). In some embodiments, a nucleic acid encoding the protein can further include one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to

about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 3. Additionally or alternatively, a nucleic acid encoding the protein can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 3 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 3.

In some embodiments, the protein comprises or is SEQ ID NO: 4 (shown below).

Exemplary Protein (SEQ ID NO: 4) (IL7RA EKV Protein, EKV sequence is underlined)

PILLTISILSFFSVEKVVILACVLW

20

Nucleic Acid Encoding IL7RA EKV (SEQ ID NO: 5, nucleic acid sequence encoding EKV sequence is underlined)

cccatcctgctgaccatcagcatcctgagcttcttcagcgtgagagaggtggtgatcctggcctgcgtgctgtgg

In some embodiments, the protein comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 4. In some embodiments, a protein includes a sequence that differs from SEQ ID NO: 4 by one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids). In some embodiments, any of the

proteins provided herein can further include one or more additional amino acids (e.g., 1 amino acid to 300 amino acids, 1 amino acid to about 250 amino acids, 1 amino acid to about 200 amino acids, 1 amino acid to about 150 amino acids, 1 amino acid to about 100 amino acids, 1 amino acid to about 50 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 250 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 50 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, or about 10 amino acids to about 50 amino acids), e.g., in addition to SEQ ID NO: 4. Additionally or alternatively, a protein can lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the N-terminus of SEQ ID NO: 4 and/or lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the C-terminus of SEQ ID NO: 4.

In some embodiments, a nucleic acid encoding the protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 5. In some embodiments, a nucleic acid encoding the protein includes a sequence that differs from SEQ ID NO: 5 by one to sixty nucleotides (e.g., 1 to 60 nucleotides, 1 to 55 nucleotides, 1 to 50 nucleotides, 1 to 45 nucleotides, 1 to 40 nucleotides, 1 to 35 nucleotides, 1 to 30 nucleotides, 1 to 25 nucleotides, 1 to 20 nucleotides, 1 to 15 nucleotides, 1 to 10 nucleotides, or 1 to 5 nucleotides). In some embodiments, a nucleic acid encoding the protein can further include one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO:

5. Additionally or alternatively, a nucleic acid encoding the protein can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 5 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 5.

In some embodiments, the protein comprises or is SEQ ID NO: 6 (shown below).

Exemplary Protein (SEQ ID NO: 6) (IL7RA CPT insert Protein, CPT sequence is underlined)

PILLTCPTISILSFFSVALLVILACVLW

Nucleic Acid Encoding IL7RA CPT insert (SEQ ID NO: 7, nucleic acid sequence encoding the CPT sequence is underlined)

cccatcctgctgacctgccccaccatcagcatcctgagcttcttcagcgtggccctgctggtgatcctggcctgcgtgctgtg
g

In some embodiments, the protein comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 6. In some embodiments, a protein includes a sequence that differs from SEQ ID NO: 6 by one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids). In some embodiments, any of the proteins provided herein can further include one or more additional amino acids (e.g.,

1 amino acid to 300 amino acids, 1 amino acid to about 250 amino acids, 1 amino acid to about 200 amino acids, 1 amino acid to about 150 amino acids, 1 amino acid to about 100 amino acids, 1 amino acid to about 50 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 250 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 50 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, or about 10 amino acids to about 50 amino acids), e.g., in addition to SEQ ID NO: 6. Additionally or alternatively, a protein can lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the N-terminus of SEQ ID NO: 6 and/or lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the C-terminus of SEQ ID NO: 6.

15 In some embodiments, a nucleic acid encoding the protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 7. In some embodiments, a nucleic acid encoding the protein includes a sequence that differs from SEQ ID NO: 7 by one to sixty nucleotides (e.g., 1 to 60 nucleotides, 1 to 55 nucleotides, 1 to 50 nucleotides, 1 to 45 nucleotides, 1 to 40 nucleotides, 1 to 35 nucleotides, 1 to 30 nucleotides, 1 to 25 nucleotides, 1 to 20 nucleotides, 1 to 15 nucleotides, 1 to 10 nucleotides, or 1 to 5 nucleotides). In some embodiments, a nucleic acid encoding the protein can further includes one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 7. Additionally or alternatively, a nucleic acid encoding the protein can lack one to

sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 7 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 7.

In some embodiments, the protein comprises or is SEQ ID NO: 8 (shown below).

Exemplary Protein (SEQ ID NO: 8) (IL7RA PPCL insert Protein, PPCL sequence is underlined)

PILLPPCLTISILSFFSVALLVILACVLW

Nucleic Acid Encoding IL7RA PPCL insert (SEQ ID NO: 9, nucleic acid sequence encoding the PPCL sequence is underlined)

cccatcctgctgccaccctgfttaaccatcagcatcctgagcttcttcagcgtggccctgctggtgatcctggcctgcgtgctgtgg

In some embodiments, the protein comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 8. In some embodiments, a protein includes a sequence that differs from SEQ ID NO: 8 by one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids). In some embodiments, any of the proteins provided herein can further include one or more additional amino acids (e.g., 1 amino acid to 300 amino acids, 1 amino acid to about 250 amino acids, 1 amino

acid to about 200 amino acids, 1 amino acid to about 150 amino acids, 1 amino acid to about 100 amino acids, 1 amino acid to about 50 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 250 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 50 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, or about 10 amino acids to about 50 amino acids), e.g., in addition to SEQ ID NO: 8.

10 Additionally or alternatively, a protein can lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the N-terminus of SEQ ID NO: 8 and/or lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the C-terminus of SEQ ID NO: 8.

In some embodiments, a nucleic acid encoding the protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 9. In some embodiments, a nucleic acid encoding the protein includes a sequence that differs from SEQ ID NO: 9 by one to sixty nucleotides (e.g., 1 to 60 nucleotides, 1 to 55 nucleotides, 1 to 50 nucleotides, 1 to 45 nucleotides, 1 to 40 nucleotides, 1 to 35 nucleotides, 1 to 30 nucleotides, 1 to 25 nucleotides, 1 to 20 nucleotides, 1 to 15 nucleotides, 1 to 10 nucleotides, or 1 to 5 nucleotides). In some embodiments, a nucleic acid encoding the protein can further include one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 9. Additionally or alternatively, a nucleic acid encoding the protein can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about

50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 9 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 9.

10 In some embodiments, the protein comprises or is SEQ ID NO: 79 (shown below).

Exemplary Protein (SEQ ID NO: 79) (IL7RA MCP insert Protein, MCP sequence is underlined)

15 PILLTMCPISILSFFSVALLVILACVLW

Nucleic Acid Encoding IL7RA MCP insert (SEQ ID NO: 80, nucleic acid sequence encoding the MCP sequence is underlined)

20 cccacctgctgaccatgtgccccatcagcatcctgagcttcttcagcgtggccctgctggtgatcctggcctgcgtgctgtg
g

In some embodiments, the protein comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 79. In some embodiments, a protein includes a sequence that differs from SEQ ID NO: 79 by one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids). In some embodiments, any of the proteins provided herein can further include one or more additional amino acids (e.g., 1 amino acid to 300 amino acids, 1 amino acid to about 250 amino acids, 1 amino acid to about 200 amino acids, 1 amino acid to about 150 amino acids, 1 amino

acid to about 100 amino acids, 1 amino acid to about 50 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 250 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 50 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, or about 10 amino acids to about 50 amino acids), e.g., in addition to SEQ ID NO: 79. Additionally or alternatively, a protein can lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the N-terminus of SEQ ID NO: 79 and/or lack one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) from the C-terminus of SEQ ID NO: 79.

In some embodiments, a nucleic acid encoding the protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 80. In some embodiments, a nucleic acid encoding the protein includes a sequence that differs from SEQ ID NO: 80 by one to sixty nucleotides (e.g., 1 to 60 nucleotides, 1 to 55 nucleotides, 1 to 50 nucleotides, 1 to 45 nucleotides, 1 to 40 nucleotides, 1 to 35 nucleotides, 1 to 30 nucleotides, 1 to 25 nucleotides, 1 to 20 nucleotides, 1 to 15 nucleotides, 1 to 10 nucleotides, or 1 to 5 nucleotides). In some embodiments, a nucleic acid encoding the protein can further include one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 80. Additionally or alternatively, a nucleic acid encoding the protein can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about

50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 80 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 80.

10 Some embodiments of any of the proteins described herein can further include an intracellular domain of an alpha chain of interleukin-7 receptor (e.g., any of the exemplary intracellular domains of an alpha chain of interleukin-7 receptor described herein or known in the art).

15 Some embodiments of any of the proteins described herein can further include an extracellular domain of an alpha chain of interleukin-7 receptor (e.g., any of the exemplary extracellular domains of an alpha chain of interleukin-7 receptor described herein or known in the art).

20 Some embodiments of any of the proteins described herein can further include a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) positioned between the transmembrane domain of an alpha chain of interleukin-7 receptor and the extracellular domain of an alpha chain of interleukin-7 receptor (e.g., any of the exemplary extracellular domains of an alpha chain of interleukin-7 receptor described herein or known in the art).

25 Some embodiments of any of the proteins described herein can further include an additional linker sequence (e.g., any of the exemplary additional linker sequences described herein or known in the art) positioned between the transmembrane domain of an alpha chain of interleukin-7 receptor and the intracellular domain of an alpha chain of interleukin-7 receptor (e.g., any of the exemplary intracellular domains of an alpha chain of interleukin-7 receptor described herein or known in the art).

30 In some embodiments, the protein further comprises a signal sequence at its N-terminus. In some embodiments, the signal sequence comprises or is the sequence MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 10). In some embodiments, the nucleic acid encoding the signal sequence comprises or is the sequence

atgctcctgctcgtgacttcacttctctctgtgaactccacaccccgcggttttgcttatecct (SEQ ID NO: 81).

Additional examples of signal sequences are known in the art. For example, a signal sequence can be about 5 amino acids to about 30 amino acids, about 5 amino acids to about 28 amino acids, about 5 amino acids to about 26 amino acids, about 5 amino acids to about 24 amino acids, about 5 amino acids to about 22 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 18 amino acids, about 5 amino acids to about 16 amino acids, about 5 amino acids to about 14 amino acids, about 5 amino acids to about 12 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 8 amino acids, about 6 amino acids to about 30 amino acids, about 6 amino acids to about 28 amino acids, about 6 amino acids to about 26 amino acids, about 6 amino acids to about 24 amino acids, about 6 amino acids to about 22 amino acids, about 6 amino acids to about 20 amino acids, about 6 amino acids to about 18 amino acids, about 6 amino acids to about 16 amino acids, about 6 amino acids to about 14 amino acids, about 6 amino acids to about 12 amino acids, about 6 amino acids to about 10 amino acids, about 6 amino acids to about 8 amino acids, about 8 amino acids to about 30 amino acids, about 8 amino acids to about 28 amino acids, about 8 amino acids to about 26 amino acids, about 8 amino acids to about 24 amino acids, about 8 amino acids to about 22 amino acids, about 8 amino acids to about 20 amino acids, about 8 amino acids to about 18 amino acids, about 8 amino acids to about 16 amino acids, about 8 amino acids to about 14 amino acids, about 8 amino acids to about 12 amino acids, about 8 amino acids to about 10 amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to about 28 amino acids, about 10 amino acids to about 26 amino acids, about 10 amino acids to about 24 amino acids, about 10 amino acids to about 22 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 18 amino acids, about 10 amino acids to about 16 amino acids, about 10 amino acids to about 14 amino acids, about 10 amino acids to about 12 amino acids, about 12 amino acids to about 30 amino acids, about 12 amino acids to about 28 amino acids, about 12 amino acids to about 26 amino acids, about 12 amino acids to about 24 amino acids, about 12 amino acids to about 22 amino acids, about 12 amino acids to about 20 amino acids, about 12 amino acids to about 18 amino acids, about 12 amino acids to about 16 amino acids, about 12 amino acids to about 14 amino acids, about 14 amino acids to about 30 amino acids, about 14 amino acids to about 28 amino

acids, about 14 amino acids to about 26 amino acids, about 14 amino acids to about 24 amino acids, about 14 amino acids to about 22 amino acids, about 14 amino acids to about 20 amino acids, about 14 amino acids to about 18 amino acids, about 14 amino acids to about 16 amino acids, about 16 amino acids to about 30 amino acids, about 16 amino acids to about 28 amino acids, about 16 amino acids to about 26 amino acids, about 16 amino acids to about 24 amino acids, about 16 amino acids to about 22 amino acids, about 16 amino acids to about 20 amino acids, about 16 amino acids to about 18 amino acids, about 18 amino acids to about 30 amino acids, about 18 amino acids to about 28 amino acids, about 18 amino acids to about 26 amino acids, about 18 amino acids to about 24 amino acids, about 18 amino acids to about 22 amino acids, about 18 amino acids to about 20 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 28 amino acids, about 20 amino acids to about 26 amino acids, about 20 amino acids to about 24 amino acids, about 20 amino acids to about 22 amino acids, about 22 amino acids to about 30 amino acids, about 22 amino acids to about 28 amino acids, about 22 amino acids to about 26 amino acids, about 22 amino acids to about 24 amino acids, about 24 amino acids to about 30 amino acids, about 24 amino acids to about 28 amino acids, about 24 amino acids to about 26 amino acids, about 26 amino acids to about 30 amino acids, about 26 amino acids to about 28 amino acids, or about 28 amino acids to about 30 amino acids, in length.

In some embodiments, the protein can further include a peptide tag. For example, a tag can be used to help facilitate purification, production, and/or identification of the chimeric transmembrane protein. In some embodiments, the tag is a histidine tag comprising at least six histidine residues. Additional examples of tags are known in the art.

Non-limiting aspects of any of the proteins provided herein are described below.

Extracellular Domains of an Alpha Chain of Interleukin-7 Receptor

Some embodiments of the proteins described herein can further include an extracellular domain of an alpha chain of interleukin-7 receptor (e.g., any of the exemplary domains of an alpha chain of interleukin-7 receptor described herein). For example, proteins described herein can include an extracellular domain of a wildtype

alpha chain of interleukin-7 receptor, from a human, a mouse, a rat, a monkey, a chimpanzee, a pig, a dog, a cat, or any other appropriate species. Non-limiting examples of extracellular domains of wildtype alpha chains of interleukin-7 receptors are described below. Additional examples of extracellular domains of wildtype alpha chains of interleukin-7 receptors are known in the art.

In some embodiments, the extracellular domain of an alpha chain of interleukin-7 receptor is or comprises an extracellular domain of a wildtype alpha chain of interleukin-7 receptor. For example, the extracellular domain of an alpha chain of interleukin-7 receptor comprises or is an extracellular domain of a wildtype human alpha chain of interleukin-7 receptor (e.g., SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15).

Exemplary Extracellular Domain of a Wildtype Human Alpha Chain of Interleukin-7 Receptor (SEQ ID NO: 11)

GESGYAQNGDLEDAELDDYSFSCYSQLEVNQSQHSLTCAFEDPDVNITNLEFEICGA
LVEVKCLNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTITIVKPEAPF
DLSVVYREGANDFVVTFNTSHLQKKYVKVLMHDAVYRQEKDENKWTHVNLSTKLTL
LQRKLQPAAMYEEKVRSIPDHYFKGFWEWSPSYFFRTPEINNSSGEMD

Nucleic Acid Encoding the Wildtype Human Extracellular Domain of an Alpha Chain of Interleukin-7 Receptor (SEQ ID NO: 12)

ggcgagagcggctacgccagaacggcgacctggaggacgccgagctggacgactacagctcagctgctacagcca
gctggaggtgaacggcagccagcacagcctgacctgcgcctcgaggaccccgacgtgaacatcaccaacctggagttc
gagatctgcggcgccctggtggaggtgaagtgcctgaacttcaggaagctgcaggagatctactcatcgagaccaagaa
gttctgctgatcggcaagagcaacatctgcgtgaaggtggcgagaagacacctgacctgcaagaagatcgacctgacc
accatcgtgaagcccgaggcccccttcgacctgagcgtggtgtacaggaggcgccaacgacttcgtggtgaccttcaa
caccagccacctgcagaagaagtacgtgaaggtgctgatgcacgacgtggcctacaggcaggagaaggacgagaaca
agtggaccacgtgaacctgagcagcaccaagctgacctgctgcagaggaagctgcagcccgcccatgtacgaga
tcaaggtgaggagcatccccgaccactacttcaagggcttctggagcgagtgagccccagctactacttcaggaccccc
gagatcaacaacagcagcggcgagatggac

In some embodiments, an extracellular domain of an alpha chain of interleukin-7 receptor comprises or is a sequence that is at least 60% identical, at least

65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to a sequence of an extracellular domain of a wildtype alpha chain of interleukin-7 receptor (e.g., a sequence of an extracellular domain of a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 11). In some embodiments, an extracellular domain of an alpha chain of interleukin-7 receptor includes a sequence that differs from SEQ ID NO: 11 by one to forty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids). In some embodiments, an extracellular domain of an alpha chain of interleukin-7 receptor can further include one to about 250 additional amino acids (e.g., 1 to about 200 amino acids, 1 to about 150 amino acids, 1 to about 100 amino acids, 1 to about 50 amino acids, about 5 to about 250 amino acids, about 5 to about 200 amino acids, about 5 to about 150 amino acids, about 5 to about 100 amino acids, about 5 to about 50 amino acids, about 10 to about 250 amino acids, about 10 to about 200 amino acids, about 10 to about 150 amino acids, about 10 to about 100 amino acids, or about 10 to about 50 amino acids), e.g., in addition to SEQ ID NO: 11. Additionally or alternatively, an extracellular domain of an alpha chain of interleukin-7 receptor is a sequence of an extracellular domain of a wildtype alpha chain of interleukin-7 receptor (e.g., a sequence of an extracellular domain of a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 11) having one or both of: one to ten amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) deleted from the N-terminus of the sequence of the extracellular domain of the wildtype alpha chain of interleukin-7 receptor and (ii) one to ten amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) deleted from the C-terminus of the sequence of the extracellular domain of the wildtype alpha chain of interleukin-7 receptor.

In some embodiments, a nucleic acid encoding the extracellular domain of an alpha chain of interleukin-7 receptor comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97%

identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 12. In some embodiments, a nucleic acid encoding the extracellular domain of an alpha chain of interleukin-7 receptor includes a sequence that differs from SEQ ID NO: 12 by one to sixty nucleotides (e.g., 1 to 55 nucleotides, 1 to 50 nucleotides, 1 to 45 nucleotides, 1 to 40 nucleotides, 1 to 35 nucleotides, 1 to 30 nucleotides, 1 to 25 nucleotides, 1 to 20 nucleotides, 1 to 15 nucleotides, 1 to 10 nucleotides, or 1 to 5 nucleotides). In some embodiments, a nucleic acid encoding the extracellular domain of an alpha chain of interleukin-7 receptor can further include one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 12. Additionally or alternatively, a nucleic acid encoding the extracellular domain of an alpha chain of interleukin-7 receptor can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 12 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 12.

Additional exemplary extracellular domains of wildtype alpha chains of interleukin-7 receptors are provided below.

Exemplary Wildtype Mouse Extracellular Domain of an Alpha Chain of Interleukin-7 Receptor Isoform 1 (SEQ ID NO: 13)

ESGNAQDGDLEDADADDHSFWCHSQLEVDGSQHLLTCAFNDSDINTANLEFQICGALLRVKC
LTLNKLQDIYFIKTSEFLIGSSNICVKLGQKNLTCKNMAINTIVKAEAPSDLKVYRKEAN

DFLVTFNAPHLKKKYLKKVKHVDVAYRPARGESNWTHTVSLFHTRTTIPQRKLRPKAMYEIKVR
SIPHNDYFKGFWSEWSPSSTFETPEPKNQGGWD

5

Nucleic Acid Encoding the Wildtype Mouse Extracellular Domain of an Alpha Chain of Interleukin-7 Receptor Isoform 1 (SEQ ID NO: 14)

gaaagtggaaatgccaggatggagacctagaagatgcagacgcggacgatcactccttctg
gtgccacagccagttggaagtggatggaagtcaacatttattgacttgctgtttaaataatgact
10 cagacatcaacacagctaatactggaatttcaaataatgtggggctcttttacgagtgaaatgc
ctaactcttaacaagctgcaagatatataatttataaagacatcagaattcttactgattgg
tagcagcaatatatgtgtgaagcttggacaaaagaatttaacttgcaaaaatatggctataa
acacaatagttaaagccgaggtccctctgacctgaaagtcgtttatcgcaagaagcaaat
gatttttttggtgacatttaaatgcacctcacttgaaaaagaaatatttaaaaaaagtaaagca
15 tgatgtggcctaccgccagcaaggggtgaaagcaactggacgcatgtatctttattccaca
caagaacaacaatcccacagagaaaactacgacaaaagcaatgtatgaaatcaaagtccga
tccattccccataacgattacttcaaaggcttctggagcgagtggagtccaagttctacctt
cgaaactccagaaccaagaatcaaggaggatgggat

20

Exemplary Wildtype Mouse Extracellular Domain of an Alpha Chain of Interleukin-7 Receptor Isoform 2 (SEQ ID NO: 15)

ESGNAQDGDLEDADADDHSFWCHSQLEVDGSQHLLTCAFNDSDINTANLEFQICGALLRV
KCLTLNKLQDIYFIKTSEFLIGSSNICVKLGQKNLTCKNMAINTIVKAEAPSDLKVVRKE
ANDFLVTFNAPHLKKKYLKKVKHVDVAYRPARGESNWTHTVSLFHTRTTIPQRKLRPKAMYEIK
25 VRSIPHNDYFKGFWSEWSPSSTFETPEPKNQGGWD

Nucleic Acid Encoding the Wildtype Mouse Extracellular Domain of an Alpha Chain of Interleukin-7 Receptor Variant 2 (SEQ ID NO: 16)

gaaagtggaaatgccaggatggagacctagaagatgcagacgcggacgatcactccttctg
30 gtgccacagccagttggaagtggatggaagtcaacatttattgacttgctgtttaaataatgact
cagacatcaacacagctaatactggaatttcaaataatgtggggctcttttacgagtgaaatgc
ctaactcttaacaagctgcaagatatataatttataaagacatcagaattcttactgattgg
tagcagcaatatatgtgtgaagcttggacaaaagaatttaacttgcaaaaatatggctataa
acacaatagttaaagccgaggtccctctgacctgaaagtcgtttatcgcaagaagcaaat
35 gatttttttggtgacatttaaatgcacctcacttgaaaaagaaatatttaaaaaaagtaaagca
tgatgtggcctaccgccagcaaggggtgaaagcaactggacgcatgtatctttattccaca

caagaacaacaatcccacagagaaaactacgacccaaaagcaatgtatgaaatcaaagtccga
 tccattccccataacgattacttcaaaggcttctggagcgagtggagtccaagttctacctt
 cgaaactccagaacccaagaatcaaggaggatgggat

5 In some embodiments, the extracellular domain of an alpha chain of interleukin-7 receptor is a sequence of a wildtype extracellular domain of an alpha chain of interleukin-7 receptor (e.g., a mature wildtype extracellular domain of an alpha chain of interleukin-7 receptor, e.g., any of the mature wildtype extracellular domains of an alpha chain of interleukin-7 receptor described herein, e.g., SEQ ID
 10 NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15) having one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the N-terminus of the sequence of the wildtype extracellular domain of an alpha chain of interleukin-7 receptor. In some embodiments, the extracellular domain of an alpha chain of interleukin-7 receptor is a sequence of a wildtype extracellular domain of an alpha
 15 chain of interleukin-7 receptor (e.g., a mature wildtype extracellular domain of an alpha chain of interleukin-7 receptor, e.g., any of the mature wildtype extracellular domains of an alpha chain of interleukin-7 receptor described herein, e.g., SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15) having one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the C-terminus of the sequence of the wildtype extracellular domain of an alpha chain of interleukin-7
 20 receptor. In some embodiments, the extracellular domain of an alpha chain of interleukin-7 receptor is a sequence of a wildtype extracellular domain of an alpha chain of interleukin-7 receptor (e.g., a mature wildtype extracellular domain of an alpha chain of interleukin-7 receptor, e.g., any of the mature wildtype extracellular domains of an alpha chain of interleukin-7 receptor described herein, e.g., SEQ ID
 25 NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15) having both one to ten amino (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) acids removed from the N-terminus of the sequence of the wildtype extracellular domain of an alpha chain of interleukin-7 receptor and one to ten (e.g., one, two, three, four, five, six, seven, eight,
 30 nine, or ten) amino acids removed from the C-terminus of the sequence of the wildtype extracellular domain of an alpha chain of interleukin-7 receptor.

As will be appreciated by those of ordinary skill in the art, when a nucleic acid encoding an extracellular domain of an alpha chain of interleukin-7 receptor includes or lacks one or more additional nucleotides as compared to SEQ ID NO: 12, SEQ ID

NO: 14, or SEQ ID NO: 16, the translational reading frame should still be maintained such that a nonsense codon is not introduced and the full protein can be translated (e.g., a nucleic acid encoding an extracellular domain of an alpha chain of interleukin-7 receptor can include or lack three nucleotides, or multiples thereof).

5 As one skilled in the art can appreciate, when amino acids that are not conserved between extracellular domains of alpha chains of interleukin-7 receptors from different species are mutated (e.g., substituted with a different amino acid) they are less likely to cause a decrease in the level of one or more activities of an extracellular domain of an alpha chain of interleukin-7 receptor. In contrast, when
10 amino acids that are conserved between extracellular domains of alpha chains of interleukin-7 receptors from different species are mutated (e.g., substituted with a different amino acid) they are more likely to cause a decrease in the level of one or more activities of an extracellular domain of an alpha chain of interleukin-7 receptor. In view of this knowledge, one skilled in the art can select which amino acid positions
15 in an extracellular domain of an alpha chain of interleukin-7 receptor (e.g., the non-conserved amino acids) can be substituted without decreasing the activity of the extracellular domain of an alpha chain of interleukin-7 receptor.

Chimeric Transmembrane Proteins

 Provided herein are chimeric transmembrane proteins that include an
20 extracellular IL-15 domain (e.g., any of the exemplary extracellular IL-15 domains described herein or known in the art), an extracellular sushi domain from an alpha chain of interleukin-15 receptor (e.g., one or more of any of the exemplary extracellular sushi domains from an alpha chain of interleukin-15 receptor described herein or known in the art), and a transmembrane domain of an alpha chain of
25 interleukin-7 receptor (e.g., any of the exemplary transmembrane domains of an alpha chain of interleukin-7 receptor described herein or known in the art). In some embodiments, the chimeric transmembrane protein comprises a linker sequence positioned between the extracellular IL-15 domain and the extracellular sushi domain from an alpha chain of interleukin-15 receptor (e.g., any of the exemplary linker
30 sequences described herein or known in the art). In some embodiments, the chimeric transmembrane protein further comprises an additional linker positioned between the extracellular sushi domain from an alpha chain of interleukin-15 receptor and the transmembrane domain of the alpha chain of the interleukin-7 receptor (e.g., any of

the exemplary linker sequences described herein or known in the art). In some
embodiments, the chimeric transmembrane protein can further include an intracellular
domain of an alpha chain of interleukin-7 receptor (e.g., any of the exemplary
intracellular domains of an alpha chain of interleukin-7 receptor described herein or
5 known in the art).

In some embodiments, the chimeric transmembrane protein comprises or is
SEQ ID NO: 17 (shown below).

Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 17)

10 NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLEELQVISLES
GDASIHDTVENLILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQ
MFINTSSGGSGGGSGGGSGGGSGGGSLQITCPPPMSEHADIWVKSYS
LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD PALVHQRP
APPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPS
15 TGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTPILLTISLL
SFFSVALLVILACVLWKKRIKPIVWPSLPDHHKTLEHLCKKPRKNLNVSNPES
FLDCQIHRVDDIQARDEVEGFLQDTFPQQLSESEKQRLGGDVQSPNCPSEDVV
ITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSLG
TTNSTLPPPFSLQSGILTLPVAQGGPILTSLSNQEEAYVTMSSFYQNQ

20

Nucleic Acid Encoding Chimeric Transmembrane Protein (SEQ ID NO: 18)

aactgggtgaatgtgatcagcgacctgaagaagatcgaggatctgatccagagcatgcacattgatgccacctgtacaca
gaatctgatgtgaccctagctgtaaagtaccgcatgaagtgtttctgctggagctgcaggtgatttctctgaaagcgg
agatgcctctatccagcacagtgaggagaatctgatcatcctggccaacaatagcctgagcagcaatggcaatgtacaga
25 gtctggctgtaaggagtgtgaggagctggaggagaagaacatcaaggagttctgcagagctttgtcacatcgtgcagat
gttcatcaatacaagcagcgggtggggctcaggcggaggaggctctggcggaggcgggaagcggggagggggctca
ggcggcgggtccttgagattacatgccctcctccaatgtctgtggagcacgccgatattgggtgaagtcctacagcctgt
acagcagagagagatacatctgcaacagcggctttaagagaaaggccggcacctcttctgacagagtgctgctgaat
aaggccacaaatgtggccactggacaacacctagcctgaagtgcattagagatcctgccctgtccaccagaggcctgc
30 cctccatctacagtgacaacagcggagtacacctcagcctgaatctctgagcccttctggaaaagaacctgccgccag
ctctcctagctctaataataccgccccacaacagccgcattgtgcctggatctcagctgatgcctagcaagtccttagca
caggcacaacagagatcagcagccacgaatctctcacggaacaccttctcagaccaccgccaagaattgggagctgac
agcctctgcctctcaccagcctcaggagtgtatcctcaggccactctgatacaacacccatcctgctgacctacagcatc

ctgagcttcttcagcgtggccctgctgggtgatcctggcctgcgtgctgtggaagaagaggatcaagcccatcgtgtggccc
 agcctgccccgaccacaagaagaccctggagcacctgtgtaagaagcccaggaagaacctgaacgtgagcttcaacccc
 gagagcttcctggactgccagatccacaggggtggacgacatccaggccagggacgaggtggagggttcctgcaggac
 acctccccagcagctggaggagagcgagaagcagaggctgggcgcgacgtgcagagcccaactgccccagcg
 5 aggacgtggtgatcacccccgagagcttcggcagggacagcagcctgacctgcctggccggcaacgtgagcgctgcg
 acgccccatcctgagcagcagcagcagcctggactgcagggagagcggcaagaacggccccacgtgtaccaggac
 ctgctgctgagcctgggcaccaccaacagcacctgccaccccccttcagcctgcagagcggcatcctgacctgaacc
 ccgtggcccgaggccagcccatcctgaccagcctgggcagcaaccaggaggagcctacgtgacctgagcagcttct
 accagaaccag

10

In some embodiments, the chimeric transmembrane protein comprises or is a
 sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at
 least 75% 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
 15 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at
 least 96% identical, at least 97% identical, at least 98% identical, at least 99%
 identical, or 100% identical to SEQ ID NO: 17. In some embodiments, a chimeric
 transmembrane protein includes a sequence that differs from SEQ ID NO: 17 by one
 to about 100 amino acids (e.g., 1 to about 95 amino acids, 1 to about 90 amino acids,
 20 1 to about 80 amino acids, 1 to about 75 amino acids, 1 to about 70 amino acids, 1 to
 about 65 amino acids, 1 to about 60 amino acids, 1 to about 55 amino acids, 1 to
 about 50 amino acids, 1 to about 45 amino acids, 1 to about 40 amino acids, 1 to
 about 35 amino acids, 1 to about 30 amino acids, 1 to about 25 amino acids, 1 to
 about 20 amino acids, 1 to about 15 amino acids, 1 to about 10 amino acids, 1 to
 25 about 5 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino
 acids to about 95 amino acids, about 5 amino acids to about 90 amino acids, about 5
 amino acids to about 85 amino acids, about 5 amino acids to about 80 amino acids,
 about 5 amino acids to about 75 amino acids, about 5 amino acids to about 70 amino
 acids, about 5 amino acids to about 65 amino acids, about 5 amino acids to about 60
 30 amino acids, about 5 amino acids to about 55 amino acids, about 5 amino acids to
 about 50 amino acids, about 5 amino acids to about 45 amino acids, about 5 amino
 acids to about 40 amino acids, about 5 amino acids to about 35 amino acids, about 5
 amino acids to about 30 amino acids, about 5 amino acids to about 25 amino acids,

about 5 amino acids to about 20 amino acids, about 5 amino acids to about 15 amino acids, or about 5 amino acids to about 10 amino acids). In some embodiments, a chimeric transmembrane protein includes 1 to about 300 additional amino acids (e.g., 1 to about 250 amino acids, 1 to about 200 amino acids, 1 to about 150 amino acids, 1 to about 100 amino acids, 1 to about 50 amino acids, 1 to about 25 amino acids, about 5 to about 300 amino acids, about 5 to about 250 amino acids, about 5 to about 200 amino acids, about 5 to about 150 amino acids, about 5 to about 100 amino acids, about 5 to about 50 amino acids, about 10 to about 300 amino acids, about 10 to about 250 amino acids, about 10 to about 200 amino acids, about 10 to about 150 amino acids, about 10 to about 100 amino acids, or about 10 to about 50 amino acids), e.g., in addition to the sequence of SEQ ID NO: 17. In some embodiments, the chimeric transmembrane protein comprises a sequence of SEQ ID NO: 17 having one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) removed from the N-terminus of SEQ ID NO: 17 and/or one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) removed from the C-terminus of SEQ ID NO: 17.

In some embodiments, a nucleic acid encoding a chimeric transmembrane protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 18. In some embodiments, a nucleic acid encoding a chimeric transmembrane protein includes a sequence that differs from SEQ ID NO: 18 by 1 to about 300 nucleotides (e.g., 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, 1 to about 50 nucleotides, about 5 nucleotides to about 300 nucleotides, about 5 nucleotides to about 250 nucleotides, about 5 nucleotides to about 200 nucleotides, about 5 nucleotides to about 150 nucleotides, about 5 nucleotides to about 100 nucleotides, about 5 nucleotides to about 50 nucleotides, about 10 nucleotides to about 300 nucleotides, about 10 nucleotides to about 250 nucleotides, about 10 nucleotides to about 200 nucleotides, about 10 nucleotides to about 150 nucleotides, about 10 nucleotides to about 100 nucleotides, or about 10 nucleotides to about 50 nucleotides). In some embodiments, a nucleic acid encoding a chimeric

transmembrane protein includes one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1 to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 18. Additionally or alternatively, a nucleic acid encoding the chimeric transmembrane protein can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to about 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 18 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to about 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 18.

In some embodiments, the chimeric transmembrane protein comprises or is one of SEQ ID NO: 19, 83, 85, 87, or 89 (shown below).

Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 19)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLES
GDASIHDTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQ
MFINTSSGGGSGGGGSGGGGSGGGGSLQITCPPPMSVEHADIWVKSYS
LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPAHVHQR
APPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAIVPGSQLMPKSPS
TGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTPILLPPCLT
ISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSN
PESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLESEKQRLGGDVQSPNCPSE
DVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL
SLGTTNSTLPPPSLQSGILTLPVAQGGQPILTSLSNQEAYVTMSSFYQNQ

Nucleic Acid Encoding Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 20)

aactgggtgaatgtgatcagcgacctgaagaagatcgaggatctgatccagagcatgcacattgatccaccctgtacaca
 5 gaatctgatgtgcaccctagctgtaaagtaccgcatgaagtgtttctgctggagctgcaggtgatttcttgaaagcgg
 agatgcctctatccacgacacagtggagaatctgatcatcctggccaacaatagcctgagcagcaatggcaatgtacaga
 gtctggctgtaaggagtgtgaggagctggaggagaagaacatcaaggagtctgagagcttctgcacatcgtgcagat
 gtcatcaatacaagcagcgggtgggggctcaggcggaggaggctctggcggaggcgggaagcggggagggggctca
 ggcggggggtccttgagattacatgccctccccaatgtctgtggagcacgccgatatgtgggtgaagtctacagcctgt
 10 acagcagagagatcatctgcaacagcggcttaagagaaaggccgcacctcttctgacagagtgcgtgctgaat
 aaggccacaaatgtggccactggacaacacctagcctgaagtgcattagagatcctgccctgtccaccagaggcctgc
 cctccatctacagtacaacagccggagtgcacctcagcctgaatctctgagcccttctggaaaagaacctgccgccag
 ctctcctagctctaataataccgccccacaacagccgcatgtgcctggatctcagctgatgcctagcaagtctcctagca
 caggcacaacagagatcagcagccacgaatctctcacggaacacctctcagaccaccgccaagaattgggagctgac
 15 agcctctgcctctcaccagcctcaggagtgtatctcagggccactctgatacaacacccatcctgctgccacctgttaa
 ccatcagcatcctgagcttctcagcgtggccctgctggtgatcctggcctgcgtgctgtggaagaagaggatcaagccat
 cgtgtggcccagcctgcccaccacaagaagacctggagcacctgtgtaagaagcccaggaagaacctgaacgtgag
 ctcaaccccgagagcttctgactgccagatccacagggtggacgacatccaggccaggagacgaggtggagggttc
 ctgcaggacaccttccccagcagctggaggagagcgagaagcagaggctggcgggcgacgtgcagagccccaactg
 20 cccagcgaggagctggtgatcccccgagagcttcggcaggagacagcagcctgacctgcctggccggcaactga
 gcgcctgcgacgccccatcctgagcagcagcaggagcctggactgcaggagagcggcaagaacggccccacgt
 gtaccaggacctgctgctgagcctgggcaccaccaacagcacctgccacccccctcagcctgcagagcggcatcctg
 acctgaaccccgctggccagggccagccatcctgaccagcctgggcagcaaccaggaggaggcctacgtgacctg
 agcagcttctaccagaaccag

25

Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 83)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLLELQVISLES
 GDASIHDTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQ
 MFINTSSGGSGGGSGGGSGGGSGGGSLQITCPPPMSVEHADIWVKSYS
 30 LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQR
 APPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPS
 TGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTPILLTCPTI
 SILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKPRKNLNVSN

PESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSE
 DVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL
 SLGTTNSTLPPFSLQSGILTLPVAQGGQPILTSLSNQEEAYVTMSSFYQNQ

5 **Nucleic Acid Encoding Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 84)**

aactgggtgaatgtgatcagcgacctgaagaagatcaggatctgatccagagcatgcacattgatgccaccctgtacaca
 gaatctgatgtgcaccctagctgtaaagtaccgccatgaagtgtttctgctggagctgcaggtgatttcttgaaagcgg
 agatgcctctatccacgacacagtggaatctgatcatcctggccaacaatagcctgagcagcaatggcaatgtacaga
 10 gtctggctgaaggagtgtgaggagctgaggagaagaacatcaaggagtcttctgagagcttctgcacatcgtgcagat
 gtcatcaataacaagcagcgggtggggctcaggcggaggaggtctggcggaggcggaggcggggagggggctca
 ggcggcgggtccttcagattacatgccctcctccaatgtctgtggagcacgcgataattgggtgaagtctacagcctgt
 acagcagagagatacatctgaacagcggcttaagagaaaggccggcaccttctctgacagagtgcgtgctgaat
 aaggccacaaatgtggccactggacaacacctagcctgaagtgcattagagatcctgccctggccaccagaggcctgc
 15 cctcctactacagtgaacaacagccggagtacacctcagcctgaatctctgagcccttctggaaaagaacctgccgacg
 ctctcctagcttaataataccgccccacaacagccgacctgtgcctggatctcagctgatgcctagaagtctcctagca
 caggcacaacagagatcagcagccacgaatctctcacggaacaccttctcagaccaccgccaagaattgggagctgac
 agcctctgcctctcaccagcctcaggagtgtatctcaggccactctgatacaacacctcctctgacgtgccccacc
 atcagcatcctgagcttctcagcgtggcctgctggatcctggcctgcgtgctgtggaagaaggagatcaagcccatc
 20 gtgtggccagcctgcccaccacaagaagacctggagcacctgtgaagaagcccaggaagaacctgaacgtgagc
 ttcaacccgagagcttctggactgccagatccacagggtggacgacatccaggccaggagcaggtggagggtcttc
 tgcaggacaccttccccagcagctggaggagagcgagaagcagaggctggcgccgacgtgcagagccccaaactgc
 cccagcgaggacgtggtgatcccccgagagcttcggcaggacagcagcctgacctgctggccgcaacgtgag
 cgctgcgacccccatcctgagcagcagcaggagcctggactgcaggagagcggcaagaacggccccacgtgt
 25 accaggacctgctgctgagcctgggcaccaccaacagcacctgccacccccctcagcctgcagagcggcatcctgac
 cctgaaccccgctggccaggccagccatcctgaccagcctgggcagcaaccaggaggagcctacgtgacctga
 gcagcttctaccagaaccag

Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 85)

30 NWNVNISDLKKIEDLIQSMHIDATLYTESDVHPSCKV TAMKCF LLELQVISLES
 GDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQ
 MFINTSSGGSGGGSGGGSGGGSGGGSLQITCPPPMSVEHADIWVKSYS
 LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTPSLKCIRDPALVHQR

APPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAIVPGSQLMPSKSPS
 TGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTPILLMCPTI
 SILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSN
 PESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLESEKQRLGGDVQSPNCPSE
 5 DVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNNGPHVYQDLLL
 SLGTTNSTLPPPSLQSGILTLPVAQGGPILTSLSNQEAYVTMSSFYQNNQ

Nucleic Acid Encoding Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 86)

10 aactgggtgaatgtgatcagcgacctgaagaagatcaggatctgatccagagcatgcacattgatgccacctgtacaca
 gaatctgatgtgaccctagctgtaaagtaccgccatgaagtgtttctgctggagctgcaggtgatttctctggaagcgg
 agatgcctctatccagcacagtgaggaaatctgatcatcctggccaacaatagcctgagcagaatggcaatgtacaga
 gtctggctgtaaggagtgtgaggagctggaggagaagaacatcaaggagttctgcagagcfttgcacatcgtgcagat
 gttcatcaatacaagcagcgggtgggggctcaggcgaggaggctctggcgaggcggaagcggggagggggctca
 15 ggcggcgggtccttcagattacatgccctccccaatgtctgtggagcacgccgataattgggtgaagtctacagcctgt
 acagcagagagatacatctgcaacagcggctttaagagaaaggccggcacctcttctgacagagtgcgtgctgaat
 aaggccacaaatgtgcccactggacaacacctagcctgaagtgcattagagatcctgccctgtccaccagaggcctgc
 cctccatctacagtacaacagccgagtacacctcagcctgaatctctgagcccttggaaaagaacctgccgccag
 ctctcctagctctaataataccgccccacaacagccgcattgtgcctggatctcagctgatgcctagcaagtctcctagca
 20 caggcacaacagagatcagcagccacgaatctctcacggaacaccttctcagaccaccgccaagaattgggagctgac
 agcctctgcctctcaccagcctcaggagtgtatctcagggccactctgatacaacacccatcctgctgatgtgcccacc
 atcagcatcctgagcttctcagcgtggccctgctggatcctggcctgcgtgctgtggaagaagaggatcaagccatc
 gtgtggccagcctgcccaccacaagaagacctggagcacctgtgtaagaagcccaggaagaacctgaacgtgagc
 ttaaccccagagcttctgactgccagatccacagggtggacgacatccaggccaggagcaggtggagggttcc
 25 tgcaggacaccttccccagcagctggaggagagcgagaagcagaggctggcgcgacgtgcagagcccaactgc
 cccagcaggacgtggtgatcccccgagagcttggcaggacagcagcctgacctgcctggccgcaacgtgag
 cgctgcgacgccccatcctgagcagcagcaggagcctggactgcaggagagcggaagaacggccccacgtgt
 accaggacctgctgctgagcctgggcaccaccaacagcacctgccacccccctcagcctgcagagcggcatcctgac
 cctgaaccccgtggcccaggccagccatcctgaccagcctgggcagcaaccaggaggaggcctacgtgacatga
 30 gcagcttctaccagaaccag

Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 87)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLEELQVISLES
 GDASIHDVTENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQ
 MFINTSSGGSGGGSGGGSGGGSGGGSLQITCPPPMSVEHADIWVKSYS
 LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRP
 5 APPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPS
 TGTTEISSHESSHGTPSQTAKNWELTASASHQPPGVYPQGHSDTTPILLTISILS
 FFSVEKAVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESF
 LDCQIHRVDDIQRDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVI
 TPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGT
 10 TNSTLPPPSLQSGILTLPVAQQQPILTSLSNQEEAYVTMSSFYQNQ

Nucleic Acid Encoding Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 88)

aactgggtgaatgtgatcagcgacctgaagaagatcaggatctgatccagagcatgcacattgatgccacctgtacaca
 15 gaatctgatgtgcaccctagctgtaaagtaccgcatgaagtgtttctgctggagctgcaggtgatttctctgaaagcgg
 agatgcctctatccagacacagtgaggagaatctgatcatcctggccaacaatagcctgagcagcaatggcaatgtgacaga
 gtctggctgtaaggagtgtgaggagctggaggagaagaacatcaaggagtttctgcagagctttgtgcacatcgtgcagat
 gtcatcaataacaagcagcgggtgggggctcaggcggaggagctctggcggaggcgggaagcgggggagggggctca
 ggcggcgggtccttgcaattacatgccctcctccaatgtctgtggagcacgccgatattgggtgaagtctacagcctgt
 20 acagcagagagatacatctgcaacagcggcttaagagaaaggccggcaccttctctgacagagtgcgtgctgaat
 aaggccacaaatgtggccactggacaacacctagcctgaagtgcattagagatcctgccctggccaccagaggcctgc
 cctccatctacagtgcacaacagccggagtgcacctcagcctgaatctctgagccctctggaaaagaacctgccgcca
 ctctcctagcttaataataccgccccacaacagccgcatgtgctggatctcagctgatgcctagcaagtctcctagca
 caggcacaacagagatcagcagccacgaatctctcacggaacaccttctcagaccaccgccaagaattgggagctgac
 25 agcctctgcctctcaccagcctcaggagtgtatcctcagggccactctgataaacacccatcctgctgacctcagcatc
 ctgagcttctcagcgtggagaagggcgtgatcctggcctgctgctgtggaagaagaggatcaagccatcgtgtggcc
 cagcctgcccaccacaagaagacctggagcacctgtgtaagaagcccagggaagaacctgaacgtgagcttaaccc
 cgagagcttctggactgccagatccacagggtggacgacatccaggccaggagcaggtggagggttctctgcagga
 caccttccccagcagctggaggagagcgaagcagaggctgggcggcgacgtgcagagcccaactgccccagc
 30 gaggacgtggtgatcccccgagagcttcggcaggagacagcagcctgacctgcctggcggcaacgtgagcgctgc
 gacccccatcctgagcagcagcaggagcctggactgcaggagagcggcaagaacggccccacgtgtaccagg
 acctgctgctgagcctgggcaccaccaacagcacctgccacccccctcagcctgcagagcggcatcctgacctgaa

ccccgtggccaggccagcccatcctgaccagcctgggcagcaaccaggaggaggcctacgtgacatgagcagctt
ctaccagaaccag

Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 89)

5 NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES
GDASIHDTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQ
MFINTSSGGSGGGSGGGSGGGSGGGSLQITCPPPMSEHADIWVKSYS
LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQR
APPSTVTTAGVTQPESLSPSGKEPAASSPSSNNTAATTAIVPGSQLMPSKSPS
10 TGTTEISSHESHGTPSQTAKNWELTASASHQPPGVYPQGHSDTTPILLTISILS
FFSVEKVVLACVLWKKRIKPIVWPSLPDHKKTLLEHLCKKPRKNLNVSFNPES
FLDCQIHRVDDIQARDEVEGFLQDTFPQQLSESEKQRLGGDVQSPNCPSEDVV
ITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSLG
TTNSTLPPPFSLQSGILTLPVAQGGPILTSLSGNQEEAYVTMSSFYQNNQ

15

Nucleic Acid Encoding Exemplary Chimeric Transmembrane Protein (SEQ ID NO: 90)

aactgggtgaatgtgatcagcgacctgaagaagatcgaggatctgatccagagcatgcacattgatccaccctgtacaca
gaatctgatgtgcaccctagctgtaaagtaccgcatgaagtgtttctgctggagctgcaggtgatttcttgaaagcgg
20 agatgcctctatccacgacacagtgaggagaatctgatcatcctggccaacaatagcctgagcagcaatggcaatgtgacaga
gtctggctgtaaggagtgtaggagctggaggagaagaacatcaaggagtctgagagctttgtgcacatcgtgcagat
gttcatcaatacaagcagcggtgggggctcaggcgaggaggctctggcgaggcggaagcgggggagggggctca
ggcggcgggtccttgagattacatgccctcctccaatgtctgtggagcacgcccgaatttgggtgaagtctacagcctgt
acagcagagagagatacatctgcaacagcggctttaagagaaaggccggcacctcttctgacagagtgctgtgctgaat
25 aaggccacaaatgtggccactggacaacacctagcctgaagtgcattagagatcctgccctggtcaccagagggcctgc
ccctccatctacagtacaacagcggagtgcacctcagcctgaatctctgagcccttctggaaaagaacctgccgccag
ctctcctagctctaataataccgccgccacaacagccgcatgtgcctggatctcagctgatgcctagcaagtctcctagca
caggcacaacagagatcagcagccacgaatctctcacggaacaccttctcagaccaccgccaagaattgggagctgac
agcctctgcctctcaccagcctcaggagtgatcctcaggccactctgatacaacacctcctgtgacctcagcatc
30 ctgagcttctcagcgtggagaaggtggtgatcctggcctgctgctgtggaagaagaggatcaagcccatcgtgtggccc
agcctgcccgaccacaagaagacctggagcacctgtgtaagaagcccaggaagaacctgaacgtgagcttcaacccc
gagagcttctggactgccagatccacagggtggacgacatccaggccaggagcaggtggagggtctcctcaggac
accttccccagcagctggaggagagcgagaagcagaggctggcgcgacgtgcagagcccaactgccccagcg

aggacgtggtgatcccccgagagcttcggcagggacagcagcctgacctgcctggccggcaacgtgagcgctgcg
 acgccccatcctgagcagcagcaggagcctggactgcagggagagcggcaagaacggccccacgtgtaccaggac
 ctgctgctgagcctgggcaccaccaacagcaccctgccaccccccttcagcctgcagagcggcatcctgaccctgaacc
 ccgtggcccagggccagccatcctgaccagcctgggcagcaaccaggaggagcctacgtgacctgagcagcttct
 5 accagaaccag

In some embodiments, the chimeric transmembrane protein comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 19, 83, 85, 87, or 89. In some embodiments, a chimeric transmembrane protein includes a sequence that differs from
 10 SEQ ID NO: 19, 83, 85, 87, or 89 by one to about 100 amino acids (e.g., 1 to about 95 amino acids, 1 to about 90 amino acids, 1 to about 80 amino acids, 1 to about 75 amino acids, 1 to about 70 amino acids, 1 to about 65 amino acids, 1 to about 60 amino acids, 1 to about 55 amino acids, 1 to about 50 amino acids, 1 to about 45 amino acids, 1 to about 40 amino acids, 1 to about 35 amino acids, 1 to about 30 amino acids, 1 to about 25 amino acids, 1 to about 20 amino acids, 1 to about 15 amino acids, 1 to about 10 amino acids, 1 to about 5 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 95 amino acids, about 5 amino acids to about 90 amino acids, about 5 amino acids to about 85 amino acids, about 5 amino acids to about 80 amino acids, about 5 amino acids to about 75 amino acids,
 25 about 5 amino acids to about 70 amino acids, about 5 amino acids to about 65 amino acids, about 5 amino acids to about 60 amino acids, about 5 amino acids to about 55 amino acids, about 5 amino acids to about 50 amino acids, about 5 amino acids to about 45 amino acids, about 5 amino acids to about 40 amino acids, about 5 amino acids to about 35 amino acids, about 5 amino acids to about 30 amino acids, about 5 amino acids to about 25 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 15 amino acids, or about 5 amino acids to about 10 amino acids). In some embodiments, , a chimeric transmembrane protein includes 1 to about 300 additional amino acids (e.g., 1 to about 250 amino acids, 1 to about 200

amino acids, 1 to about 150 amino acids, 1 to about 100 amino acids, 1 to about 50 amino acids, 1 to about 25 amino acids, about 5 to about 300 amino acids, about 5 to about 250 amino acids, about 5 to about 200 amino acids, about 5 to about 150 amino acids, about 5 to about 100 amino acids, about 5 to about 50 amino acids, about 10 to about 300 amino acids, about 10 to about 250 amino acids, about 10 to about 200 amino acids, about 10 to about 150 amino acids, about 10 to about 100 amino acids, or about 10 to about 50 amino acids), e.g., in addition to the sequence of SEQ ID NO: 19, 83, 85, 87, or 89. In some embodiments, the chimeric transmembrane protein comprises a sequence of SEQ ID NO: 19, 83, 85, 97, or 89 having one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) removed from the N-terminus of SEQ ID NO: 19, 83, 85, 87, or 89 and/or one to twenty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) removed from the C-terminus of SEQ ID NO: 19, 83, 85, 87, or 89.

In some embodiments, a nucleic acid encoding a chimeric transmembrane protein comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 20, 84, 86, 88, or 90. In some embodiments, a nucleic acid encoding a chimeric transmembrane protein includes a sequence that differs from SEQ ID NO: 20, 84, 86, 88, or 90 by 1 to about 300 nucleotides (e.g., 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, 1 to about 50 nucleotides, about 5 nucleotides to about 300 nucleotides, about 5 nucleotides to about 250 nucleotides, about 5 nucleotides to about 200 nucleotides, about 5 nucleotides to about 150 nucleotides, about 5 nucleotides to about 100 nucleotides, about 5 nucleotides to about 50 nucleotides, about 10 nucleotides to about 300 nucleotides, about 10 nucleotides to about 250 nucleotides, about 10 nucleotides to about 200 nucleotides, about 10 nucleotides to about 150 nucleotides, about 10 nucleotides to about 100 nucleotides, or about 10 nucleotides to about 50 nucleotides). In some embodiments, a nucleic acid encoding a chimeric transmembrane protein includes one or more additional nucleotides (e.g., 1 to about 900 nucleotides, 1 to about 850 nucleotides, 1

to about 800 nucleotides, 1 to about 750 nucleotides, 1 to about 700 nucleotides, 1 to about 650 nucleotides, 1 to about 600 nucleotides, 1 to about 550 nucleotides, 1 to about 500 nucleotides, 1 to about 450 nucleotides, 1 to about 400 nucleotides, 1 to about 350 nucleotides, 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, or 1 to about 50 nucleotides), e.g., in addition to SEQ ID NO: 20, 84, 86, 88, or 90.

Additionally or alternatively, a nucleic acid encoding the chimeric transmembrane protein can lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 20, 84, 86, 88, or 90 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 20, 84, 86, 88, or 90.

In some embodiments, the chimeric transmembrane protein (e.g., mature or precursor protein) can be about 210 amino acids to about 650 amino acids, about 210 amino acids to about 640 amino acids, about 210 amino acids to about 620 amino acids, about 210 amino acids to about 600 amino acids, about 210 amino acids to about 580 amino acids, about 210 amino acids to about 560 amino acids, about 210 amino acids to about 540 amino acids, about 210 amino acids to about 520 amino acids, about 210 amino acids to about 500 amino acids, about 210 amino acids to about 480 amino acids, about 210 amino acids to about 460 amino acids, about 210 amino acids to about 440 amino acids, about 210 amino acids to about 420 amino acids, about 210 amino acids to about 400 amino acids, about 210 amino acids to about 380 amino acids, about 210 amino acids to about 360 amino acids, about 210 amino acids to about 340 amino acids, about 210 amino acids to about 320 amino acids, about 210 amino acids to about 300 amino acids, about 210 amino acids to about 280 amino acids, about 210 amino acids to about 260 amino acids, about 210 amino acids to about 240 amino acids, about 220 amino acids to about 650 amino acids, about 220 amino acids to about 640 amino acids, about 220 amino acids to

[illegible]

about 380 amino acids, about 260 amino acids to about 360 amino acids, about 260
amino acids to about 340 amino acids, about 260 amino acids to about 320 amino
acids, about 260 amino acids to about 300 amino acids, about 260 amino acids to
about 280 amino acids, about 280 amino acids to about 650 amino acids, about 280
5 amino acids to about 640 amino acids, about 280 amino acids to about 620 amino
acids, about 280 amino acids to about 600 amino acids, about 280 amino acids to
about 580 amino acids, about 280 amino acids to about 560 amino acids, about 280
amino acids to about 540 amino acids, about 280 amino acids to about 520 amino
acids, about 280 amino acids to about 500 amino acids, about 280 amino acids to
10 about 480 amino acids, about 280 amino acids to about 460 amino acids, about 280
amino acids to about 440 amino acids, about 280 amino acids to about 420 amino
acids, about 280 amino acids to about 400 amino acids, about 280 amino acids to
about 380 amino acids, about 280 amino acids to about 360 amino acids, about 280
amino acids to about 340 amino acids, about 280 amino acids to about 320 amino
15 acids, about 280 amino acids to about 300 amino acids, about 300 amino acids to
about 650 amino acids, about 300 amino acids to about 640 amino acids, about 300
amino acids to about 620 amino acids, about 300 amino acids to about 600 amino
acids, about 300 amino acids to about 580 amino acids, about 300 amino acids to
about 560 amino acids, about 300 amino acids to about 540 amino acids, about 300
20 amino acids to about 520 amino acids, about 300 amino acids to about 500 amino
acids, about 300 amino acids to about 480 amino acids, about 300 amino acids to
about 460 amino acids, about 300 amino acids to about 440 amino acids, about 300
amino acids to about 420 amino acids, about 300 amino acids to about 400 amino
acids, about 300 amino acids to about 380 amino acids, about 300 amino acids to
25 about 360 amino acids, about 300 amino acids to about 340 amino acids, about 300
amino acids to about 320 amino acids, about 320 amino acids to about 650 amino
acids, about 320 amino acids to about 640 amino acids, about 320 amino acids to
about 620 amino acids, about 320 amino acids to about 600 amino acids, about 320
amino acids to about 580 amino acids, about 320 amino acids to about 560 amino
30 acids, about 320 amino acids to about 540 amino acids, about 320 amino acids to
about 520 amino acids, about 320 amino acids to about 500 amino acids, about 320
amino acids to about 480 amino acids, about 320 amino acids to about 460 amino
acids, about 320 amino acids to about 440 amino acids, about 320 amino acids to

[illegible]

about 560 amino acids, about 400 amino acids to about 540 amino acids, about 400
amino acids to about 520 amino acids, about 400 amino acids to about 500 amino
acids, about 400 amino acids to about 480 amino acids, about 400 amino acids to
about 460 amino acids, about 400 amino acids to about 440 amino acids, about 400
5 amino acids to about 420 amino acids, about 420 amino acids to about 650 amino
acids, about 420 amino acids to about 640 amino acids, about 420 amino acids to
about 620 amino acids, about 420 amino acids to about 600 amino acids, about 420
amino acids to about 580 amino acids, about 420 amino acids to about 560 amino
acids, about 420 amino acids to about 540 amino acids, about 420 amino acids to
10 about 520 amino acids, about 420 amino acids to about 500 amino acids, about 420
amino acids to about 480 amino acids, about 420 amino acids to about 460 amino
acids, about 420 amino acids to about 440 amino acids, about 440 amino acids to
about 650 amino acids, about 440 amino acids to about 640 amino acids, about 440
amino acids to about 620 amino acids, about 440 amino acids to about 600 amino
15 acids, about 440 amino acids to about 580 amino acids, about 440 amino acids to
about 560 amino acids, about 440 amino acids to about 540 amino acids, about 440
amino acids to about 520 amino acids, about 440 amino acids to about 500 amino
acids, about 440 amino acids to about 480 amino acids, about 440 amino acids to
about 460 amino acids, about 460 amino acids to about 650 amino acids, about 460
20 amino acids to about 640 amino acids, about 460 amino acids to about 620 amino
acids, about 460 amino acids to about 600 amino acids, about 460 amino acids to
about 580 amino acids, about 460 amino acids to about 560 amino acids, about 460
amino acids to about 540 amino acids, about 460 amino acids to about 520 amino
acids, about 460 amino acids to about 500 amino acids, about 460 amino acids to
25 about 480 amino acids, about 480 amino acids to about 650 amino acids, about 480
amino acids to about 640 amino acids, about 480 amino acids to about 620 amino
acids, about 480 amino acids to about 600 amino acids, about 480 amino acids to
about 580 amino acids, about 480 amino acids to about 560 amino acids, about 480
amino acids to about 540 amino acids, about 480 amino acids to about 520 amino
30 acids, about 480 amino acids to about 500 amino acids, about 500 amino acids to
about 650 amino acids, about 500 amino acids to about 640 amino acids, about 500
amino acids to about 620 amino acids, about 500 amino acids to about 600 amino
acids, about 500 amino acids to about 580 amino acids, about 500 amino acids to

about 560 amino acids, about 500 amino acids to about 540 amino acids, about 500 amino acids to about 520 amino acids, about 520 amino acids to about 650 amino acids, about 520 amino acids to about 640 amino acids, about 520 amino acids to about 620 amino acids, about 520 amino acids to about 600 amino acids, about 520 amino acids to about 580 amino acids, about 520 amino acids to about 560 amino acids, about 520 amino acids to about 540 amino acids, about 540 amino acids to about 650 amino acids, about 540 amino acids to about 640 amino acids, about 540 amino acids to about 620 amino acids, about 540 amino acids to about 600 amino acids, about 540 amino acids to about 580 amino acids, about 540 amino acids to about 560 amino acids, about 560 amino acids to about 650 amino acids, about 560 amino acids to about 640 amino acids, about 560 amino acids to about 620 amino acids, about 560 amino acids to about 600 amino acids, about 560 amino acids to about 580 amino acids, about 580 amino acids to about 650 amino acids, about 580 amino acids to about 640 amino acids, about 580 amino acids to about 620 amino acids, about 580 amino acids to about 600 amino acids, about 600 amino acids to about 650 amino acids, about 600 amino acids to about 640 amino acids, about 600 amino acids to about 620 amino acids, about 620 amino acids to about 650 amino acids, about 620 amino acids to about 640 amino acids, or about 630 amino acids to about 650 amino acids, in length.

In some embodiments, the chimeric transmembrane protein further comprises a signal sequence at its N-terminus. In some embodiments, the signal sequence comprises or is the sequence of MDWTWILFLVAAATRVHS (SEQ ID NO: 21). In some embodiments, the nucleic acid encoding the signal sequence comprises or is the sequence atggattggacctggattctgtttctggtggccgctgccacaagagtgcacagc (SEQ ID NO: 91). Additional examples of signal sequences are known in the art. For example, a signal sequence can be about 5 amino acids to about 30 amino acids, about 5 amino acids to about 28 amino acids, about 5 amino acids to about 26 amino acids, about 5 amino acids to about 24 amino acids, about 5 amino acids to about 22 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 18 amino acids, about 5 amino acids to about 16 amino acids, about 5 amino acids to about 14 amino acids, about 5 amino acids to about 12 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 8 amino acids, about 6 amino acids to about 30 amino acids, about 6 amino acids to about 28 amino acids, about 6 amino

[illegible]

22 amino acids, about 18 amino acids to about 20 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 28 amino acids, about 20 amino acids to about 26 amino acids, about 20 amino acids to about 24 amino acids, about 20 amino acids to about 22 amino acids, about 22 amino acids to about 30 amino acids, about 22 amino acids to about 28 amino acids, about 22 amino acids to about 26 amino acids, about 22 amino acids to about 24 amino acids, about 24 amino acids to about 30 amino acids, about 24 amino acids to about 28 amino acids, about 24 amino acids to about 26 amino acids, about 26 amino acids to about 30 amino acids, about 26 amino acids to about 28 amino acids, or about 28 amino acids to about 30 amino acids, in length.

In some embodiments, the chimeric transmembrane protein can further include a peptide tag. For example, a tag can be used to help facilitate purification, production, and/or identification of the chimeric transmembrane protein. In some embodiments, the tag is a histidine tag comprising at least six histidine residues. Additional examples of tags are known in the art.

Non-limiting aspects of any of the chimeric transmembrane proteins provided herein are described below.

Extracellular IL-15 Domains

In some embodiments of any of the chimeric transmembrane proteins described herein, the extracellular IL-15 domain comprises or is a sequence of a wildtype IL-15 protein (e.g., a mature wildtype IL-15 protein). For example, the wildtype IL-15 protein can be a wildtype human IL-15 protein (e.g., a mature wildtype human IL-2 protein), a wildtype mouse IL-15 protein (e.g., a mature wildtype mouse IL-15 protein), a wildtype chimpanzee IL-15 protein (e.g., a mature wildtype chimpanzee IL-15 protein), or a wildtype monkey IL-15 protein (e.g., a mature wildtype monkey IL-15 protein). Non-limiting examples of wildtype IL-15 protein sequences and nucleic acids encoding these exemplary IL-15 protein sequences are provided below.

Exemplary Human IL-15 Isoform 1 (SEQ ID NO: 22)

NWNVVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLES GDASIHDT
VENLIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINTS

Nucleic Acid Encoding Human IL-15 Isoform 1 (SEQ ID NO: 23)

5 aactgggtgaatgtaataagtgatttgaaaaaattgaagatcttattcaatctatgcatat
 tgatgctactttatatacggaagtgatgttcacccagttgcaaagtaacagcaatgaagt
 gctttctcttgaggttacaagttatttcacttgagtcggagatgcaagtattcatgataca
 gtagaaaatctgatcatcctagcaaacaacagtttgcttctaatgggaatgtaacagaatc
 tggatgcaaagaatgtgaggaactggaggaaaaaataattaagaatTTTTgcagagttttg
 10 tacatattgtccaaatgttcatcaacacttct

Exemplary Human IL-15 Isoform 2 (SEQ ID NO: 24)

MVLGTIDLCSFSGAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK
 CFLLELQVISLES GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
 15 VHIVQMFINTS

Nucleic Acid Encoding Human IL-15 Isoform 2 (SEQ ID NO: 25)

atggatattggaaccatagatttgtgcagctgtttcagtgagggcttcctaaacagaagc
 caactgggtgaatgtaataagtgatttgaaaaaattgaagatcttattcaatctatgcata
 20 ttgatgctactttatatacggaagtgatgttcacccagttgcaaagtaacagcaatgaag
 tgctttctcttgaggttacaagttatttcacttgagtcggagatgcaagtattcatgatac
 agtagaaaatctgatcatcctagcaaacaacagtttgcttctaatgggaatgtaacagaat
 ctggatgcaaagaatgtgaggaactggaggaaaaaataattaagaatTTTTgcagagtttt
 gtacatattgtccaaatgttcatcaacacttcttga
 25

Exemplary Mouse IL-15 Variant A (SEQ ID NO: 26)

NWIDVRYDLEKIESLIQSIHIDTTLYTDSDFHPSCKVTAMNCFLELQVILHEYSNMTLNET
 VRNVLYLANSTLSSKNVAESGCKECEELEEKTFTEFLQSFIRIVQMFINTS

Nucleic Acid Encoding Mouse IL-15 Variant A (SEQ ID NO: 27)

aactggatagatgtaagatatgacctggagaaaattgaaagccttattcaatctattcat
 attgacaccactttatacactgacagtgactttcatccagttgcaaagttactgcaatgaa
 ctgctttctcctggaattgcagggtatttttacatgagtacagtaacatgactcttaataaaa
 cagtaagaaacgtgctctaccttgcaaacagcactctgtcttctaacaagaatgtagcagaa
 35 tctggctgcaaggaatgtgaggagctggaggagaaaaccttcacagagtttttgcaaagctt
 tatacgcatattgtccaaatgttcatcaacacgtcc

Exemplary Mouse IL-15 Variant B (SEQ ID NO: 28)

5 NWIDVRYDLEKIESLIQSIHIDTTLTYTDSDFHPSCCKVTAMNCFLELQVILHEYSNMTLNET
 VRNVLYLANSTLSSNKNVAESGCKECELEEKTFTEFLQSFIRIVQMFINTS

Nucleic Acid Encoding Mouse IL-15 Variant B (SEQ ID NO: 29)

10 aactggatagatgtaagatatgacctggagaaaattgaaagccttattcaatctattcatat
 tgacaccactttatacactgacagtgcactttcatcccagttgcaaagttactgcaatgaact
 gctttctcctggaattgcagggtattttacatgagtacagtaacatgactcttaatgaaaca
 gtaagaaacgtgctctaccttgcaaacagcactctgtcttctaacaagaatgtagcagaatc
 tggctgcaaggaatgtgaggagctggaggagaaaaccttcacagagtttttgcaaagcttta
 tacgcattgtccaaatgttcacacacgtcc

15

 In some embodiments, the extracellular IL-15 domain comprises or is a
 sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at
 least 75% identical, at least 80% identical, at least 82% identical, at least 84%
 identical, at least 85%, at least 86% identical, at least 88% identical, at least 90%
 identical, at least 91% identical, at least 92% identical, at least 93% identical, at least
 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at
 least 98% identical, at least 99% identical, or 100% identical to a wildtype IL-15
 protein (e.g., a wildtype mature IL-15 protein, e.g., SEQ ID NO: 22, 24, 26, or 28). In
 some embodiments, an extracellular IL-15 domain includes a sequence that differs
 from SEQ ID NO: 22, 24, 26, or 28 by one to 25 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7,
 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids). In
 some embodiments, an extracellular IL-15 domain includes one or more additional
 amino acids (e.g., 1 to about 100 amino acids, 1 to about 80 amino acids, 1 to about
 60 amino acids, 1 to about 40 amino acids, 1 to about 20 amino acids, 1 to about 10
 amino acids, about 5 to about 100 amino acids, about 5 to about 80 amino acids, about
 5 to about 60 amino acids, about 5 to about 40 amino acids, about 5 to about 20 amino
 acids, about 10 to about 100 amino acids, about 10 to about 80 amino acids, about 10
 to about 60 amino acids, about 10 to about 40 amino acids, or about 10 to about 20
 amino acids), e.g., in addition to the sequence of SEQ ID NO: 22, 24, 26, or 28.

Additionally or alternatively, an extracellular IL-15 domain can lack one to about 25 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids) as compared to the sequence of SEQ ID NO: 22, 24, 26, or 28.

5 In some embodiments, a nucleic acid encoding an extracellular IL-15 domain comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 23, 25, 27, or 29. In some
10 embodiments, a nucleic acid encoding an extracellular IL-15 domain includes a sequence that differs from SEQ ID NO: 23, 25, 27, or 29 by one to about 75 nucleotides (e.g., 1 to about 70 nucleotides, 1 to about 60 nucleotides, 1 to about 50 nucleotides, 1 to about 40 nucleotides, 1 to about 30 nucleotides, 1 to about 20
15 nucleotides, 1 to about 10 nucleotides, about 5 nucleotides to about 75 nucleotides, about 5 nucleotides to about 70 nucleotides, about 5 nucleotides to about 60 nucleotides, about 5 nucleotides to about 50 nucleotides, about 5 nucleotides to about 40 nucleotides, about 5 nucleotides to about 30 nucleotides, about 5 nucleotides to about 20 nucleotides, about 5 nucleotides to about 10 nucleotides). In some
20 embodiments, a nucleic acid encoding an extracellular IL-15 domain includes one or more additional nucleotides (e.g., 1 to about 300 nucleotides, 1 to about 250 nucleotides, 1 to about 200 nucleotides, 1 to about 150 nucleotides, 1 to about 100 nucleotides, 1 to about 50 nucleotides, about 5 nucleotides to about 300 nucleotides, about 5 nucleotides to about 250 nucleotides, about 5 nucleotides to about 200
25 nucleotides, about 5 nucleotides to about 150 nucleotides, about 5 nucleotides to about 100 nucleotides, or about 5 nucleotides to about 50 nucleotides), e.g., in addition to the sequence of SEQ ID NO: 23, 25, 27, or 29. Additionally or alternatively, a nucleic acid encoding the extracellular IL-15 domain can lack one to
30 75 nucleotides (e.g., 1 to about 70 nucleotides, 1 to about 65 nucleotides, 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to about 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 5'-end of SEQ ID NO: 23,

25, 27, or 29 and/or lack one to sixty nucleotides (e.g., 1 to about 60 nucleotides, 1 to about 55 nucleotides, 1 to about 50 nucleotides, 1 to about 45 nucleotides, 1 to 40 nucleotides, 1 to about 35 nucleotides, 1 to about 30 nucleotides, 1 to about 25 nucleotides, 1 to about 20 nucleotides, 1 to about 15 nucleotides, 1 to about 10 nucleotides, or 1 to about 5 nucleotides) from the 3'-end of SEQ ID NO: 23, 25, 27, or 29.

As one skilled in the art can appreciate, when amino acids that are not conserved between wildtype IL-15 proteins from different species are mutated (e.g., substituted with a different amino acid) they are less likely to cause a decrease in the level of one or more activities of an IL-15 protein. In contrast, when amino acids that are conserved between wildtype IL-15 proteins from different species are mutated (e.g., substituted with a different amino acid) they are more likely to cause a decrease in the level of one or more activities of an IL-15 protein. In view of this knowledge, one skilled in the art can select which amino acid positions in a wildtype IL-15 protein (e.g., the non-conserved amino acids) can be substituted without decreasing the activity of the IL-15 protein.

In some embodiments, the extracellular IL-15 domain is a sequence of a wildtype IL-15 protein (e.g., a mature wildtype IL-15 protein, e.g., any of the mature wildtype IL-15 proteins described herein, e.g., SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28) having one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the N-terminus of the sequence of the wildtype IL-15 protein. In some embodiments, the extracellular IL-15 domain is a sequence of a wildtype IL-15 protein (e.g., a mature wildtype IL-15 protein, e.g., any of the mature wildtype IL-15 proteins described herein, e.g., mature wildtype human IL-15 protein, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28) having one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the C-terminus of the sequence of the wildtype IL-15 protein. In some embodiments, the extracellular IL-15 domain is a sequence of a wildtype IL-15 protein (e.g., a mature wildtype IL-15 protein, e.g., any of the mature wildtype IL-15 proteins described herein, e.g., mature wildtype human IL-15 protein, e.g., SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28) having both one to ten amino (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) acids removed from the N-terminus of the sequence of the wildtype IL-15 protein and

one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the C-terminus of the sequence of the wildtype IL-15 protein.

As will be appreciated by those of ordinary skill in the art, when a nucleic acid encoding a chimeric transmembrane protein having an extracellular IL-15 domain
 5 includes or lacks one or more additional nucleotides as compared to SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, or SEQ ID NO: 29, the translational reading frame should still be maintained such that a nonsense codon is not introduced and the full protein can be translated (e.g., a nucleic acid encoding a chimeric transmembrane protein having an extracellular IL-15 domain can include or lack three nucleotides, or
 10 multiples thereof, within the extracellular IL-15 portion of the chimeric transmembrane protein).

Alpha Chain of an IL-7 Receptor

Some embodiments of the chimeric transmembrane proteins described herein
 15 can further include a sequence of an alpha chain of an IL-7 receptor protein, or a portion thereof. For example, chimeric transmembrane proteins described herein can include a sequence of a wildtype alpha chain of an IL-7 receptor protein from a human, a mouse, a rat, a monkey, a chimpanzee, a pig, a dog, a cat, or any other appropriate species, or a portion thereof. Non-limiting examples of wildtype alpha
 20 chains of IL-7 receptors are described below. Additional examples of alpha chains of IL-7 receptors are known in the art. In some embodiments, chimeric transmembrane proteins including a sequence of an alpha chain of an IL-7 receptor, or a portion thereof, include a transmembrane domain of an alpha chain of an IL-7 receptor (e.g., any of the transmembrane domains of an alpha chain of an IL-7 receptor described
 25 herein).

Exemplary Wildtype Human IL-7 Receptor Alpha Chain (SEQ ID NO: 30)

ESGYAQNGDLEDAELDDYSFSCYSQLEVNQSQHSLTCAFEDPDVNITNLEFEICGALVEVKC
 LNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTITIVKPEAPFDLSVVYREGAN
 30 DFVVTENTSHLQKKYVKVLMHDVAYRQEKDENKWTHVNLSTKLTLLQRKLQPAAMYEIKVR
 SIPDHYFKGFSEWSPSYFRTPEINNSSGEMDPILLTISILSFFSVALLVILACVLWKKRI
 KPIVWPSLPDHKKTLLEHLCKPRKLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQ
 QLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRE

SGKNGPHVYQDLLLSLGTNSTLPPFSLQSGILTLNPVAQGQPILTSLGSNQEEAYVTMSS
FYQNQ

Nucleic Acid Encoding Wildtype Human IL-7 Receptor Alpha Chain (SEQ ID NO: 31)

5 gaaagtggctatgctcaaaatggagacttggagatgcagaactggatgactactcattctc
atgctatagccagttggaagtgaatggatcgagcactcactgacctgtgcttttgaggacc
cagatgtcaacatcaccaatctggaatttgaaatatgtggggccctcgtggaggtaaagtgc
ctgaatttcaggaaactacaagagatatatttcacgagacaaagaaattcttactgattgg
aaagagcaatatatgtgtgaaggttggagaaaagagtctaacctgcaaaaaatagacctaa
10 ccactatagttaaacctgaggctccttttgacctgagtgctcgtctatcggaaggagccaat
gactttgtggtgacatttaatacatcacacttgcaaaagaagtatgtaaaagttttaatgca
cgatgtagcttaccgccaggaaaaggatgaaaacaaatggacgcatgtgaatttatccagca
caaagctgacactcctgcagagaaagctccaaccggcagcaatgtatgagattaaagttcga
tccatccctgatcactattttaaaggcttctggagtgaatggagtccaagttattacttcag
15 aactccagagatcaataatagctcaggggagatggatcctatcttactaaccatcagcattt
tgagttttttctctgctcgtctgttggtcatcttggcctgtgtgttatggaaaaaaggatt
aagcctatcgtagtggccagctctcccgatcataagaagactctggaacatctttgtaagaa
accaagaaaaatttaaatgtgagtttcaatcctgaaagtttctggactgccagattcata
gggtggatgacattcaagctagagatgaagtgaagggttttctgcaagatacgtttctcag
20 caactagaagaatctgagaagcagaggcttggaggggatgtgcagagcccaactgcccatc
tgaggatgtagtcatcactccagaaagctttggaagagattcatccctcacatgcctggctg
ggaatgtcagtgcatgtgacgccccatttctctcctcttccaggtccctagactgcaggag
agtggcaagaatgggcctcatgtgtaccaggacctcctgcttagccttgggactacaaacag
cacgctgccccctccattttctctccaatctggaatcctgacattgaaccagttgctcagg
25 gtcagcccattcttacttccctgggatcaaatcaagaagaagcatatgtcaccatgtccagc
ttctaccaaaccag

Exemplary Wildtype Mouse IL-7 Receptor Alpha Chain Variant 1 (SEQ ID NO: 32)

30 ESGNAQDGDLEDADADDHSFWCHSQLEVDGSQHLLTCAFNDSDINTANLEFQICGALLRVKC
LTLNKLQDIYFIKTSEFLIGSSNICVKLGQKNLTCKNMAINTIVKAEAPSDLKVVRKEAN
DFLVTFNAPHLKKKYLKKVKHDVAYRPARGESNWTHTVSLFHTRTTIPQRKLRPKAMYKVR
SIPHNDYFKGFWSEWSPSSTFETPEPKNQGGWDPVLPVSVTILSLFSVFLLVILAHVLWKKRI
KPVVWPSLPDHKKTLQLCKPKTSLNVSFNPESFLDCQIHEVKGVEARDEVESFLPNDLPA
35 QPEELETQGHRAAVHSANRSPETSVSPPETVRRESPLRCLARNLSTCNAPLLSSRSPDYRD
GDRNRPPVYQDLLPNSGNTNVPVPVQPLPFQSGILIPVSQRQPISTSSVLNQEEAYVTMSS
FYQNK

Nucleic Acid Encoding Wildtype Mouse IL-7 Receptor Alpha Chain Variant 1 (SEQ ID NO: 33)

40

gaaagtggaaatgccaggatggagacctagaagatgcagacgcggacgatcactccttctg
 gtgccacagccagttggaagtggatggaagtcaacatttattgacttgtgcttttaaatgact
 cagacatcaacacagctaactctggaatttcaaatatgtggggctcttttacgagtgaatgc
 ctaactcttaacaagctgcaagatatataattttataaagacatcagaattcttactgattgg
 5 tagcagcaatatatgtgtgaagcttggacaaaagaatttaacttgcaaaaatatggctataa
 acacaatagttaaagccgaggtccctctgacctgaaagtcgtttatcgcaaagaagcaaat
 gatTTTTTgtgacatttaaatgcacctcacttgaaaaagaaatatTTAAAAAagtaaagca
 tgatgtggcctaccgccagcaaggggtgaaagcaactggacgcatgtatctttattccaca
 caagaacaacaatcccacagagaaaactacgaccaaagcaatgtatgaaatcaaagtccga
 10 tccattccccataacgattacttcaaaggcttctggagcgagtggagtccaagttctacctt
 cgaaactccagaacccaagaatcaaggaggatgggatcctgtcttgccaagtgtcaccattc
 tgagtttgttctctgtgtttttgttggtcatcttagcccatgtgctatggaaaaaaggatt
 aaacctgtcgatggcctagtctccccgatcataagaaaactctggaacaactatgtaagaa
 gccaaaaacgagtctgaatgtgagtttcaatcccgaagtttcttgactgccagattcatg
 15 aggtgaaaggcgttgaagccaggagcaggtggaagttttctgcccaatgatcttctctgca
 cagccagaggagttggagacacagggacacagagccgctgtacacagtgc aaaccgctcgcc
 tgagacttcagtcagcccaccagaaacagttagaagagagtcacccttaagatgcctggcta
 gaaatctgagtacctgcaatgccccctcactcctttcctctaggtccccctgactacagagat
 ggtgacagaaataggcctcctgtgtatcaagacttgctgcaaactctggaaacacaaatgt
 20 cctgtccctgtccctcaaccattgcctttccagtcgggaatcctgataccagtttctcaga
 gacagcccactctccacttcctcagtactgaatcaagaagaagcgtatgtcaccatgtctagt
 ttttaccaaaacaaa

Exemplary Wildtype Mouse IL-7 Receptor Alpha Chain Variant 2 (SEQ ID NO:

25 **34)**
 ESGNAQDGDLEDADADDHSFWSQLEVDGSQHLLTCAFNDSDINTANLEFQICGALLRVKC
 LTLNKLQDIYFIKTSEFLLIGSSNICVKGQKNLTCKNMAINTIVKAEAPSDLKVVRKEAN
 DFLVTFNAPHLKKKYLKKVKHDVAYRPARGESNWTHTVSLFHTRTTPQRKLRPKAMYEIKVR
 SIPHNDYFKGFWSSEWSPSTFETPEPKNQGGWDPVLPSTILSLFSVFLLVILAHVLWKKRI
 30 KPVVWPSLPDHKKTTLEQL

Nucleic Acid Encoding Mouse IL-7 Receptor Alpha Chain Variant 2 (SEQ ID NO: 35)

35 atgatggctctgggtagagctttcgctatagttttctgcttaattcaagctgtttctggagaaagtgga
 aatgccaggatggagacctagaagatgcagacgcggacgatcactccttctggtgccacagccagttg
 gaagtggatggaagtcaacatttattgacttgtgcttttaagtactcagacatcaacacagctaactctg
 gaatttcaaatatgtggggctcttttacgagtgaatgcctaactcttaacaagctgcaagatatatat

tttataaagacatcagaattcttactgattggtagcagcaatataatgtgtgaagcttggacaaaagaat
 ttaacttgcaaaaatatggctataaacacaatagttaaagccgaggtccctctgacctgaaagtcgtt
 tatcgcaaagaagcaaatgattttttggtgacatttaatgcacctcacttgaaaaagaatatttaaaa
 aaagtaaagcatgatgtggcctaccgcccagcaaggggtgaaagcaactggacgcatgtatctttattc
 5 cacacaagaacaacaatcccacagagaaaaactacgaccaaagcaatgtatgaaatcaaagtccgatcc
 attccccataacgattacttcaaaggcttctggagcagtgagggtccaagttctaccttcgaaactcca
 gaacccaagaatcaaggaggatgggatcctgtcttgccaagtgtcaccattctgagtttggtctctgtg
 tttttgttggtcatcttagcccatgtgctatggaaaaaaggattaaacctgtcgatggcctagtctc
 cccgatcataagaaaaactctggaacaactatag

10

Some embodiments of any of the chimeric transmembrane proteins provided
 herein can include, e.g., a sequence that is at least 60% identical, at least 65%
 identical, at least 70% identical, at least 75% identical, at least 80% identical, at least
 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88%
 15 identical, at least 90% identical, at least 91% identical, at least 92% identical, at least
 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to
 SEQ ID NO: 30, 32 or 34, or a portion thereof. Some embodiments of any of the
 chimeric transmembrane proteins provided herein can include, e.g., a sequence that
 20 differs from SEQ ID NO: 30, 32 or 34 by one to 60 amino acids (e.g., 1, 2, 3, 4, 5, 6,
 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,
 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53,
 54, 55, 56, 57, 58, 59, or 60 amino acids). Some embodiments of any of the chimeric
 transmembrane proteins described herein can further include, e.g., a sequence that
 25 differs from SEQ ID NO: 30, 32 or 34 by lacking one to 60 amino acids (e.g., 1, 2, 3,
 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51,
 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids).

In some embodiments, a nucleic acid encoding an alpha chain of an IL-7
 30 receptor comprises or is a sequence that is 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 91% identical, at least 92% identical, at least 94% identical, at
 least 95% identical, at least 96% identical, at least 97% identical, at least 98%
 identical, at least 99% identical, or 100% identical to SEQ ID NO: 31, 33, or 35. In
 35 some embodiments, a nucleic acid encoding an alpha chain of an IL-7 receptor
 includes a sequence that differs from SEQ ID NO: 31, 33, or 35 by one or more

nucleotides (e.g., 1 to about 180 nucleotides, 1 to about 160 nucleotides, 1 to about 140 nucleotides, 1 to about 120 nucleotides, 1 to about 100 nucleotides, 1 to about 80 nucleotides, 1 to about 60 nucleotides, 1 to about 40 nucleotides, 1 to about 20 nucleotides, about 5 to about 180 nucleotides, about 5 to about 180 nucleotides, about 5 to about 160 nucleotides, about 5 to about 160 nucleotides, about 5 to about 140 nucleotides, about 5 to about 120 nucleotides, about 5 to about 100 nucleotides, about 5 to about 80 nucleotides, about 5 to about 60 nucleotides, about 5 to about 40 nucleotides, or about 5 to about 20 nucleotides). Additionally or alternatively, a nucleic acid encoding an alpha chain of an IL-7 receptor can lack one or more nucleotides (e.g., 1 to about 180 nucleotides, 1 to about 160 nucleotides, 1 to about 140 nucleotides, 1 to about 120 nucleotides, 1 to about 100 nucleotides, 1 to about 80 nucleotides, 1 to about 60 nucleotides, 1 to about 40 nucleotides, 1 to about 20 nucleotides, about 5 to about 180 nucleotides, about 5 to about 180 nucleotides, about 5 to about 160 nucleotides, about 5 to about 160 nucleotides, about 5 to about 140 nucleotides, about 5 to about 120 nucleotides, about 5 to about 100 nucleotides, about 5 to about 80 nucleotides, about 5 to about 60 nucleotides, about 5 to about 40 nucleotides, or about 5 to about 20 nucleotides) as compared to the sequence of SEQ ID NO: 31, 33, or 35.

As one skilled in the art can appreciate, when amino acids that are not conserved between wildtype alpha chains of IL-7 receptor proteins from different species are mutated (e.g., substituted with a different amino acid) they are less likely to cause a decrease in the level of one or more activities of an alpha chain of an IL-7 receptor protein. In contrast, when amino acids that are conserved between wildtype alpha chains of alpha chains of IL-7 receptor proteins from different species are mutated (e.g., substituted with a different amino acid) they are more likely to cause a decrease in the level of one or more activities of an alpha chain of an alpha chain of an IL-7 receptor protein. In view of this knowledge, one skilled in the art can select which amino acid positions in a wildtype alpha chain of an alpha chain of an IL-7 receptor protein (e.g., the non-conserved amino acids) can be substituted without decreasing the activity of the alpha chain of an alpha chain of an IL-7 receptor protein.

Some embodiments of any of the chimeric transmembrane proteins described herein can include the sequence of a wildtype alpha chain of IL-7 receptor protein

(e.g., a mature wildtype alpha chain of IL-7 receptor protein, e.g., any of the mature wildtype alpha chains of IL-7 receptor described herein, e.g., mature wildtype human alpha chain of IL-7 receptor protein, e.g., SEQ ID NO: 30) having one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the N-terminus of the sequence of the wildtype alpha chain of IL-7 receptor protein. Some embodiments of any of the chimeric transmembrane proteins described herein can include the sequence of a wildtype alpha chain of IL-7 receptor protein (e.g., a mature wildtype alpha chain of IL-7 receptor protein, e.g., any of the mature wildtype alpha chains of IL-7 receptor described herein, e.g., mature wildtype human alpha chain of IL-7 receptor protein, e.g., SEQ ID NO: 30) having one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the C-terminus of the sequence of the wildtype alpha chain of IL-7 receptor protein. Some embodiments of any of the chimeric transmembrane proteins described herein can include the sequence of a wildtype alpha chain of IL-7 receptor protein (e.g., a mature wildtype alpha chain of IL-7 receptor protein, e.g., any of the mature wildtype alpha chains of IL-7 receptor described herein, e.g., mature wildtype human alpha chain of IL-7 receptor protein, e.g., SEQ ID NO: 30) having both one to ten amino (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) acids removed from the N-terminus of the sequence of the wildtype alpha chain of IL-7 receptor protein and one to ten (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) amino acids removed from the C-terminus of the sequence of the wildtype alpha chain of IL-7 receptor protein.

As will be appreciated by those of ordinary skill in the art, when a nucleic acid encoding a chimeric transmembrane protein having an alpha chain of an IL-7 receptor protein domain includes or lacks one or more additional nucleotides as compared to SEQ ID NO: 31, SEQ ID NO: 33, or SEQ ID NO: 35, the translational reading frame should still be maintained such that a nonsense codon is not introduced and the full protein can be translated (e.g., a nucleic acid encoding a chimeric transmembrane protein having a domain encoding an alpha chain of an IL-7 receptor protein can include or lack three nucleotides, or multiples thereof, within the domain encoding the alpha chain of the IL-7 receptor).

Extracellular Sushi Domain of an Alpha Chain of IL-15 Receptor

In some embodiments, the chimeric transmembrane receptors described herein can include one or more (e.g., one, two, three, or four) sushi domains from an alpha chain of IL-15 receptor. For example, chimeric transmembrane proteins described herein can include a sushi domain from a wildtype alpha chain of a IL-15 receptor (e.g., a wildtype human alpha chain of a IL-15 receptor, a wildtype mouse alpha chain of a IL-15 receptor, a wildtype rat alpha chain of a IL-15 receptor, a wildtype monkey alpha chain of an IL-15 receptor, a wildtype chimpanzee alpha chain of an IL-15 receptor, a wildtype pig alpha chain of an IL-15 receptor, a wildtype dog alpha chain of an IL-15 receptor, or a wildtype cat alpha chain of an IL-15 receptor). Non-limiting examples of sushi domains from IL-15 receptors are described below. Additional examples of sushi domains from IL-15 receptors are known in the art.

A sushi domain, also known as a short consensus repeat or type 1 glycoprotein motif, is a common motif in protein-protein interaction. Sushi domains have been identified on a number of protein-binding molecules, including complement components C1r, C1s, factor H, and C2m, as well as the nonimmunologic molecules factor XIII and 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, a sushi domain can be about 25 amino acids to about 90 amino acids, about 25 amino acids to about 85 amino acids, about 25 amino acids to about 80 amino acids, about 25 amino acids to about 75 amino acids, about 25 amino acids to about 70 amino acids, about 25 amino acids to about 65 amino acids, about 25 amino acids to about 60 amino acids, about 25 amino acids to about 55 amino acids, about 25 amino acids to about 50 amino acids, about 25 amino acids to about 45 amino acids, about 25 amino acids to about 40 amino acids, about 25 amino acids to about 35 amino acids, about 25 amino acids to about 30 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 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 90 amino acids, about 35 amino acids to about 85 amino acids, about 35 amino
5 acids to about 80 amino acids, about 35 amino acids to about 75 amino acids, about
35 amino acids to about 70 amino acids, about 35 amino acids to about 65 amino
acids, about 35 amino acids to about 60 amino acids, about 35 amino acids to about
55 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
10 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 to about 75
amino acids, about 40 amino acids to about 70 amino acids, about 40 amino acids to
about 65 amino acids, about 40 amino acids to about 60 amino acids, about 40 amino
acids to about 55 amino acids, about 40 amino acids to about 50 amino acids, about
15 40 amino acids to about 45 amino acids, about 45 amino acids to about 90 amino
acids, about 45 amino acids to about 85 amino acids, about 45 amino acids to about
80 amino acids, about 45 amino acids to about 75 amino acids, about 45 amino acids
to about 70 amino acids, about 45 amino acids to about 65 amino acids, about 45
amino acids to about 60 amino acids, about 45 amino acids to about 55 amino acids,
20 about 45 amino acids to about 50 amino acids, about 50 amino acids to about 90
amino acids, about 50 amino acids to about 85 amino acids, about 50 amino acids to
about 80 amino acids, about 50 amino acids to about 75 amino acids, about 50 amino
acids to about 70 amino acids, about 50 amino acids to about 65 amino acids, about
50 amino acids to about 60 amino acids, about 50 amino acids to about 55 amino
25 acids, about 55 amino acids to about 90 amino acids, about 55 amino acids to about
85 amino acids, about 55 amino acids to about 80 amino acids, about 55 amino acids
to about 75 amino acids, about 55 amino acids to about 70 amino acids, about 55
amino acids to about 65 amino acids, about 55 amino acids to about 60 amino acids,
about 60 amino acids to about 90 amino acids, about 60 amino acids to about 85
30 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to
about 75 amino acids, about 60 amino acids to about 70 amino acids, about 60 amino
acids to about 65 amino acids, about 65 amino acids to about 90 amino acids, about
65 amino acids to about 85 amino acids, about 65 amino acids to about 80 amino

acids, about 65 amino acids to about 75 amino acids, about 65 amino acids to about 70 amino acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about 85 amino acids, about 70 amino acids to about 80 amino acids, about 70 amino acids to about 75 amino acids, about 75 amino acids to about 90 amino acids, about 75 amino acids to about 85 amino acids, about 75 amino acids to about 80 amino acids, about 80 amino acids to about 90 amino acids, about 80 amino acids to about 85 amino acids, or about 85 amino acids to about 90 amino acids, in length.

In some embodiments, an extracellular sushi domain comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an extracellular portion of a mature wildtype alpha chain of IL-2 receptor, e.g., a mature wildtype human alpha chain of IL-2 receptor, e.g., SEQ ID NO: 36 or 37.

Sushi Domain from Wildtype Human Alpha Chain of IL-15 Isoform 1 (SEQ ID NO: 36)

CPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCI

Sushi Domain from Wildtype Human Alpha Chain of IL-15 Isoform 2 (SEQ ID NO: 37)

CPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCI

Sushi Domain from Wildtype Mouse Alpha Chain of IL-15 Isoform 1 (SEQ ID NO: 38)

CPPPVSIHADIRVKNYSVNSRERYVCNSGFKRKAGTSTLIECVINKNTNVAHWTTPSLKCI

Sushi Domain from Wildtype Mouse Alpha Chain of IL-15 Isoform 4 (SEQ ID NO: 39)

CPPPVSIHADIRVKNYSVNSRERYVCNSGFKRKAGTSTLIECVINKNTNVAHWTTPSLKCI

Sushi Domain from Wildtype Chicken Alpha Chain of IL-15 Protein (SEQ ID NO: 40)

CPRLSTTEFADVAAETYPLKTKLRYECDSGYRRRSGNTLTIRCQNVSGTASWVHDELVC

**Extracellular Portion from Wildtype Human Alpha Chain of IL-15 Isoform 1
(SEQ ID NO: 41)**

ITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWT
TPSLKCIKIRDPALVHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTA
5 AIVPGSQLMPSKSPSTGTTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGH
SDTT

In some embodiments, an extracellular sushi domain is an extracellular portion of a mature wildtype alpha chain of IL-15 receptor (e.g., an extracellular portion of a mature wildtype human alpha chain of IL-15 receptor, e.g., SEQ ID NO: 41). In some
10 embodiments, an extracellular sushi domain is sequence that is at least 80%, at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or 100% identical to an extracellular portion of a mature wildtype alpha chain of IL-15 receptor (e.g.,
15 SEQ ID NO: 41). In some embodiments, an extracellular sushi domain is a sequence of an extracellular portion of a mature wildtype alpha chain of IL-15 receptor (e.g., SEQ ID NO: 41) having one to twenty (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from the N-terminus of the sequence of the extracellular portion of the mature wildtype alpha chain of IL-15 receptor In
20 some embodiments, an extracellular sushi domain is a sequence of an extracellular portion of a mature wildtype alpha chain of IL-15 receptor (e.g., SEQ ID NO: 41) having one to twenty (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from the C-terminus of the sequence of the extracellular portion of the mature wildtype alpha chain of IL-15 receptor. In some
25 embodiments, an extracellular sushi domain is a sequence of an extracellular portion of a mature wildtype alpha chain of IL-15 receptor (e.g., SEQ ID NO: 41) having one to twenty (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from the N-terminus of the sequence of the extracellular portion of the mature wildtype alpha chain of IL-15 receptor and one to twenty (e.g., 1, 2, 3,
30 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from the C-terminus of the sequence of the extracellular portion of the mature wildtype alpha chain of IL-15 receptor.

In some embodiments, an extracellular sushi domain comprises or is a sequence of a sushi domain from a wildtype alpha-chain of IL-15 receptor (e.g., any
35 of the sushi domains from wildtype alpha-chains of IL-15 receptor described herein,

e.g., a sushi domain from wildtype human alpha-chain of IL-15 receptor, e.g., a sequence comprising one or both of SEQ ID NO: 36 or 37).

In some embodiments, the extracellular sushi domain of the chimeric transmembrane protein comprises a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to a sushi domain of a wildtype alpha chain of IL-15 receptor (e.g., SEQ ID NO: 36, 37, 38, 39, or 40).

As one skilled in the art can appreciate, when amino acids that are not conserved between sushi domains from wildtype alpha chains of IL-15 receptor protein from different species are mutated (e.g., substituted with a different amino acid) they are less likely to cause a decrease in the level of IL-15 binding activity of the sushi domain from an alpha chain of IL-15 receptor protein. In contrast, when amino acids that are conserved between the sushi domains from wildtype alpha chains of IL-15 receptor protein from different species are mutated (e.g., substituted with a different amino acid) they are more likely to cause a decrease in the level of IL-15 binding activity of the sushi domain from an alpha chain of IL-15 receptor protein. In view of this knowledge, one skilled in the art can select which amino acid positions in a sushi domain of an alpha chain of an IL-15 receptor protein (e.g., the non-conserved amino acids) can be substituted without decreasing the activity of the sushi domain of the alpha chain of an IL-15 receptor protein.

In some embodiments, the extracellular sushi domain of the chimeric transmembrane protein is a sequence of a sushi domain from a wildtype alpha chain of an IL-15 receptor (e.g., any of SEQ ID NO: 36, 37, 38, 39, or 40) having one to five (e.g., one, two, three, four, or five) amino acids removed from the N-terminus of the sushi domain from the wildtype alpha chain of an IL-15 receptor. In some embodiments, the extracellular sushi domain of the chimeric transmembrane protein is a sequence of a sushi domain from a wildtype alpha chain of an IL-15 receptor (e.g., any of SEQ ID NO: 36, 37, 38, 39, or 40) having one to five (e.g., one, two, three, four, or five) amino acids removed from the C-terminus of the sushi domain

from the wildtype alpha chain of an IL-15 receptor. In some embodiments, the extracellular sushi domain of the chimeric transmembrane protein is a sequence of a sushi domain from a wildtype alpha chain of an IL-15 receptor (e.g., any of SEQ ID NO: 36, 37, 38, 39, or 40) having one to five (e.g., one, two, three, four, or five) amino acids removed from the N-terminus of the sushi domain from the wildtype alpha chain of an IL-15 receptor, and one to five (e.g., one, two, three, four, or five) amino acids removed from the C-terminus of the sushi domain from the wildtype alpha chain of an IL-15 receptor.

In some embodiments, the chimeric transmembrane protein comprises two sushi domains. In some embodiments, each of the sushi domains can independently be any of the sushi domains described herein.

Transmembrane Domains of an Alpha-Chain of an IL-7 Receptor

Chimeric transmembrane proteins described herein can include a transmembrane domain of a wildtype alpha chain of an IL-7 receptor from a human, a mouse, a rat, a monkey, a chimpanzee, a pig, a dog, a cat, or any other appropriate species. Non-limiting examples of transmembrane domains of alpha chains of IL-7 receptors are described below. Additional examples of transmembrane domains of alpha chain of IL-7 receptors are known in the art.

20

Transmembrane Domain of Wildtype Human Alpha Chain of IL-7 Receptor (SEQ ID NO: 1)

PILLTISILSFFSVALLVILACVLW

Transmembrane Domain of Wildtype Mouse Alpha Chain of IL-7 Receptor Isoform 1 (SEQ ID NO: 42)

PVLPSVTILSLFSVFLVILAHVLW

Transmembrane Domain of Wildtype Mouse Alpha Chain of IL-7 Receptor Isoform 2 (SEQ ID NO: 42)

PVLPSVTILSLFSVFLVILAHVLW

30

Transmembrane Domain of Wildtype Macaca fascicularis Alpha Chain of IL-7 Receptor (SEQ ID NO: 44)

PILLTISLLSFFSVALLVILACVLW

In some embodiments, the transmembrane domain from an alpha chain of an IL-2 receptor protein can be about 10 amino acids to about 50 amino acids, about 10 amino acids to about 45 amino acids, about 10 amino acids to about 40 amino acids, about 10 amino acids to about 35 amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to about 25 amino acids, about 10 amino acids to about 22 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 18 amino acids, about 10 amino acids to about 15 amino acids, about 15 amino acids to about 50 amino acids, about 15 amino acids to about 45 amino acids, about 15 amino acids to about 40 amino acids, about 15 amino acids to about 35 amino acids, about 15 amino acids to about 30 amino acids, about 15 amino acids to about 25 amino acids, about 15 amino acids to about 22 amino acids, about 15 amino acids to about 20 amino acids, about 15 amino acids to about 18 amino acids, about 18 amino acids to about 50 amino acids, about 18 amino acids to about 45 amino acids, about 18 amino acids to about 40 amino acids, about 18 amino acids to about 35 amino acids, about 18 amino acids to about 30 amino acids, about 18 amino acids to about 25 amino acids, about 18 amino acids to about 22 amino acids, about 18 amino acids to about 20 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 45 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 35 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 25 amino acids, about 20 amino acids to about 22 amino acids, about 22 amino acids to about 50 amino acids, about 22 amino acids to about 45 amino acids, about 22 amino acids to about 40 amino acids, about 22 amino acids to about 35 amino acids, about 22 amino acids to about 30 amino acids, about 22 amino acids to about 25 amino acids, about 25 amino acids to about 50 amino acids, about 25 amino acids to about 45 amino acids, about 25 amino acids to about 40 amino acids, about 25 amino acids to about 35 amino acids, about 25 amino acids to about 30 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 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 50

amino acids, about 40 amino acids to about 45 amino acids, or about 45 amino acids to about 50 amino acids, in length.

In some embodiments of any of the chimeric transmembrane proteins described herein, the transmembrane domain of an alpha chain of an IL-7 receptor can be a transmembrane domain from a wildtype alpha chain of an IL-7 receptor (e.g., a transmembrane domain of a wildtype human alpha chain of an IL-7 receptor, e.g., SEQ ID NO: 1) (e.g., any of the exemplary transmembrane domains of a wildtype alpha chain of an IL-7 receptor listed below or known in the art, e.g., SEQ ID NO: 1, 42, 43, or 44).

In some embodiments, the transmembrane domain of an alpha chain of IL-7 receptor comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to a transmembrane domain from a wildtype alpha chain of IL-7 receptor (e.g., any one of SEQ ID NO: 1, 42, 43, or 44). In some embodiments, a transmembrane domain of an alpha chain of an IL-7 receptor includes a sequence that differs from a transmembrane domain of a wildtype alpha chain of IL-7 receptor (e.g., any one of SEQ ID NO: 1, 42, 43, or 44) by one to five amino acids (e.g., 1, 2, 3, 4, or 5 amino acids). In some embodiments, a transmembrane domain of an alpha chain of an IL-7 receptor includes one or twenty additional amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids), e.g., in addition to the sequence of SEQ ID NO: 1, 42, 43, or 44. In some embodiments, a transmembrane domain of an alpha chain of an IL-7 receptor can have a sequence of any one of SEQ ID NOs: 1, 42, 43, or 44, but lack one to five amino acids (e.g., 1, 2, 3, 4, or 5 amino acids).

As one skilled in the art can appreciate, when amino acids that are not conserved between transmembrane domains from wildtype alpha chains of IL-7 receptor protein from different species are mutated (e.g., substituted with a different amino acid) they are less likely to cause a decrease in the activity of the transmembrane domain from an alpha chain of IL-7 receptor protein. In contrast, when amino acids that are conserved between the transmembrane domains from

wildtype alpha chains of IL-7 receptor protein from different species are mutated (e.g., substituted with a different amino acid) they are more likely to cause a decrease in the activity of the transmembrane domain from an alpha chain of IL-7 receptor protein. In view of this knowledge, one skilled in the art can select which amino acid positions in a transmembrane domain of an alpha chain of an IL-7 receptor protein (e.g., the non-conserved amino acids) can be substituted without decreasing the activity of the transmembrane domain from the alpha chain of an IL-7 receptor protein.

In some embodiments, the transmembrane domain of an alpha chain of an IL-7 receptor protein is a sequence of a transmembrane domain of a wildtype alpha chain of IL-7 receptor protein (e.g., SEQ ID NO: 1, 42, 43, or 44) having one to five (e.g., one, two, three, four, or five) amino acids removed from the N-terminus of the transmembrane domain of the alpha chain of the IL-7 receptor protein. In some embodiments, the transmembrane domain of an alpha chain of an IL-7 receptor protein is a sequence of a transmembrane domain of a wildtype alpha chain of an IL-7 receptor protein (e.g., SEQ ID NO: 1, 42, 43, or 44) having one to five (e.g., one, two, three, four, or five) amino acids removed from the C-terminus of the transmembrane domain of the alpha chain of the IL-7 receptor protein. In some embodiments, the transmembrane domain of an alpha chain of an IL-7 receptor protein is a sequence of a transmembrane domain of a wildtype alpha chain of IL-7 receptor protein (e.g., a transmembrane domain of a wildtype human alpha chain of IL-7 receptor protein, e.g., SEQ ID NO: 1, 42, 43, or 44) having one to five (e.g., one, two, three, four, or five) amino acids removed from the N-terminus of the transmembrane domain of the alpha chain of the IL-7 receptor protein, and one to five (e.g., one, two, three, four, or five) amino acids removed from the C-terminus of the transmembrane domain of the alpha chain of the IL-7 receptor protein.

In some embodiments of any of the chimeric transmembrane proteins described herein, the transmembrane domain of an alpha chain of interleukin-7 receptor includes the sequence of PILLTISILSFFSVALLVILACVLW (SEQ ID NO: 1) with one or more (e.g., one, two, three, or four) of the following modifications: (i) alanine-leucine-leucine at amino acids positions 15 through 17 in SEQ ID NO: 1 has been replaced with a different three-amino acid sequence (e.g., has been replaced with glutamic acid-lysine-valine or glutamic acid-lysine-alanine); (ii) one to three (e.g.,

one, two, or three) amino acids (e.g., cysteine-proline-threonine) has been inserted between amino acid positions 5 and 6 in SEQ ID NO: 1; and (iii) one to four (e.g., one, two, three, or four) amino acids (e.g., proline-proline-cysteine-leucine) has been inserted between amino acid positions 4 and 5 in SEQ ID NO: 1. In some
5 embodiments, the transmembrane domain is modified to have a sequence of SEQ ID NO: 2, 4, 6 or 8.

In some embodiments, the transmembrane domain includes the sequence of SEQ ID NO: 1 with the alanine-leucine-leucine at amino acid positions 15 through 17 in SEQ ID NO: 1 replaced with glutamic acid-lysine-valine or glutamic acid-lysine-
10 alanine. In some embodiments, the transmembrane domain includes the sequence of SEQ ID NO: 1 with cysteine-proline-threonine inserted between amino acid positions 5 and 6 in SEQ ID NO: 1. In some embodiments, the transmembrane domain includes the sequence of SEQ ID NO: 1 with a proline-proline-cysteine-leucine inserted between amino acid positions 4 and 5 in SEQ ID NO: 1. In some
15 embodiments, the transmembrane domain is modified to have a sequence of SEQ ID NO: 2, 4, 6 or 8.

Intracellular Domains of an Alpha Chain of IL-7 Receptor

Some embodiments of the chimeric transmembrane proteins described herein
20 can further include an intracellular domain of an alpha chain of an IL-7 receptor, or a portion thereof. For example, chimeric transmembrane proteins described herein can include an intracellular domain of a wildtype alpha chain of an IL-7 receptor from a human, a mouse, a rat, a monkey, a chimpanzee, a pig, a dog, a cat, or any other appropriate species. Non-limiting examples of intracellular domains of alpha chains
25 of IL-7 receptors are described below. Additional examples of intracellular domains of alpha chain of IL-7 receptors are known in the art.

Any of the chimeric transmembrane proteins described herein can comprise an intracellular domain of an alpha chain of an interleukin-7 receptor (e.g., any of the alpha chains of an interleukin-7 receptor described herein). In some embodiments,
30 the intracellular domain of an alpha chain of an interleukin-7 receptor can be about 100 amino acids to about 225 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 180 amino acids, about 100 amino acids to about 160 amino acids, about 100 amino acids to about 140 amino acids, about 100

amino acids to about 120 amino acids, about 120 amino acids to about 225 amino acids, about 120 amino acids to about 200 amino acids, about 120 amino acids to about 180 amino acids, about 120 amino acids to about 160 amino acids, about 120 amino acids to about 140 amino acids, about 140 amino acids to about 225 amino acids, about 140 amino acids to about 200 amino acids, about 140 amino acids to about 180 amino acids, about 140 amino acids to about 160 amino acids, about 160 amino acids to about 225 amino acids, about 160 amino acids to about 200 amino acids, about 160 amino acids to about 180 amino acids, about 180 amino acids to about 225 amino acids, about 180 amino acids to about 200 amino acids, or about 200 amino acids to about 225 amino acids, in length.

Any of the chimeric transmembrane proteins described herein can comprise an intracellular domain of an alpha chain of interleukin-7 receptor (e.g., an intracellular domain of a wildtype alpha chain of interleukin-7 receptor (e.g., an intracellular domain of a wildtype human alpha chain of interleukin-7 receptor, e.g., SEQ ID NO: 45) (e.g., an intracellular domain of a wildtype human alpha chain of interleukin-7 of SEQ ID NO: 46).

Intracellular Domain of Wildtype Human Alpha Chain of IL-7 Receptor (SEQ ID NO: 45, the BOX1 motif is underlined, the tyrosines that are phosphorylated are in large bold font)

KKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQD
TFPQQLEESEKQRLGGDVQSPNCPSSEDDVITPESFGRDSSLTCLAGNVSACDAPILSSSRSL
DCRESGKNGPHV**Y**QDLLLSLGTNTSTLPPFSLQSGILTLNPVAQGQPILTSLGSNQEEA**Y**V
TMSSF**Y**QNQ

25

Nucleic Acid Encoding an Intracellular Domain of Wildtype Human Alpha Chain of IL-7 Receptor (SEQ ID NO: 82, nucleic acid sequence encoding the BOX1 motif is underlined)

aagaagaggatcaagcccatcgtgtgcccagcctgcccaccacaagaagaccctggagcacctgtgtaagaagccc
aggaagaacctgaacgtgagcttcaaccccgagagcttctggactgccagatccacagggtggacgacatccaggcca
gggacgaggtggaggcttctgcaggacaccttccccagcagctggaggagagcgagaagcagaggctggcgcg
cgacgtgcagagcccaactgccccagcagagctggtgatcccccagagcttcggcaggagacagcagcctga
cctgcctggccggcaacgtgagcgctgcgacgccccatcctgagcagcagcaggagcctggactgcaggagagc

30

ggcaagaacggccccacgtgtaccaggacctgctgctgagcctgggcaccaccaacagcacctgccaccccccttca
gcctgcagagcggcacatcctgacctgaaccccgaggccagccatcctgaccagcctgggcagcaaccagg
aggaggcctacgtgacatgagcagcttctaccagaaccag

5 Intracellular Domain of Wildtype Mouse Alpha Chain of IL-7 Receptor Isoform 1 (SEQ ID NO: 46)

KKRIKPVVWPSLPDHKKTLEQLCKKPKTSLNVSFNPESFLDCQIHEVKGVEARDEVESFLPN
DLPAQPEELETQGHRAAVHSANRSPETSVSPPETVRRESPLRCLARNLSTCNAPPLLSSRSP
DYRDGDRNRPPVYQDLLPNSGNTNVPVPVQPLPFQSGILIPVSQRQPISTSSVLNQEEAYV
10 TMSSFYQNK

In some embodiments, the intracellular domain of an alpha chain of IL-7 receptor comprises or is a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the intracellular domain of a wildtype alpha chain of IL-7 receptor (e.g., SEQ ID NO: 45 or 46). In some embodiments, an intracellular domain of an alpha chain of an IL-7 receptor includes a sequence that differs the sequence of an intracellular domain of a wildtype alpha chain of IL-7 receptor (e.g., SEQ ID NO: 45 or 46) by one to 40 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids). In some embodiments, an intracellular domain of an alpha chain of an IL-7 receptor includes one or more additional amino acids (e.g., 1 to about 100 amino acids, 1 to about 95 amino acids, 1 to about 90 amino acids, 1 to about 85 amino acids, 1 to about 80 amino acids, 1 to about 75 amino acids, 1 to about 70 amino acids, 1 to about 65 amino acids, 1 to about 60 amino acids, 1 to about 55 amino acids 1 to about 50 amino acids, 1 to about 45 amino acids, 1 to about 40 amino acids, 1 to about 35 amino acids, 1 to about 30 amino acids, 1 to about 25 amino acids, 1 to about 20 amino acids, 1 to about 15 amino acids, 1 to about 10 amino acids, or 1 to about 5 amino acids), e.g., in addition to the sequence of SEQ ID NO: 45 or 46. In some embodiments, an intracellular domain of an alpha chain of an IL-7 receptor can have a

sequence of any one of SEQ ID NOs: 45 or 46, but lack one to forty amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids).

As one skilled in the art can appreciate, when amino acids that are not
5 conserved between intracellular domains from wildtype alpha chains of IL-7 receptor protein from different species are mutated (e.g., substituted with a different amino acid) they are less likely to cause a decrease in the activity of the intracellular domain from an alpha chain of IL-7 receptor protein. In contrast, when amino acids that are conserved between the intracellular domains from wildtype alpha chains of IL-7
10 receptor protein from different species are mutated (e.g., substituted with a different amino acid) they are more likely to cause a decrease in the activity of the intracellular domain from an alpha chain of IL-7 receptor protein. In view of this knowledge, one skilled in the art can select which amino acid positions in an intracellular domain of an alpha chain of an IL-7 receptor protein (e.g., the non-conserved amino acids) can
15 be substituted without decreasing the activity of the intracellular domain from the alpha chain of an IL-7 receptor protein.

In some embodiments, the intracellular domain of an alpha chain of an IL-7 receptor protein is a sequence of an intracellular domain of a wildtype alpha chain of IL-7 receptor protein (e.g., SEQ ID NO: 45 or 46) having one to three (e.g., one, two,
20 or three) amino acids removed from the N-terminus of the intracellular domain of the alpha chain of the IL-7 receptor protein. In some embodiments, the intracellular domain of an alpha chain of an IL-7 receptor protein is a sequence of an intracellular domain of a wildtype alpha chain of an IL-7 receptor protein (e.g., SEQ ID NO: 45 or 46) having one to three (e.g., one, two, or three) amino acids removed from the C-
25 terminus of the intracellular domain of the alpha chain of the IL-7 receptor protein. In some embodiments, the intracellular domain of an alpha chain of an IL-7 receptor protein is a sequence of an intracellular domain of a wildtype alpha chain of IL-7 receptor protein (e.g., SEQ ID NO: 45 or 46) having one to three (e.g., one, two, or three) amino acids removed from the N-terminus of the intracellular domain of the
30 alpha chain of the IL-7 receptor protein, and one to three (e.g., one, two, or three) amino acids removed from the C-terminus of the intracellular domain of the alpha chain of the IL-7 receptor protein.

Linker Sequences

In some embodiments, the chimeric transmembrane protein comprises a linker sequence positioned between the extracellular IL-15 domain and the extracellular sushi domain. In some embodiments, the chimeric transmembrane protein comprises an additional linker sequence positioned between extracellular sushi domain and the transmembrane domain of an alpha chain of an IL-15 receptor. When the chimeric transmembrane protein comprises a linker sequence and an additional linker sequence, each the linker sequences can be the same or different from each other.

In some embodiments, the linker sequence or the additional linker sequence is 1 amino acid to about 50 amino acids, 1 amino acid to about 48 amino acids, 1 amino acid to about 46 amino acids, 1 amino acid to about 44 amino acids, 1 amino acid to about 42 amino acids, 1 amino acid to about 40 amino acids, 1 amino acid to about 38 amino acids, 1 amino acid to about 36 amino acids, 1 amino acid to about 34 amino acids, 1 amino acid to about 32 amino acids, 1 amino acid to about 30 amino acids, 1 amino acid to about 28 amino acids, 1 amino acid to about 26 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, 1 amino acid to about 3 amino acids, about 2 amino acids to about 50 amino acids, about 2 amino acids to about 48 amino acids, about 2 amino acids to about 46 amino acids, about 2 amino acids to about 44 amino acids, about 2 amino acids to about 42 amino acids, about 2 amino acids to about 40 amino acids, about 2 amino acids to about 38 amino acids, about 2 amino acids to about 36 amino acids, about 2 amino acids to about 34 amino acids, about 2 amino acids to about 32 amino acids, about 2 amino acids to about 30 amino acids, about 2 amino acids to about 28 amino acids, about 2 amino acids to about 26 amino acids, about 2 amino acids to about 24 amino acids, about 2 amino acids to about 22 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 18 amino acids, about 2 amino acids to about 16 amino acids, about 2 amino acids to about 14 amino acids, about 2 amino acids to about 12 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 6 amino acids, about 2 amino acids

[illegible]

[illegible]

[illegible]

amino acids, about 22 amino acids to about 36 amino acids, about 22 amino acids to
about 34 amino acids, about 22 amino acids to about 32 amino acids, about 22 amino
acids to about 30 amino acids, about 22 amino acids to about 28 amino acids, about
22 amino acids to about 26 amino acids, about 22 amino acids to about 24 amino
5 acids, about 24 amino acids to about 50 amino acids, about 24 amino acids to about
48 amino acids, about 24 amino acids to about 46 amino acids, about 24 amino acids
to about 44 amino acids, about 24 amino acids to about 42 amino acids, about 24
amino acids to about 40 amino acids, about 24 amino acids to about 38 amino acids,
about 24 amino acids to about 36 amino acids, about 24 amino acids to about 34
10 amino acids, about 24 amino acids to about 32 amino acids, about 24 amino acids to
about 30 amino acids, about 24 amino acids to about 28 amino acids, about 24 amino
acids to about 26 amino acids, about 26 amino acids to about 50 amino acids, about
26 amino acids to about 48 amino acids, about 26 amino acids to about 46 amino
acids, about 26 amino acids to about 44 amino acids, about 26 amino acids to about
15 42 amino acids, about 26 amino acids to about 40 amino acids, about 26 amino acids
to about 38 amino acids, about 26 amino acids to about 36 amino acids, about 26
amino acids to about 34 amino acids, about 26 amino acids to about 32 amino acids,
about 26 amino acids to about 30 amino acids, about 26 amino acids to about 28
amino acids, about 28 amino acids to about 50 amino acids, about 28 amino acids to
20 about 48 amino acids, about 28 amino acids to about 46 amino acids, about 28 amino
acids to about 44 amino acids, about 28 amino acids to about 42 amino acids, about
28 amino acids to about 40 amino acids, about 28 amino acids to about 38 amino
acids, about 28 amino acids to about 36 amino acids, about 28 amino acids to about
34 amino acids, about 28 amino acids to about 32 amino acids, about 28 amino acids
25 to about 30 amino acids, about 30 amino acids to about 50 amino acids, about 30
amino acids to about 48 amino acids, about 30 amino acids to about 46 amino acids,
about 30 amino acids to about 44 amino acids, about 30 amino acids to about 42
amino acids, about 30 amino acids to about 40 amino acids, about 30 amino acids to
about 38 amino acids, about 30 amino acids to about 36 amino acids, about 30 amino
30 acids to about 34 amino acids, about 30 amino acids to about 32 amino acids, about
32 amino acids to about 50 amino acids, about 32 amino acids to about 48 amino
acids, about 32 amino acids to about 46 amino acids, about 32 amino acids to about
44 amino acids, about 32 amino acids to about 42 amino acids, about 32 amino acids

to about 40 amino acids, about 32 amino acids to about 38 amino acids, about 32 amino acids to about 36 amino acids, about 32 amino acids to about 34 amino acids, about 34 amino acids to about 50 amino acids, about 34 amino acids to about 48 amino acids, about 34 amino acids to about 46 amino acids, about 34 amino acids to about 44 amino acids, about 34 amino acids to about 42 amino acids, about 34 amino acids to about 40 amino acids, about 34 amino acids to about 38 amino acids, about 34 amino acids to about 36 amino acids, about 36 amino acids to about 50 amino acids, about 36 amino acids to about 48 amino acids, about 36 amino acids to about 46 amino acids, about 36 amino acids to about 44 amino acids, about 36 amino acids to about 42 amino acids, about 36 amino acids to about 40 amino acids, about 36 amino acids to about 38 amino acids, about 38 amino acids to about 50 amino acids, about 38 amino acids to about 48 amino acids, about 38 amino acids to about 46 amino acids, about 38 amino acids to about 44 amino acids, about 38 amino acids to about 42 amino acids, about 38 amino acids to about 40 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 48 amino acids, about 40 amino acids to about 46 amino acids, about 40 amino acids to about 44 amino acids, about 40 amino acids to about 42 amino acids, about 42 amino acids to about 50 amino acids, about 42 amino acids to about 48 amino acids, about 42 amino acids to about 46 amino acids, about 42 amino acids to about 44 amino acids, about 44 amino acids to about 50 amino acids, about 44 amino acids to about 48 amino acids, about 44 amino acids to about 46 amino acids, about 46 amino acids to about 50 amino acids, about 46 amino acids to about 48 amino acids, or about 48 amino acids to about 50 amino acids.

In some embodiments, the linker sequence and/or the additional linker sequence comprises a sequence of (SG)_n, where n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25. In some embodiments, the linker sequence and/or the additional linker sequence comprises a sequence of (GS)_n, where n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25.

In some embodiments, the linker sequence and/or the additional linker sequence comprises a sequence of (SGGS)_n [SGGS = SEQ ID NO: 47], where n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In some embodiments, the linker sequence and/or the additional linker sequence comprises a sequence of (SGGS)_n

[SGGGS = SEQ ID NO: 48], where n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the linker sequence and/or the additional linker sequence comprises a sequence of (SGGGGS)_n [SGGGGS = SEQ ID NO: 49], where n can be 1, 2, 3, 4, 5, 6, 7, 8, or 9.

5 In some embodiments, the linker sequence and/or the additional linker
sequence can be or include the sequence of SGGGSGGGGSGGGG (SEQ ID NO:
50). In some embodiments, the linker sequence and/or the additional linker sequence
can be or include the sequence of ASTKGPSVFPLAPSSSGSG (SEQ ID NO: 51). In
some embodiments, the linker sequence and/or the additional linker sequence can be
10 or include the sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 52). In some
embodiments, the linker sequence and/or the additional linker sequence can be or
include the sequence of GGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 53). In
some embodiments, the linker sequence and/or the additional linker sequence can be
or include the sequence of SGGGSGGGGSGGGGSGGGGSGGGSLQ (SEQ ID NO:
15 92).

In some embodiments, the linker sequence and/or the additional linker sequence can be a Whitlow linker. In some embodiments, the Whitlow linker has the amino acid sequence of GSTSGSGKPGSGEGSTKG (SEQ ID NO: 54) or the nucleotide sequence encoding the Whitlow linker sequence is

ggcagcaccagcggcagcggcgaaccgggcagcggcgaaggcagcaccaaaaggc (SEQ ID NO: 55).

In some embodiments, the linker sequence and/or the additional linker sequence can be a (G₄S)₅ linker. In some embodiments, the (G₄S)₅ linker has the amino acid sequence of GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 56) or the nucleotide sequence encoding the (G₄S)₅ linker sequence is

25 ggccggtggtggttctggaggcgggtggcagcgggtggaggtggctcaggaggaggaggtagcggcggcgaggaggat
(SEQ ID NO: 57).

In some embodiments, the linker sequence and/or the additional linker sequence can be or can include one or more of an IgG1, IgG2, IgG3, or IgG4 CH1, CH2, and CH3 domain. In some embodiments, the linker sequence and/or the additional linker sequence can be or can include CH2-CH3 human IgG1 domains. In some embodiments, the CH2-CH3 human IgG1 domains have a sequence of:

AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCWVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEOYNSTYRVVSVLTVLHQDWLNGKEYK

CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFCFV
MHEALHNHYTQKSLSLSPGKKD (SEQ ID NO: 58).

5 In some embodiments, the linker sequence and/or the additional linker
sequence can be or include a portion of the human CD8 extracellular sequence that is
proximal to the human CD8 transmembrane domain. For example, the linker
sequence and/or the additional linker sequence can be or include human CD8
sequence of: TTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDI
(SEQ ID NO: 59).

10 In some embodiments, the linker sequence and/or the additional linker
sequence can be or include a human IgG1 hinge sequence. In some embodiments, the
human IgG1 hinge sequence is AEPKSPDKTHTCPPCKDPK (SEQ ID NO: 60).

In some embodiments, the linker sequence and/or the additional linker
sequence has an alpha helix structure. In some embodiments, the linker sequence
15 and/or the additional linker sequence is a coiled coil domain.

In some embodiments, the linker sequence and/or the additional linker
sequence is a naturally-occurring amino acid sequence. In some embodiments, the
linker sequence and/or the additional linker sequence is not a naturally-occurring
amino acid sequence. In some embodiments, the linker sequence and/or the
20 additional linker sequence comprises a sequence of SEQ ID NO: 92. In some
embodiments, the linker sequence and/or the additional linker sequence consists of a
sequence of SEQ ID NO: 92. Additional aspects and examples of linkers are known
in the art.

Chimeric Antigen Receptors

25 A chimeric antigen receptor (CAR) is a protein that includes an extracellular
antigen-binding domain (e.g., any of the antigen-binding domains described herein or
known in the art), a transmembrane domain (e.g., any of the transmembrane domains
described herein or known in the art), a costimulatory domain (e.g., any of the
costimulatory domains described herein or known in the art), and an immunoreceptor
30 tyrosine-based activation motif (ITAM). In some embodiments, a CAR comprises
additional sequences such as, without limitation an extracellular stalk sequence (e.g.,
a CD28 stalk sequence). In some embodiments of a CAR comprising an extracellular
stalk sequence (e.g., a CD28 stalk sequence), the stalk sequence is coterminal with the

transmembrane domain. In some embodiments of a CAR comprising an extracellular stalk sequence (e.g., a CD28 stalk sequence), the extracellular stalk sequence is from the same protein as the transmembrane domain. In some embodiments of a CAR comprising an extracellular stalk sequence (e.g., a CD28 stalk sequence), the extracellular stalk sequence is from a different protein as the transmembrane domain. Non-limiting aspects of chimeric antigen receptors are described in, e.g., Kershaw et al., *Nature Reviews Immunol.* 5(12):928-940, 2005; Eshhar et al., *Proc. Natl. Acad. Sci. U.S.A.* 90(2):720-724, 1993; Sadelain et al., *Curr. Opin. Immunol.* 21(2): 215-223, 2009; WO 2015/142675; WO 2015/150526; and WO 2014/134165, the disclosures of each of which are incorporated herein by reference in their entirety.

In some embodiments, a chimeric antigen receptor can include one or more (e.g., two, three, four, or five) costimulatory domain(s) (e.g., any combination of any of the exemplary costimulatory domains described herein or known in the art). Some embodiments of these chimeric antigen receptors include one or both of a 4-1BB costimulatory domain and a CD28 costimulatory domain.

In some embodiments, a chimeric antigen receptor can include one or more (e.g., two, three, four, or five) ITAMs (e.g., any of the ITAMs described herein or known in the art). In some embodiments of these chimeric antigen receptors, the ITAM includes a cytoplasmic signaling sequence from CD3 ζ (e.g., human CD3 ζ).

In some embodiments, one or more amino acids between the extracellular antigen-binding domain and the transmembrane domain is a sequence from the same endogenous single-chain polypeptide from which the transmembrane domain is derived. In some embodiments, one or more amino acids between the extracellular antigen-binding domain and the transmembrane domain is or includes a hinge region sequence of an antibody such as, without limitation, a human antibody (e.g., IgG1, IgG2, IgG3, or IgG4). In some embodiments, one or more amino acids between the extracellular antigen-binding domain and the transmembrane domain is or comprises a linker sequence (e.g., a non-naturally occurring linker sequence, e.g., GS or any of the other linker sequences described herein).

In some examples of any of the CARs described herein, going in the N-terminal to the C-terminal direction, the intracellular portion of the CAR includes a co-stimulatory domain and an intracellular signaling domain. In some examples of any of the CARs described herein, going in the N-terminal to the C-terminal direction,

the intracellular portion of the CAR includes an intracellular signaling domain and a co-stimulatory domain.

In some examples of any of the CARs described herein, going in the C-terminal to the N-terminal direction, the intracellular portion of the CAR includes a co-stimulatory domain and an intracellular signaling domain. In some examples of any of the CARs described herein, going in the C-terminal to the N-terminal direction, the intracellular portion of the CAR includes an intracellular signaling domain and a co-stimulatory domain.

In some embodiments of any of the CARs described herein, the transmembrane domain is or includes a transmembrane domain of CD28, CD3 epsilon, CD4, CD5, CD6, CD8a, CD9, CD16, CD22, CD33, CD37, CD45, CD64, CD80, CD86, CD134, 4-1BB, or CD154. Additional examples and aspects of transmembrane domains are described herein.

In some embodiments of any of the CARs described herein, the co-stimulatory domain is or includes the co-stimulatory domain of 4-1BB, CD28, CD2, CD4 or CD8. Additional examples and aspects of co-stimulatory domains are described herein.

In some embodiments of any of the CARs described herein, the CAR is generated from a CAR precursor, which CAR precursor include a signal peptide sequence for targeting the CAR to the cell membrane. In some embodiments, a CAR signal peptide comprises or is a sequence of SEQ ID NO: 93:

Exemplary CAR Signal Sequence Peptide (SEQ ID NO: 93)

MLLLVTSLLLCELPHPAFLIP

Exemplary Nucleic Acid Sequence Encoding an Exemplary CAR Signal Sequence Peptide (SEQ ID NO: 94)

atgcttctcctggtgacaagcctctgctctgtgagttaccacaccagcattcctcctgattcct

In some embodiments, a CAR signal peptide comprises a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least

95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the polypeptide sequence of SEQ ID NO: 93.

In some embodiments, a nucleic acid sequence encoding a CAR signal peptide comprises a sequence that is at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the nucleic acid sequence of SEQ ID NO: 94.

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

Some embodiments of any of the chimeric antigen receptors described herein can further include a dimerization domain and/or a peptide tag.

Antigen-Binding Domains

In some embodiments of chimeric antigen receptors, the antigen-binding domain can be selected from a scFv, a scFv-Fc, a VHH domain, a VNAR domain, a (scFv)₂, and a BiTE. Additional examples of antigen-binding domains that can be used with chimeric antigen receptors described herein are known in the art.

A single-chain Fv or scFv fragment includes a V_H domain and a V_L domain in a single polypeptide chain. The V_H and V_L are generally linked by a peptide linker. In other examples, the linker can be a single amino acid. In some examples, the linker can be a chemical bond. See, e.g., Pluckthun, *Antibodies from E. coli*. In Rosenberg M. & Moore G.P. (Eds.), *The Pharmacology of Monoclonal Antibodies*, Vol. 113, pp. 269-315, Springer-Verlag, New York, 1994.

ScFv-Fc fragments include an scFv attached to an Fc domain. For example, an Fc domain can be attached, e.g., to the C-terminus of the scFv. The Fc domain can follow the V_L or V_H, depending on the orientation of the variable domains in the scFv. The Fc domain can be any Fc domain known in the art. In some examples, the Fc

domain is an IgG1, IgG2, IgG3, or IgG4 Fc domain (e.g., a human IgG1, IgG2, IgG3, or IgG4 Fc domain).

BiTEs are antigen-binding domains that include two V_L and two V_H in a single polypeptide that together form two scFvs, which can each bind to different epitopes on the same antigen or each bind to different antigens. See, e.g., Baeuerle et al., *Curr. Opin. Mol. Ther.* 11:22-30, 2009; Wolf et al., *Drug Discovery Today* 10:1237-1244, 2005; and Huehls et al., *Immunol. Cell Biol.* 93:290-296, 2015.

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

In some embodiments, an antigen-binding domain of a CAR is a sequence that comprises or is SEQ ID NO: 95:

Exemplary anti-CD19 Antigen Binding Domain (SEQ ID NO: 95)

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSR
LHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITG
GGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR
QPPRKGLEWLGVWGETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTA
IYYCAKHYYYGGSYAMDYWGQGTSTVTVSS

Nucleic Acid Encoding an Exemplary anti-CD19 Antigen Binding Domain (SEQ ID NO: 96)

Gacatccagatgaccagaccaccagcagcctgagcgccagcctggcgatagagtgacatcagctgcagagccag
 ccaggacatcagcaagtacctgaactggtatcagcagaacccgacggcaccgtgaagctgctgatctaccacaccagc
 5 agactgcacagcggcggtgccagcagattttctggcagcggctccggcaccgactacagcctgaccatctccaacctgga
 acaggaagatatcgtactacttctgtcagcaaggcaaacacctgccctacaccttcggcggaggcaccaagctggaaa
 tcacaggcggcgaggatctggcggaggcgggaagtggcggagggggatctgaagtgaactgcaggaaagcggccc
 tggcctgtggccccatctcagctctgtgagcgtgacctgtaccgtgtccggcgtgtccctgcctgactatggcgtgtcctgg
 atcagacagccccccagaaaggcctggaatggctgggagtgatctggggcagcgagacaacctactacaacagcgcc
 10 ctgaagtccggctgaccatcatcaaggacaactccaagagccaggtgttctgaagatgaacagcctgcagaccgagc
 acaccgccatctactactcgccaagcactactactacggcgagctacgcatggactactggggccagggcacaag
 cgtgaccgtgtctagc

In some embodiments, an antigen binding domain comprises a sequence that is
 15 at least 60% identical, at least 65% identical, at least 70% identical, at least 75%
 identical, at least 80% identical, at least 82% identical, at least 84% identical, at least
 85%, at least 86% identical, at least 88% identical, at least 90% identical, at least 91%
 identical, at least 92% identical, at least 93% identical, at least 94% identical, at least
 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at
 20 least 99% identical, or 100% identical to the polypeptide sequence of SEQ ID NO: 95.
 In some embodiments, a nucleic acid sequence encoding an antigen binding domain
 comprises a sequence that is at least 60% identical, at least 65% identical, at least
 70% identical, at least 75% identical, at least 80% identical, at least 82% identical, at
 least 84% identical, at least 85%, at least 86% identical, at least 88% identical, at least
 25 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at
 least 94% identical, at least 95% identical, at least 96% identical, at least 97%
 identical, at least 98% identical, at least 99% identical, or 100% identical to the
 nucleic acid sequence of SEQ ID NO: 96.

30 Any of the antigen-binding domains described herein can bind to an antigen
 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

complexes provided herein can bind to a first and/or second antigen 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, or about 1×10^{-9} M to about 1×10^{-11} M (inclusive). A variety of different methods known in the art can be used to determine the K_D value of an antigen-binding domain (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

Antigens

In some embodiments, a chimeric antigen receptor described herein can bind to a single antigen (e.g., any of the exemplary antigens described herein or known in the art). In some embodiments, an antigen-binding domain described herein can bind to two or more different antigens (e.g., two or more of any of the exemplary antigens described herein or known in the art). Non-limiting examples of antigens include:

glypican-3, HER2, A33 antigen, 9-O-acetyl-GD3, CA19-9 marker, BhCG, CA-125 marker, carboanhydrase IX (MN/CA IX), calreticulin, CCR5, CCR8, CD2, CD3, CD5, CD16, CD19, CD20, CD22, CD24, CD25, CD27, CD28, CD30, CD33, CD38, CD40L, CD44, CD44V6, CD63, CD70, CD84, CD96, CD100, CC123, CD133, CD137, CD138, CD150, CD152 (CTLA-4), CD160, CRTAM, CS1 (CD319), DNAM-1 (CD226), CD229, CD244, CD272 (BTLA), CD274 (PDL-1, B7H1), CD279 (PD-1), CD319, CD352, CRTAM (CD355), CD358, DR3, GITR (TNFRSF18), HVEM, ICOS, LIGHT, LTBR, OX40, activating forms of KIR, NKG2C, NKG2D, NKG2E, one or more natural cytotoxicity receptors, NTB-A, PEN-5, carcinoma embryonic antigen (CEA; CD66e), desmoglein 4, E-cadherin neoepitope, endosialin, ephrin A2 (EphA2), epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), fucosyl GM1, GD2, GD3, GM2, ganglioside GM3, Globo H, glycoprotein 100, HER2/neu, HER3, HER4, insulin-like growth factor receptor 1, Lewis-Y, LG, Ly-6, melanoma-specific chondroitin-sulfate proteoglycan (MCSCP), mesothelin, MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5b, MUC7, MUC16, Mullerian inhibitory substance (MIS) receptor type II, plasma cell antigen, poly SA, PSCA, PSMA, sonic hedgehog (SHH), SAS, STEAP, sTn antigen, TNF- α precursor, 2B4 (CD244), β 2-integrins, KIR, KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, KIR-L, KLRG1, LAIR-1, NKG2A, NKR-P1A, Siglec-3, Siglec-7, Siglec-

9, TCR α , TCR β , TCR δ , TIM1, LAG3, LAIR1, PD-1H, TIGIT, TIM2, and TIM3.

Additional examples of antigens are known in the art.

CAR Transmembrane Domains

5 In some embodiments, a chimeric antigen receptor includes a transmembrane domain, or portion thereof, from an endogenous polypeptide, where the endogenous polypeptide is selected from the group of: an α chain of a T cell receptor, a β chain of the T cell receptor, a ζ chain of the T cell receptor, CD28 (also known as Tp44), CD3 ϵ , CD3 δ , CD3 γ , CD33, CD37 (also known as GP52-40 or TSPAN26), CD64
10 (also known as FCGR1A), CD80 (also known as B7, B7-1, B7.1, BB1, CD28LG, CD28LG1, and LAB7), CD45 (also known as PTPRC, B220, CD45R, GP180, L-CA, LCA, LY5, T200, and protein tyrosine phosphatase, receptor type C), CD4, CD5 (also known as LEU1 and T1), CD8 α (also known as Leu2, MAL, and p32), CD9 (also known as BTCC-1, DRAP-27, MIC3, MRP-1, TSPAN-29, and TSPAN29), CD16
15 (also known as FCGR3 and FCG3), CD22 (also known as SIGLEC-2 and SIGLEC2), CD86 (also known as B7-2, B7.2, B70, CD28LG2, and LAB72), CD134 (also known as TNFRSF4, ACT35, RP5-902P8.3, IMD16, OX40, TXGP1L, and tumor necrosis factor receptor superfamily member 4), CD137 (also known as TNFRSF9, 4-1BB, CDw137, ILA, and tumor necrosis factor receptor superfamily member 9), CD27
20 (also known as S152, S152.LPFS2, T14, TNFRSF7, and Tp55), CD152 (also known as CTLA4, ALPS5, CELIAC3, CTLA-4, GRD4, GSE, IDDM12, and cytotoxic T-lymphocyte associated protein 4), PD1 (also known as PDCD1, CD279, PD-1, SLEB2, hPD-1, hPD-L, hSLE1, and Programmed cell death 1), ICOS (also known as AILIM, CD278, and CVID1), CD272 (also known as BTLA and BTLA1), CD30
25 (also known as TNFRSF8, D1S166E, and Ki-1), GITR (also known as TNFRSF18, RP5-902P8.2, AITR, CD357, and GITR-D), HVEM (also known as TNFRSF14, RP3-395M20.6, ATAR, CD270, HVEA, HVEM, LIGHTR, and TR2), DAP10, and CD154 (also known as CD40LG, CD40L, HIGM1, IGM, IMD3, T-BAM, TNFSF5, TRAP, gp39, hCD40L, and CD40 ligand). The letters “CD” is the previous sentence
30 stand for “Cluster of Differentiation.” E.g., CD3 stands for “Cluster of Differentiation 3.” In some embodiments, a chimeric antigen receptor includes a transmembrane domain, or portion thereof, from an endogenous mammalian (e.g., human)

polypeptide (e.g., a mammalian or human homolog of any of the polypeptides listed above).

Any transmembrane domain, or portion thereof, that serves to anchor an endogenous polypeptide in a lipid bilayer (e.g., plasma membrane) of a mammalian cell is suitable for use in accordance with compositions and methods disclosed herein. In some embodiments, a chimeric antigen receptor includes a transmembrane domain, or portion thereof, from human CD28, e.g., GenBank Accession No. P01747, e.g., amino acids 153 to 179 of SEQ ID NO: 61. In some embodiments, a chimeric antigen receptor includes a transmembrane domain that is at least 80% (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, or at least 99% identical) to amino acids 153 to 179 of SEQ ID NO: 61, or a portion thereof.

SEQ ID NO: 61

MLRLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLCKYSYNLFSREFR
ASLHKGLDSAVEVCVVYGNYSQQLQVYSKTGFNC DGKLGNESVTFYLQNL
VNQTDIYFCKIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLV
VVGGLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPY
APPRDFAAYRS

20

In some embodiments, a CAR comprises a transmembrane domain and extracellular stalk domain having the sequence of SEQ ID NO: 97. In some embodiments, a chimeric antigen receptor includes transmembrane domain and extracellular stalk domain that is or includes a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, or at least 99% identical) to SEQ ID NO: 97.

25

Exemplary Polypeptide Sequence for CD28 Stalk and TMD (SEQ ID NO: 97)

LDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVGGLACYSLLVTVAFI
FWV

30

In some embodiments, a nucleic acid encoding a CAR comprises a nucleic acid sequence encoding a transmembrane domain and extracellular stalk domain having the sequence of SEQ ID NO: 98. In some embodiments, a nucleic acid encoding a chimeric antigen receptor includes a nucleic acid encoding a transmembrane domain and extracellular stalk domain that is or includes a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, or at least 99% identical) to SEQ ID NO: 98.

Exemplary Nucleic Acid Sequence Encoding a Polypeptide Sequence for CD28 Stalk and TMD (SEQ ID NO: 98)

ctagacaatgagaagagcaatggaaccattatccatgtgaaagggaaacaccttgtccaagtcctattcccgaccttc
taagccctttgggtgctggtggtggtggtggagtctgcttgctatagcttgctagtaacagtggccttattttctgggt
g

In some embodiments, a chimeric antigen receptor includes a transmembrane domain, or portion thereof, from human CD3, e.g., GenBank Accession No. P20963, e.g., amino acids 31 to 51 of SEQ ID NO: 62. In some embodiments, a chimeric antigen receptor includes a transmembrane domain that is or includes a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, or at least 99% identical) to amino acids 31 to 51 of SEQ ID NO: 62.

SEQ ID NO: 62

MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPQRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

In some embodiments, a chimeric antigen receptor includes a transmembrane domain, or portion thereof, of any one of SEQ ID NOs. 63-69.

LGLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO: 63);

VAAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO: 64);
 ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO: 65);
 LCYLLDGILFIYGVILTALFLRV (SEQ ID NO: 66);
 WVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 67);
 5 IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 68); and
 ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO: 69).

In some embodiments, a chimeric antigen receptor includes a transmembrane domain that is or includes a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 85%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%,
 10 at least 95%, at least 96%, at least 98%, or at least 99% identical) to any one of SEQ ID NOs. 63-69.

As will be appreciated by those of ordinary skill in the art, certain endogenous polypeptides have two or more isoforms that differ at least in their primary polypeptide sequence. A chimeric antigen receptor disclosed herein can include a
 15 transmembrane domain that includes a sequence of amino acids from any isoform of an endogenous transmembrane protein (e.g., an endogenous mammalian, e.g., human, transmembrane protein) including, e.g., an isoform (e.g., a human isoform) of: an α chain of a T cell receptor, a β chain of the T cell receptor, a ζ chain of the T cell receptor, CD28, CD3 ϵ , CD3 δ , CD3 γ , CD33, CD37, CD64, CD80, CD45, CD4, CD5,
 20 CD8 α , CD9, CD16, CD22, CD86, CD134, CD137, CD27, CD152, PD1, or CD154.

In some embodiments, a transmembrane domain, or portion thereof, of a chimeric antigen receptor includes a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the transmembrane domains from one or more of the
 25 following endogenous mammalian (e.g., human) transmembrane proteins: an α chain of a T cell receptor, a β chain of the T cell receptor, a ζ chain of the T cell receptor, CD28, CD3 ϵ , CD3 δ , CD3 γ , CD33, CD37, CD64, CD80, CD45, CD4, CD5, CD8 α , CD9, CD16, CD22, CD86, CD134, CD137, CD27, CD152, PD1, or CD154. In some embodiments, a transmembrane domain, or portion thereof, of a chimeric antigen
 30 receptor includes a sequence of amino acids having one or more amino acid substitutions, deletions, or additions as compared to the transmembrane domain of an endogenous mammalian (e.g., human) transmembrane protein: an α chain of a T cell receptor, a β chain of the T cell receptor, a ζ chain of the T cell receptor, CD28,

CD3 ϵ , CD3 δ , CD3 γ , CD33, CD37, CD64, CD80, CD45, CD4, CD5, CD8 α , CD9, CD16, CD22, CD86, CD134, CD137, CD27, CD152, PD1, or CD154.

In some embodiments, a chimeric antigen receptor includes a synthetic transmembrane domain. In some cases, a synthetic transmembrane domain can include predominantly hydrophobic residues such as, without limitation, leucine and valine. In some embodiments, a synthetic transmembrane domain includes a triplet of phenylalanine, tryptophan, and valine at each end of the domain.

In some embodiments, a chimeric antigen receptor includes a transmembrane domain that is a chimeric transmembrane domain having portions of a transmembrane domain from two or more endogenous mammalian (e.g., human) transmembrane polypeptides such as, without limitation, an α chain of a T cell receptor, a β chain of the T cell receptor, a ζ chain of the T cell receptor, CD28, CD3 ϵ , CD3 δ , CD3 γ , CD33, CD37, CD64, CD80, CD45, CD4, CD5, CD8 α , CD9, CD16, CD22, CD86, CD134, CD137, CD27, CD152, PD1, and CD154, such that the two or more portions of transmembrane domains together constitute a functional transmembrane domain. In some embodiments, such a portion of a chimeric transmembrane domain can include one or more amino acid substitutions, deletions, or additions as compared to a corresponding portion of a wild type transmembrane domain.

A transmembrane domain can include one, two, three, four, five, six, seven, eight, nine, or ten contiguous amino acid sequences that each traverse a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell. As is known in the art, a transmembrane domain can, e.g., include at least one (e.g., two, three, four, five, six, seven, eight, nine, or ten) contiguous amino acid sequence (that traverses a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell) that has α -helical secondary structure in the lipid bilayer. In some embodiments, a transmembrane domain can include two or more contiguous amino acid sequences (that each traverse a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell) that form a β -barrel secondary structure in the lipid bilayer. Additional examples and features of transmembrane domains are known in the art.

Costimulatory Domains

In normal lymphocytes, T cell activation is mediated by two classes of intracellular signaling domains. Primary signaling is initiated via MHC-mediated antigen-dependent activation via the T cell receptor (e.g., a TCR/CD3 complex). A
 5 secondary or costimulatory signal is provided by a different receptor that includes a costimulatory signaling domain, which acts in an antigen-independent manner. Signals generated through the signaling domain of the TCR alone are insufficient for complete T cell activation; a co-stimulatory signal is also required.

In some embodiments, a chimeric antigen receptor includes a costimulatory
 10 domain, or portion thereof, from an endogenous mammalian (e.g., human) transmembrane polypeptide selected from the group of: CD27 (also known as S152, S152.LPFS2, T14, TNFRSF7, and Tp55), CD28 (also known as Tp44), 4-1BB (also known as TNFRSF9, CD137, CDw137, ILA, and tumor necrosis factor receptor superfamily member 9), OX40 (also known as TNFRSF4, ACT35, RP5-902P8.3,
 15 IMD16, CD134, TXGP1L, and tumor necrosis factor receptor superfamily member 4), CD30 (also known as TNFRSF8, D1S166E, and Ki-1), CD40L (also known as CD40LG, CD154, HIGM1, IGM, IMD3, T-BAM, TNFSF5, TRAP, gp39, hCD40L, and CD40 ligand), CD40 (also known as Bp50, CDW40, TNFRSF5, p50, CD40 (protein), and CD40 molecule), PD-1 (also known as PDCD1, CD279, PD-1, SLEB2,
 20 hPD-1, hPD-1, hSLE1, and Programmed cell death 1), PD-L1 (also known as CD274, B7-H, B7H1, PD-L1, PDCD1L1, PDCD1LG1, PDL1, CD274 molecule, and Programmed cell death 1 ligand 1), ICOS (also known as AILIM, CD278, and CVID1), LFA-1 (also known as Lymphocyte function-associated antigen 1), CD2 (also known as LFA-2, SRBC, T11, and CD2 molecule), CD7 (also known as GP40,
 25 LEU-9, TP41, Tp40, and CD7 molecule), CD160 (also known as BY55, NK1, NK28, and CD160 molecule), LIGHT (also known as TNFSF14, CD258, HVEM, LIGHT, LTg, TR2, TNLG1D, and tumor necrosis factor superfamily member 14), BTLA (also known as CD272 and BTLA1), TIM3 (also known as HAVCR2, HAVcr-2, KIM-3, TIM3, TIMD-3, TIMD3, Tim-3, CD366, and hepatitis A virus cellular receptor 2),
 30 CD244 (also known as 2B4, NAIL, NKR2B4, Nmrk, SLAMF4, and CD244 molecule), CD80 (also known as B7, B7-1, B7.1, BB1, CD28LG, CD28LG1, LAB7, and CD80 molecule), LAG3 (also known as CD223 and lymphocyte activating 3), NKG2C (also known as CD314, D12S2489E, KLR, NKG2-D, NKG2D, and killer

cell lectin like receptor K1), GITR (also known as TNFRSF18, RP5-902P8.2, AITR, CD357, and GITR-D), HVEM (also known as TNFRSF14, RP3-395M20.6, ATAR, CD270, HVEA, HVEM, LIGHTR, and TR2), TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD54 (ICAM), CD83, DAP10, LAT, SLP76, TRIM, ZAP70, and B7-H3 (also known as CD276, 4Ig-B7-H3, B7H3, B7RP-2, and CD276 molecule). In some embodiments, a single-chain chimeric polypeptide, a single-chain chimeric antigen receptor, or a multi-chain chimeric antigen receptor includes a costimulatory domain, or portion thereof, from an endogenous mammalian (e.g., human) transmembrane polypeptide (e.g., a mammalian or human homolog of any of the polypeptides listed above).

Any costimulatory domain, or portion thereof, that serves to provide a costimulatory signal is suitable for use in accordance with compositions and methods disclosed herein. In some embodiments, a chimeric antigen receptor includes a costimulatory domain, or portion thereof, from human CD28 (e.g. GenBank Accession No. P01747, e.g., from amino acids 180 to 220 of SEQ ID NO: 70). In some embodiments, a costimulatory domain is or includes a sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical (or is identical) to amino acids 180 to 220 of SEQ ID NO: 70, or a fragment thereof.

20

SEQ ID NO: 70 (Amino acids 180 to 220 are underlined)

MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSCKYSYNLFSREFR
 ASLHKGLDSAVEVCVVYGNYSQQLQVYSKTGFNC DGKLGNESVTFYLQONLY
 VNQTDIYFCKIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFWVLV
 VVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPY
APPRDFAAYRS

Exemplary Nucleic Acid Encoding Amino Acids a Human CD28 Costimulatory Domain (SEQ ID NO: 99)

aggagtaagaggagcaggctcctgcacagtgactacatgaacatgactccccgccgccccggcccccccaagcatt
 accagccctatgcccccaccacgcgacttcgcagcctatcgctcc

In some embodiments, a chimeric antigen receptor includes a costimulatory domain, or portion thereof, from human 4-1BB (e.g. GenBank Accession No. Q07011, e.g., from amino acids 214 to 255 of SEQ ID NO: 71). In some embodiments, a costimulatory domain is or includes a sequence that is at least 80%,
 5 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to amino acids 214 to 255 of SEQ ID NO: 75, or a portion thereof.

SEQ ID NO: 71

10 MGNSCYNIVATLLLVNFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFS
 SAGGQRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDC
 KQGQELTKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVV
 CGPSPADLSPGASSVTPAPAREPGHSPQIISFFLALTSTALLFLLFLLTLRFSVV
 KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

15

A chimeric antigen receptor disclosed herein can include a costimulatory domain that includes a sequence of amino acids from any isoform of an endogenous mammalian (e.g., human) transmembrane polypeptide having a costimulatory domain including, e.g., an isoform of: CD27, CD28, 4-1BB, OX40, CD30, CD40L, CD40,
 20 PD-1, PD-L1, ICOS, LFA-1, CD2, CD7, CD160, LIGHT, BTLA, TIM3, CD244, CD80, LAG3, NKG2C, or B7-H3 (including, without limitation, a mammalian or human homolog of any of these polypeptides).

In some embodiments, a costimulatory domain, or portion thereof, of a chimeric antigen receptor includes a sequence of amino acids that exhibits at least
 25 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a costimulatory domain from one or more of a mammalian (e.g., human) CD27, CD28, 4-1BB, OX40, CD30, CD40L, CD40, PD-1, PD-L1, ICOS, LFA-1, CD2, CD7, CD160, LIGHT, BTLA, TIM3, CD244, CD80, LAG3, NKG2C, or B7-H3. In some embodiments, a costimulatory domain, or
 30 portion thereof, of a chimeric antigen receptor includes a sequence of amino acids having one or more amino acid substitutions, deletions, or additions as compared to a costimulatory domain of one or more of an endogenous mammalian (e.g., human) transmembrane polypeptide: an α chain of a T cell receptor, a β chain of the T cell

receptor, a ζ chain of the T cell receptor, CD27, CD28, 4-1BB, OX40, CD30, CD40L, CD40, PD-1, PD-L1, ICOS, LFA-1, CD2, CD7, CD160, LIGHT, BTLA, TIM3, CD244, CD80, LAG3, NKG2C, or B7-H3 (including, without limitation, a mammalian or human homolog of any of these polypeptides).

5 In some embodiments, a chimeric antigen receptor includes a costimulatory domain that is a chimeric costimulatory domain having portions of a costimulatory domain from two or more endogenous mammalian (e.g., human) transmembrane polypeptides including, without limitation, CD27, CD28, 4-1BB, OX40, CD30, CD40L, CD40, PD-1, PD-L1, ICOS, LFA-1, CD2, CD7, CD160, LIGHT, BTLA,
10 TIM3, CD244, CD80, LAG3, NKG2C, or B7-H3 (including, without limitation, a mammalian or human homolog of any of these polypeptides), such that the two or more portions of the transmembrane domains together constitute a functional costimulatory domain. In some embodiments, such a portion of a chimeric costimulatory domain can include one or more amino acid substitutions, deletions, or
15 additions as compared to a corresponding portion of a wildtype costimulatory domain.

A costimulatory domain of a chimeric antigen receptor disclosed herein can be of any suitable length. For example, a costimulatory domain can have a length of about 20 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160 amino acids, about 150 amino acids,
20 about 140 amino acids, about 130 amino acids, about 120 amino acids, about 110 amino acids, about 100 amino acids, about 95 amino acids, about 90 amino acids, about 85 amino acids, about 80 amino acids, about 75 amino acids, about 70 amino acids, about 65 amino acids, about 60 amino acids, about 55 amino acids, about 50 amino acids, about 45 amino acids, about 40 amino acids, about 35 amino acids, about
25 30 amino acids, or about 25 amino acids (inclusive); about 25 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160 amino acids, about 150 amino acids, about 140 amino acids, about 130 amino acids, about 120 amino acids, about 110 amino acids, about 100 amino acids, about 95 amino acids, about 90 amino acids, about 85 amino acids, about 80
30 amino acids, about 75 amino acids, about 70 amino acids, about 65 amino acids, about 60 amino acids, about 55 amino acids, about 50 amino acids, about 45 amino acids, about 40 amino acids, about 35 amino acids, or about 30 amino acids (inclusive); about 30 amino acids to about 200 amino acids, about 190 amino acids, about 180

amino acids, about 170 amino acids, about 160 amino acids, about 150 amino acids,
about 140 amino acids, about 130 amino acids, about 120 amino acids, about 110
amino acids, about 100 amino acids, about 95 amino acids, about 90 amino acids,
about 85 amino acids, about 80 amino acids, about 75 amino acids, about 70 amino
5 acids, about 65 amino acids, about 60 amino acids, about 55 amino acids, about 50
amino acids, about 45 amino acids, about 40 amino acids, or about 35 amino acids
(inclusive); about 35 amino acids to about 200 amino acids, about 190 amino acids,
about 180 amino acids, about 170 amino acids, about 160 amino acids, about 150
amino acids, about 140 amino acids, about 130 amino acids, about 120 amino acids,
10 about 110 amino acids, about 100 amino acids, about 95 amino acids, about 90 amino
acids, about 85 amino acids, about 80 amino acids, about 75 amino acids, about 70
amino acids, about 65 amino acids, about 60 amino acids, about 55 amino acids, about
50 amino acids, about 45 amino acids, or about 40 amino acids (inclusive); about 40
amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids,
15 about 170 amino acids, about 160 amino acids, about 150 amino acids, about 140
amino acids, about 130 amino acids, about 120 amino acids, about 110 amino acids,
about 100 amino acids, about 95 amino acids, about 90 amino acids, about 85 amino
acids, about 80 amino acids, about 75 amino acids, about 70 amino acids, about 65
amino acids, about 60 amino acids, about 55 amino acids, about 50 amino acids, or
20 about 45 amino acids (inclusive); about 45 amino acids to about 200 amino acids,
about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160
amino acids, about 150 amino acids, about 140 amino acids, about 130 amino acids,
about 120 amino acids, about 110 amino acids, about 100 amino acids, about 95
amino acids, about 90 amino acids, about 85 amino acids, about 80 amino acids, about
25 75 amino acids, about 70 amino acids, about 65 amino acids, about 60 amino acids,
about 55 amino acids, or about 50 amino acids (inclusive); about 50 amino acids to
about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170
amino acids, about 160 amino acids, about 150 amino acids, about 140 amino acids,
about 130 amino acids, about 120 amino acids, about 110 amino acids, about 100
30 amino acids, about 95 amino acids, about 90 amino acids, about 85 amino acids, about
80 amino acids, about 75 amino acids, about 70 amino acids, about 65 amino acids,
about 60 amino acids, or about 55 amino acids (inclusive); about 55 amino acids to
about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170

amino acids, about 160 amino acids, about 150 amino acids, about 140 amino acids,
about 130 amino acids, about 120 amino acids, about 110 amino acids, about 100
amino acids, about 95 amino acids, about 90 amino acids, about 85 amino acids, about
80 amino acids, about 75 amino acids, about 70 amino acids, about 65 amino acids, or
5 about 60 amino acids (inclusive); about 60 amino acids to about 200 amino acids,
about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160
amino acids, about 150 amino acids, about 140 amino acids, about 130 amino acids,
about 120 amino acids, about 110 amino acids, about 100 amino acids, about 95
amino acids, about 90 amino acids, about 85 amino acids, about 80 amino acids, about
10 75 amino acids, about 70 amino acids, or about 65 amino acids (inclusive); about 65
amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids,
about 170 amino acids, about 160 amino acids, about 150 amino acids, about 140
amino acids, about 130 amino acids, about 120 amino acids, about 110 amino acids,
about 100 amino acids, about 95 amino acids, about 90 amino acids, about 85 amino
15 acids, about 80 amino acids, about 75 amino acids, or about 70 amino acids
(inclusive); about 70 amino acids to about 200 amino acids, about 190 amino acids,
about 180 amino acids, about 170 amino acids, about 160 amino acids, about 150
amino acids, about 140 amino acids, about 130 amino acids, about 120 amino acids,
about 110 amino acids, about 100 amino acids, about 95 amino acids, about 90 amino
20 acids, about 85 amino acids, or about 80 amino acids (inclusive); about 80 amino
acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about
170 amino acids, about 160 amino acids, about 150 amino acids, about 140 amino
acids, about 130 amino acids, about 120 amino acids, about 110 amino acids, about
100 amino acids, about 95 amino acids, or about 90 amino acids (inclusive); about 90
25 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids,
about 170 amino acids, about 160 amino acids, about 150 amino acids, about 140
amino acids, about 130 amino acids, about 120 amino acids, about 110 amino acids,
or about 100 amino acids (inclusive); about 100 amino acids to about 200 amino
acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, about
30 160 amino acids, about 150 amino acids, about 140 amino acids, about 130 amino
acids, about 120 amino acids, or about 110 amino acids (inclusive); about 110 amino
acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about
170 amino acids, about 160 amino acids, about 150 amino acids, about 140 amino

acids, about 130 amino acids, or about 120 amino acids (inclusive); about 120 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160 amino acids, about 150 amino acids, about 140 amino acids, or about 130 amino acids (inclusive); about 130 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160 amino acids, about 150 amino acids, or about 140 amino acids (inclusive); about 140 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, about 160 amino acids, or about 150 amino acids (inclusive); about 150 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, about 170 amino acids, or about 160 amino acids (inclusive); about 160 amino acids to about 200 amino acids, about 190 amino acids, about 180 amino acids, or about 170 amino acids (inclusive); about 170 amino acids to about 200 amino acids, about 190 amino acids, or about 180 amino acids (inclusive); about 180 amino acids to about 200 amino acids or about 190 amino acids (inclusive); or about 190 amino acids to about 200 amino acids (inclusive).

In some embodiments, a chimeric antigen receptor includes two or more costimulatory domains, e.g., two, three, four, five, or more costimulatory domains. In some embodiments, the two or more costimulatory domains are identical (e.g., they have the same amino acid sequence). In some embodiments, the costimulatory domains are not identical. For example, the costimulatory domains can be selected from different endogenous mammalian (e.g., human) transmembrane polypeptides including, without limitation, CD27, CD28, 4-1BB, OX40, CD30, CD40L, CD40, PD-1, PD-L1, ICOS, LFA-1, CD2, CD7, CD160, LIGHT, BTLA, TIM3, CD244, CD80, LAG3, NKG2C, or B7-H3 (including, without limitation, a mammalian or human homolog of any of these polypeptides). In some embodiments, the two or more costimulatory domains can differ from each other by one or more (e.g., two, three, four, or five) amino acid substitutions, deletions, or additions. In some embodiments, the two or more costimulatory domains exhibit at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to each other.

Immunoreceptor Tyrosine-Based Activation Motifs (ITAMs)

ITAMs are typically repeated (e.g., two or more times) in the cytoplasmic tails of certain cell surface proteins of the immune system, and are typically separated by between six and eight amino acids.

5 In some embodiments, a chimeric antigen receptor includes an ITAM, or portion thereof, from an endogenous mammalian (e.g., human) polypeptide, wherein endogenous mammalian (e.g., human) polypeptide is selected from the group of: CD3 ζ (also referred to as CD3 zeta), CD3 δ (CD3 delta), CD3 ϵ (CD3 epsilon), CD3 γ (CD3 gamma), DAP12, FC ϵ R1 γ (Fc epsilon receptor I gamma chain), FcR γ , FcR δ ,
10 CD35, CD22, CD79A (antigen receptor complex-associated protein alpha chain), CD79B (antigen receptor complex-associated protein beta chain), and CD66d.

Any ITAM, or portion thereof, that serves to mediate signaling in an endogenous mammalian (e.g., human) transmembrane protein suitable for use in accordance with compositions and methods disclosed herein. In some embodiments,
15 a chimeric antigen receptor includes an ITAM, or portion thereof, from human CD3 zeta (e.g. GenBank Accession No. P20963, e.g., an ITAM present in amino acids 52 – 164 of SEQ ID NO: 72, or a portion thereof; or SEQ ID NO: 73 or a portion thereof). In some embodiments, an ITAM comprises a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least
20 94%, at least 96%, at least 98%, at least 99%, or 100% identical to: the sequence of amino acids 52-164 of SEQ ID NO: 72 (or a portion thereof), or the sequence of SEQ ID NO: 73 (or a portion thereof).

SEQ ID NO: 72

25 MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPKRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

30 **SEQ ID NO: 73** (Human CD3 zeta signaling domain)

LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPKR
RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPR

SEQ ID NO: 74 (cDNA encoding human CD3 zeta signaling domain of SEQ ID NO: 73)

ctgagagtgaagttcagcaggagcgcagacgccccgcgtaccagcaggccagaaccagctctataacgagctcaat
 ctaggacgaagagaggagtacgatgttttggacaagagacgtggccgggaccctgagatggggggaaagccgagaag
 5 gaagaaccctcaggaaggcctgtacaatgaactgcagaaagataagatggcggaggcctacagtgagattgggatgaaa
 ggcgagcgccggaggggcaaggggcacgatggcctttaccagggtctcagtacagccaccaaggacacctacgacgc
 ccttcacatgcaggccctgccccctcgc

As will be appreciated by those of ordinary skill in the art, certain
 10 polypeptides have two or more isoforms that differ at least in their primary
 polypeptide sequence. For example, different isoforms can be generated as a result of
 alternative splicing. A chimeric antigen receptor disclosed herein can include an
 ITAM that includes a sequence of amino acids from any isoform of an endogenous
 mammalian transmembrane polypeptide having an ITAM including, e.g., a
 15 mammalian (e.g., human) isoform of: CD3 ζ , CD3D, CD3E, CD3G, DAP12,
 FCER1G, FcR γ , FcR δ , CD35, CD22, CD79A, CD79B, or CD66d.

In some embodiments, an ITAM, or portion thereof, of a chimeric antigen
 receptor includes a sequence of amino acids having one or more (e.g., two, three, four,
 or five) amino acid substitutions, deletions, or additions as compared to an ITAM of
 20 one or more of an ITAM in an endogenous mammalian (e.g., human) transmembrane
 protein, such as, CD3 ζ , CD3D, CD3E, CD3G, DAP12, FCER1G, FcR γ , FcR δ , CD35,
 CD22, CD79A, CD79B, or CD66d. For example, the tyrosine and leucine or
 isoleucine of an ITAM could be retained, while the two amino acids separating them
 could be replaced with different amino acids.

25 In some embodiments, a chimeric antigen receptor includes an ITAM that is a
 chimeric ITAM having portions of an ITAM from two or more endogenous
 mammalian (e.g., human) transmembrane polypeptides including, without limitation,
 CD3 ζ , CD3D, CD3E, CD3G, DAP12, FCER1G, FcR γ , FcR δ , CD35, CD22, CD79A,
 CD79B, or CD66d (including, without limitation, a mammalian or human homolog of
 30 any of these polypeptides), such that the two or more ITAM portions together
 constitute a functional ITAM. In some embodiments, such a portion of a chimeric
 ITAM can include one or more amino acid substitutions, deletions, or additions as
 compared to a corresponding portion of a wild type ITAM.

In some embodiments, a chimeric antigen receptor includes two or more ITAMs, e.g., two, three, four, five, or more ITAMs. In some embodiments, the two or more ITAMs are identical (e.g., they have the same amino acid sequence). In some embodiments, the two or more ITAMs are not identical. For example, the ITAMs can be selected from different endogenous mammalian (e.g., human) transmembrane polypeptides including, without limitation, CD3 ζ , CD3D, CD3E, CD3G, DAP12, FCER1G, FcR γ , FcR β , CD35, CD22, CD79A, CD79B (including, without limitation, a mammalian or human homolog of any of these polypeptides). In some embodiments, the two or more ITAMs can differ from each other by one or more amino acid substitutions, deletions, or additions.

CAR-Linker Sequences

Any two neighboring domains of a chimeric antigen receptor can be separated by a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art).

In some embodiments, the linker sequence between the antigen-binding domain and the transmembrane domain can be 1 amino acid to about 250 amino acids, 1 amino acid to about 240 amino acids, 1 amino acid to about 230 amino acids, 1 amino acid to about 220 amino acids, 1 amino acid to about 210 amino acids, 1 amino acid to about 200 amino acids, 1 amino acid to about 190 amino acids, 1 amino acid to about 180 amino acids, 1 amino acid to about 170 amino acids, 1 amino acid to about 160 amino acids, 1 amino acid to about 150 amino acids, 1 amino acid to about 140 amino acids, 1 amino acid to about 130 amino acids, 1 amino acid to about 120 amino acids, 1 amino acid to about 110 amino acids, 1 amino acid to about 100 amino acids, 1 amino acid to about 95 amino acids, 1 amino acid to about 90 amino acids, 1 amino acid to about 85 amino acids, 1 amino acid to about 80 amino acids, 1 amino acid to about 75 amino acids, 1 amino acid to about 70 amino acids, 1 amino acid to about 65 amino acids, 1 amino acid to about 60 amino acids, 1 amino acid to about 55 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 20 amino acids, 1 amino acid to about 15 amino acids, 1 amino acid to about 10 amino acids, 1 amino acid to about 5 amino acids, about 5 amino

acids to about 250 amino acids, about 5 amino acids to about 240 amino acids, about
5 amino acids to about 230 amino acids, about 5 amino acids to about 220 amino
acids, about 5 amino acids to about 210 amino acids, about 5 amino acids to about
200 amino acids, about 5 amino acids to about 190 amino acids, about 5 amino acids
5 to about 180 amino acids, about 5 amino acids to about 170 amino acids, about 5
amino acids to about 160 amino acids, about 5 amino acids to about 150 amino acids,
about 5 amino acids to about 140 amino acids, about 5 amino acids to about 130
amino acids, about 5 amino acids to about 120 amino acids, about 5 amino acids to
about 110 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino
10 acids to about 95 amino acids, about 5 amino acids to about 90 amino acids, about 5
amino acids to about 85 amino acids, about 5 amino acids to about 80 amino acids,
about 5 amino acids to about 75 amino acids, about 5 amino acids to about 70 amino
acids, about 5 amino acids to about 65 amino acids, about 5 amino acids to about 60
amino acids, about 5 amino acids to about 55 amino acids, about 5 amino acids to
15 about 50 amino acids, about 5 amino acids to about 45 amino acids, about 5 amino
acids to about 40 amino acids, about 5 amino acids to about 35 amino acids, about 5
amino acids to about 30 amino acids, about 5 amino acids to about 25 amino acids,
about 5 amino acids to about 20 amino acids, about 5 amino acids to about 15 amino
acids, about 5 amino acids to about 10 amino acids, about 10 amino acids to about
20 250 amino acids, about 10 amino acids to about 240 amino acids, about 10 amino
acids to about 230 amino acids, about 10 amino acids to about 220 amino acids, about
10 amino acids to about 210 amino acids, about 10 amino acids to about 200 amino
acids, about 10 amino acids to about 190 amino acids, about 10 amino acids to about
180 amino acids, about 10 amino acids to about 170 amino acids, about 10 amino
25 acids to about 160 amino acids, about 10 amino acids to about 150 amino acids, about
10 amino acids to about 140 amino acids, about 10 amino acids to about 130 amino
acids, about 10 amino acids to about 120 amino acids, about 10 amino acids to about
110 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino
acids to about 95 amino acids, about 10 amino acids to about 90 amino acids, about
30 10 amino acids to about 85 amino acids, about 10 amino acids to about 80 amino
acids, about 10 amino acids to about 75 amino acids, about 10 amino acids to about
70 amino acids, about 10 amino acids to about 65 amino acids, about 10 amino acids
to about 60 amino acids, about 10 amino acids to about 55 amino acids, about 10

amino acids to about 50 amino acids, about 10 amino acids to about 45 amino acids,
about 10 amino acids to about 40 amino acids, about 10 amino acids to about 35
amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to
about 25 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino
5 acids to about 15 amino acids, about 15 amino acids to about 250 amino acids, about
15 amino acids to about 240 amino acids, about 15 amino acids to about 230 amino
acids, about 15 amino acids to about 220 amino acids, about 15 amino acids to about
210 amino acids, about 15 amino acids to about 200 amino acids, about 15 amino
acids to about 190 amino acids, about 15 amino acids to about 180 amino acids, about
10 15 amino acids to about 170 amino acids, about 15 amino acids to about 160 amino
acids, about 15 amino acids to about 150 amino acids, about 15 amino acids to about
140 amino acids, about 15 amino acids to about 130 amino acids, about 15 amino
acids to about 120 amino acids, about 15 amino acids to about 110 amino acids, about
15 amino acids to about 100 amino acids, about 15 amino acids to about 95 amino
15 acids, about 15 amino acids to about 90 amino acids, about 15 amino acids to about
85 amino acids, about 15 amino acids to about 80 amino acids, about 15 amino acids
to about 75 amino acids, about 15 amino acids to about 70 amino acids, about 15
amino acids to about 65 amino acids, about 15 amino acids to about 60 amino acids,
about 15 amino acids to about 55 amino acids, about 15 amino acids to about 50
20 amino acids, about 15 amino acids to about 45 amino acids, about 15 amino acids to
about 40 amino acids, about 15 amino acids to about 35 amino acids, about 15 amino
acids to about 30 amino acids, about 15 amino acids to about 25 amino acids, about
15 amino acids to about 20 amino acids, about 20 amino acids to about 250 amino
acids, about 20 amino acids to about 240 amino acids, about 20 amino acids to about
25 230 amino acids, about 20 amino acids to 220 amino acids, about 20 amino acids to
about 210 amino acids, about 20 amino acids to about 200 amino acids, about 20
amino acids to about 190 amino acids, about 20 amino acids to about 180 amino
acids, about 20 amino acids to about 170 amino acids, about 20 amino acids to about
160 amino acids, about 20 amino acids to about 150 amino acids, about 20 amino
30 acids to about 140 amino acids, about 20 amino acids to about 130 amino acids, about
20 amino acids to about 120 amino acids, about 20 amino acids to about 110 amino
acids, about 20 amino acids to about 100 amino acids, about 20 amino acids to about
95 amino acids, about 20 amino acids to about 90 amino acids, about 20 amino acids

[illegible]

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
5 amino acids, about 30 amino acids to about 60 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 250 amino
acids, about 35 amino acids to about 240 amino acids, about 35 amino acids to about
10 230 amino acids, about 35 amino acids to about 220 amino acids, about 35 amino
acids to about 210 amino acids, about 35 amino acids to about 200 amino acids, about
35 amino acids to about 190 amino acids, about 35 amino acids to about 180 amino
acids, about 35 amino acids to about 170 amino acids, about 35 amino acids to about
160 amino acids, about 35 amino acids to about 150 amino acids, about 35 amino
15 acids to about 140 amino acids, about 35 amino acids to about 130 amino acids, about
35 amino acids to about 120 amino acids, about 35 amino acids to about 110 amino
acids, about 35 amino acids to about 100 amino acids, about 35 amino acids to about
95 amino acids, about 35 amino acids to about 90 amino acids, about 35 amino acids
to about 85 amino acids, about 35 amino acids to about 80 amino acids, about 35
20 amino acids to about 75 amino acids, about 35 amino acids to about 70 amino acids,
about 35 amino acids to about 65 amino acids, about 35 amino acids to about 60
amino acids, about 35 amino acids to about 55 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 250 amino acids, about
25 40 amino acids to about 240 amino acids, about 40 amino acids to about 230 amino
acids, about 40 amino acids to about 220 amino acids, about 40 amino acids to about
210 amino acids, about 40 amino acids to about 200 amino acids, about 40 amino
acids to about 190 amino acids, about 40 amino acids to about 180 amino acids, about
40 amino acids to about 170 amino acids, about 40 amino acids to about 160 amino
30 acids, about 40 amino acids to about 150 amino acids, about 40 amino acids to about
140 amino acids, about 40 amino acids to about 130 amino acids, about 40 amino
acids to about 120 amino acids, about 40 amino acids to about 110 amino acids, about
40 amino acids to about 100 amino acids, about 40 amino acids to about 95 amino

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acids to about 180 amino acids, about 65 amino acids to about 170 amino acids, about
65 amino acids to about 160 amino acids, about 65 amino acids to about 150 amino
acids, about 65 amino acids to about 140 amino acids, about 65 amino acids to about
130 amino acids, about 65 amino acids to about 120 amino acids, about 65 amino
5 acids to about 110 amino acids, about 65 amino acids to about 100 amino acids, about
65 amino acids to about 95 amino acids, about 65 amino acids to about 90 amino
acids, about 65 amino acids to about 85 amino acids, about 65 amino acids to about
80 amino acids, about 65 amino acids to about 75 amino acids, about 65 amino acids
to about 70 amino acids, about 70 amino acids to about 250 amino acids, about 70
10 amino acids to about 240 amino acids, about 70 amino acids to about 230 amino
acids, about 70 amino acids to about 220 amino acids, about 70 amino acids to about
210 amino acids, about 70 amino acids to about 200 amino acids, about 70 amino
acids to about 190 amino acids, about 70 amino acids to about 180 amino acids, about
70 amino acids to about 170 amino acids, about 70 amino acids to about 160 amino
15 acids, about 70 amino acids to about 150 amino acids, about 70 amino acids to about
140 amino acids, about 70 amino acids to about 130 amino acids, about 70 amino
acids to about 120 amino acids, about 70 amino acids to about 110 amino acids, about
70 amino acids to about 100 amino acids, about 70 amino acids to about 95 amino
acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about
20 85 amino acids, about 70 amino acids to about 80 amino acids, about 70 amino acids
to about 75 amino acids, about 75 amino acids to about 250 amino acids, about 75
amino acids to about 240 amino acids, about 75 amino acids to about 230 amino
acids, about 75 amino acids to about 220 amino acids, about 75 amino acids to about
210 amino acids, about 75 amino acids to about 200 amino acids, about 75 amino
25 acids to about 190 amino acids, about 75 amino acids to about 180 amino acids, about
75 amino acids to about 170 amino acids, about 75 amino acids to about 160 amino
acids, about 75 amino acids to about 150 amino acids, about 75 amino acids to about
140 amino acids, about 75 amino acids to about 130 amino acids, about 75 amino
acids to about 120 amino acids, about 75 amino acids to about 110 amino acids, about
30 75 amino acids to about 100 amino acids, about 75 amino acids to about 95 amino
acids, about 75 amino acids to about 90 amino acids, about 75 amino acids to about
85 amino acids, about 75 amino acids to about 80 amino acids, about 80 amino acids
to about 250 amino acids, about 80 amino acids to about 240 amino acids, about 80

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acids, about 220 amino acids to about 240 amino acids, about 220 amino acids to about 230 amino acids, about 230 amino acids to about 250 amino acids, about 230 amino acids to about 240 amino acids, or about 240 amino acids to about 250 amino acids.

5 In some embodiments, a linker sequence between the antigen-binding domain and the transmembrane domain can be or can include one or more of an IgG1, IgG2, IgG3, or IgG4 CH1, CH2, and CH3 domain. In some embodiments, the linker between the antigen-binding domain and the transmembrane domain can be or can include CH2-CH3 human IgG1 domains. In some embodiments, the CH2-CH3
10 human IgG1 domains have a sequence of:

AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCWVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG
15 SFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGKKD (SEQ ID NO: 75).

In some embodiments, the linker sequence between the antigen-binding domain and the transmembrane domain can be or include a portion of the human CD8 extracellular sequence that is proximal to the human CD8 transmembrane domain.
20 For example, the linker sequence between the antigen-binding domain and the transmembrane domain can be or include human CD8 sequence of

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI (SEQ ID NO: 76).

In some embodiments, the linker sequence between the antigen-binding domain and the transmembrane domain can be or include a human IgG1 hinge sequence. In some embodiments, the human IgG1 hinge sequence is
25 AEPKSPDKTHTCPPCPKDPK (SEQ ID NO: 77).

In some embodiments, a linker sequence (e.g., any of the linker sequences described herein or known in the art) can be present between the transmembrane domain and a costimulatory domain. In some embodiments, a linker sequence (e.g.,
30 any of the linker sequences described herein or known in the art) can be present between the costimulatory domain and the ITAM.

Nucleic Acids

Also provided herein are nucleic acids that encode any of the variety of polypeptides having a transmembrane domain of an alpha chain of interleukin-7 receptor having one or more amino acid modifications, chimeric transmembrane proteins, or other proteins described herein.

Vectors

Provided herein are vectors that include any of the nucleic acids that encode any of the variety of polypeptides having a transmembrane domain of an alpha chain of interleukin-7 receptor having one or more amino acid modifications, chimeric transmembrane proteins, or other proteins provided herein. A “vector” according to the present disclosure is a polynucleotide capable of inducing the expression of a recombinant protein (e.g., a chimeric transmembrane protein, a protein, and/or a chimeric antigen receptor) in a mammalian cell. A vector provided herein can be, e.g., in circular or linearized form. Non-limiting examples of vectors include plasmids, SV40 vectors, adenoviral viral vectors, and adeno-associated virus (AAV) vectors. Non-limiting examples of vectors include lentiviral vectors or retroviral vectors, e.g., gamma-retroviral vectors. See, e.g., Carlens et al., *Exp. Hematol.* 28(10):1137-1146, 2000; Park et al., *Trends Biotechnol.* 29(11):550-557, 2011; and Alonso-Camino et al., *Mol. Ther. Nucleic Acids* 2:e93, 2013. Non-limiting examples of retroviral vectors include those derived from Moloney murine leukemia virus, myeloproliferative sarcoma virus, murine embryonic stem cell virus, murine stem cell virus, spleen focus forming virus, or adeno-associated virus. Non-limiting examples of retroviral vectors are described in, e.g., U.S. Patent Nos. 5,219,740 and 6,207,453; Miller et al., *BioTechniques* 7:980-990, 1989; Miller, *Human Gene Therapy* 1:5-14, 1990; Scarpa et al., *Virology* 180:849-852, 1991; Burns et al., *Proc. Natl. Acad. Sci. U.S.A.* 90:8033-8037, 1993; and Boris-Lawrie et al., *Cur. Opin. Genet. Develop.* 3:102-109, 1993. Exemplary lentiviral vectors are described in, e.g., Wang et al., *J. Immunother.* 35(9):689-701, 2003; Cooper et al., *Blood* 101:1637-1644, 2003; Verhoeven et al., *Methods Mol. Biol.* 506:97-114, 2009; and Cavalieri et al., *Blood* 102(2):497-505, 2003.

Exemplary vectors, in which any of the nucleic acids provided herein can be inserted, are described in, e.g., Ausubel et al., Eds. “Current Protocols in Molecular

Biology” Current Protocols, 1993; and Sambrook et al., Eds. “Molecular Cloning: A Laboratory Manual,” 2nd ed., Cold Spring Harbor Press, 1989.

In some embodiments, the vectors further include a promoter sequence and/or enhancer sequence operably linked to any of the nucleic acids described herein. Non-limiting examples of promoters include promoters from human cytomegalovirus (CMV), mouse phosphoglycerate kinase 1, polyoma adenovirus, thyroid stimulating hormone α , vimentin, simian virus 40 (SV40), tumor necrosis factor, β -globin, α -fetoprotein, γ -globin, β -interferon, γ -glutamyl transferase, human ubiquitin C (UBC), mouse mammary tumor virus (MMTV), Rous sarcoma virus, glyceraldehyde-3-phosphate dehydrogenase, β -actin, metallothionein II (MT II), amylase, human EF1 α , cathepsin, MI muscarinic receptor, retroviral LTR (e.g. human T-cell leukemia virus HTLV), AAV ITR, interleukin-2, collagenase, platelet-derived growth factor, adenovirus E2, stromelysin, murine MX, rat insulin, glucose regulated protein 78, human immunodeficiency virus, glucose regulated protein 94, α -2-macroglobulin, MHC class I, HSP70, proliferin, immunoglobulin light chain, T-cell receptor, HLA DQ α , HLA DQ β , interleukin-2 receptor, MHC class II, prealbumin (transthyretin), elastase I, albumin, c-fos, neural cell adhesion molecule (NCAM), H2B histone, rat growth hormone, human serum amyloid (SAA), muscle creatinine kinase, troponin I (TN I), and Gibbon Ape Leukemia Virus (GALV). In some embodiments, the promoter may be an inducible promoter or a constitutive promoter. Additional examples of promoters are known in the art.

In some examples, the vectors provided herein further include a poly(A) sequence, which is operably linked and positioned 3' to the sequence encoding the chimeric transmembrane protein, the protein, or the chimeric antigen receptor. Non-limiting examples of a poly(A) sequence include those derived from bovine growth hormone (Woychik et al., *Proc. Natl. Acad. Sci. U.S.A.* 81(13): 3944-3948, 1984, and U.S. Patent No. 5,122, 458), mouse- β -globin, mouse- α -globin (Orkin et al., *EMBO J.* 4(2): 453-456, 1985), human collagen, polyoma virus (Batt et al., *Mol. Cell Biol.* 15(9):4783-4790, 1995), the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy chain gene polyadenylation signal (U.S. Patent Application Publication No. 2006/0040354), human growth hormone (hGH) (Szymanski et al., *Mol. Therapy* 15(7):1340-1347, 2007), SV40 poly(A) site, e.g., SV40 late and early poly(A) site (Schek et al., *Mol. Cell Biol.* 12(12):5386-5393, 1992). In some embodiments, the

poly(A) sequence includes a highly conserved upstream element (AATAAA). The this AATAAA sequence can, e.g., be substituted with other hexanucleotide sequences with homology to AATAAA which are capable of signaling polyadenylation, including, e.g., ATTAAA, AGTAAA, CATAAA, TATAAA, GATAAA, ACTAAA,
 5 AATATA, AAGAAA, AATAAT, AAAAAA, AATGAA, AATCAA, AACAAA, AATCAA, AATAAC, AATAGA, AATTAA, and AATAAG. See, e.g., WO 06012414 A2).

A poly(A) sequence can, e.g., be a synthetic polyadenylation site. See, e.g., Levitt et al, *Genes Dev.* 3(7): 1019-1025, 1989). In some examples, a poly(A)
 10 sequence can be the polyadenylation signal of soluble neuropilin-1: AAATAAAATACGAAATG (SEQ ID NO: 78). Additional examples of poly(A) sequences are known in the art. Additional examples and aspects of vectors are also known in the art.

In some embodiments of any of the vectors described herein, the vector can
 15 further include a sequence encoding a chimeric antigen receptor. In some embodiments, the chimeric antigen receptor can bind specifically to a tumor antigen (e.g., a tumor antigen selected from the group of glypican-3, BCMA, MAGE, MUC16, CD19, WT-1, CD22, LI-CAM, ROR-1, CEA, 4-1BB, ETA, 5T4, adenocarcinoma antigen, alpha-fetoprotein (AFP), BAFF, B-lymphoma cell, C242
 20 antigen, CA-125, carbonic anhydrase 9 (CA- IX), C-MET, CCR4, CD152, CD20, CD125 CD200, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor
 25 receptor kinase, IGF-1 receptor, IGF-I, IgG1, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha \nu\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88,
 30 VEGF-A, VEGFR-1, VEGFR2, and vimentin). In some embodiments, the chimeric antigen receptor comprises one or more co-stimulatory signaling domains selected from the group of 4-1BB, CD27, OX40, CD40, CD28, GITR, CD2, CD5, ICAM-1, CD11a, Lck, TNFR-I, TNFR-II, FasR, CD30, ICOS, LIGHT, NKG2C, B7-H3, DAP-

10, and DAP-12. In some examples of any of the vectors described herein, the vector is a lentiviral or an adenoviral vector.

Also provided herein are sets of vectors that include a first vector that includes a sequence that encodes any of the chimeric transmembrane proteins described herein (e.g., any of the vectors that includes a sequence that encodes any of the chimeric transmembrane proteins described herein), and a second vector that includes a sequence that encodes a chimeric antigen receptor (e.g., any of the chimeric antigen receptors described herein). In some embodiments, one or both of the first vector and the second vector is a lentiviral or an adenoviral vector. In some embodiments, the second vector further includes a promoter sequence and/or an enhancer sequence that is operably linked to the sequence encoding the chimeric antigen receptor. In some embodiments, the second vector further includes a poly(A) sequence operably linked to the sequence encoding the chimeric antigen receptor.

Methods of Introducing a Nucleic Acid or Vectors into a Mammalian Cell

A variety of different methods known in the art can be used to introduce any of the nucleic acids and vectors disclosed herein into a mammalian cell (e.g., any of the mammalian cells described herein, e.g., any of the T cells (e.g., human T cells) described herein). Non-limiting examples of methods that can be used to introduce a nucleic acid or vector into a mammalian cell include lipofection, transfection, electroporation, microinjection, calcium phosphate transfection, dendrimer-based transfection, cationic polymer transfection, cell squeezing, sonoporation, optical transfection, impalection, hydrodynamic delivery, magnetofection, viral transduction (e.g., adenoviral and lentiviral transduction), and nanoparticle transfection. Additional methods of introducing a nucleic acid or vector into a mammalian cell are known in the art.

Mammalian Cells

Also provided herein are mammalian cells that include any of the nucleic acids or vectors described herein. Also provided herein are mammalian cells that include any of the sets of vectors described herein.

In some embodiments, the mammalian cell is previously obtained from a subject (e.g., a human subject, e.g., a human subject identified or diagnosed as having a cancer) or is a daughter cell of a mammalian cell that was previously obtained from

a subject (e.g., a human subject, e.g., a human subject identified or diagnosed as having a cancer). In some embodiments, the mammalian cell is an immune cell. In some embodiments, the mammalian cell is a human cell.

Non-limiting examples of immune cells include a T cell (e.g., a human T cell).

5 Non limiting examples of T cells (e.g., human T cells) include, e.g., an immature thymocyte, a peripheral blood lymphocyte, a helper T cell, a naïve T cell, a pluripotent T_H cell precursor, a lymphoid progenitor cell, a T_{reg} cell, a memory T cell, a T_H17 cell, a T_H22 cell, a T_H9 cell, a T_H2 cell, a T_H1 cell, a T_H3 cell, $\gamma\delta$ T cell, an $\alpha\beta$ T cell, a regulatory T cell (Treg cell), and a tumor-infiltrating T cell. Additional
10 examples of a T cell (e.g., a human T cell) include a CD8⁺ T cell, a CD4⁺ T cell, a memory T cell, a Treg cell, natural killer cell, B cell, and a monocyte. Additional examples of mammalian cells include a mast cell, a macrophage, a neutrophil, a dendritic cell, a basophil, an eosinophil, and a natural killer cell.

Compositions and Kits

15 Also provided herein are compositions (e.g., pharmaceutical compositions) that include any of the nucleic acids, vectors, sets of nucleic acids, sets of vectors, or mammalian cells described herein. For example, provided herein is a composition that includes any of the nucleic acids or sets of nucleic acids described herein, or any of the vectors or sets of vectors provided herein, and a pharmaceutically acceptable
20 solvent or carrier.

In some embodiments, a composition can be any of the mammalian cells described herein (e.g., any of the mammalian cells described herein previously obtained from a subject, e.g., a subject identified or diagnosed as having a cancer) comprising a nucleic acid encoding any of the chimeric transmembrane proteins
25 and/or any of the chimeric antigen receptors described herein. In a composition including any of the mammalian cells described herein, the composition can further include a cell culture medium or a pharmaceutically acceptable buffer (e.g., phosphate-buffered saline). A composition that includes any of the mammalian cells described herein can be formulated for intravenous or intraarterial administration.

30 Also provided are kits that include one or more of any of the compositions (e.g., pharmaceutical compositions) described herein. In some embodiments, a kit can further include instructions for performing any of the methods described herein.

Methods of Treating a Cancer in a Subject

Also provided herein are methods of treating a cancer in a subject (e.g., a human, a mouse, a rabbit, a rat, a horse, a dog, a monkey, or an ape) that include administering a therapeutically effective amount of any of the mammalian cells including a nucleic acid encoding a polypeptide having a transmembrane domain of an alpha chain of interleukin-7 receptor having one or more amino acid modifications as described herein, a chimeric transmembrane protein as described herein, or both (and optionally a nucleic acid including any of the chimeric antigen receptors described herein). In some examples of these methods, the mammalian cell is a T cell (e.g., a CD8⁺ T cell, a CD4⁺ T cell, a memory T cell, a Treg cell, and a natural killer T cell). In some examples, the mammalian cell (e.g., any of the mammalian cells described herein) is a mammalian cell previously obtained from a subject (e.g., a subject that has been identified or diagnosed as having a cancer, e.g., any of the cancers described herein). Some embodiments of these methods further include obtaining the mammalian cell from the subject.

Some embodiments of these methods further include introducing a nucleic acid encoding a single-chain chimeric antigen receptor described herein or a multi-chain chimeric antigen receptor described herein into a mammalian cell (e.g., any of the mammalian cells described herein or known in the art) to generate the mammalian cell that is administered to the subject.

Non-limiting examples of cancer that can be treated using any of the methods provided herein include: hepatocellular carcinoma, acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, Kaposi sarcoma, lymphoma, anal cancer, appendix cancer, teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, bronchial tumor, carcinoid tumor, cardiac tumor, cervical cancer, chordoma, chronic lymphocytic leukemia, chronic myeloproliferative neoplasm, colon cancer, colorectal cancer, craniopharyngioma, bile duct cancer, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing sarcoma, eye cancer, fallopian tube cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, germ cell tumor, hairy cell leukemia, head and neck cancer, heart cancer, liver cancer, hypopharyngeal cancer, pancreatic cancer, kidney cancer, laryngeal cancer, chronic myelogenous leukemia, lip and oral cavity cancer, lung

cancer, melanoma, Merkel cell carcinoma, mesothelioma, mouth cancer, oral cancer, osteosarcoma, ovarian cancer, penile cancer, pharyngeal cancer, prostate cancer, rectal cancer, salivary gland cancer, skin cancer, small intestine cancer, soft tissue sarcoma, gastric cancer, testicular cancer, throat cancer, thyroid cancer, urethral
5 cancer, uterine cancer, vaginal cancer, and vulvar cancer.

In some embodiments of any of these methods, the methods result in a decrease in the tumor burden (e.g., a decrease in tumor mass and/or volume of a solid tumor) in a subject. For example, any of the methods described herein can result in at least about 1% to about 99% (e.g., about 1% to about 98%, about 96%, about 94%,
10 about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 2% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%,
15 about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 3% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
20 about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 5% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%,
25 about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, or about 10% (inclusive); about 10% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%,
30 about 40%, about 35%, about 30%, about 25%, about 20%, or about 15% (inclusive); about 15% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%,

about 45%, about 40%, about 35%, about 30%, about 25%, or about 20% (inclusive);
about 20% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%,
about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%,
about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%,
5 about 45%, about 40%, about 35%, about 30%, or about 25% (inclusive); about 25%
to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%,
about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%,
about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%,
about 40%, about 35%, or about 30% (inclusive); about 30% to about 99%, about
10 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about
84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about
70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, or about
35% (inclusive); about 35% to about 99%, about 98%, about 96%, about 94%, about
92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about
15 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about
55%, about 50%, about 45%, or about 40% (inclusive); about 40% to about 99%,
about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%,
about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%,
about 70%, about 65%, about 60%, about 55%, about 50%, or about 45% (inclusive);
20 about 45% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%,
about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%,
about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, or about 50%
(inclusive); about 50% to about 99%, about 98%, about 96%, about 94%, about 92%,
about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
25 about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, or about 55%
(inclusive); about 55% to about 99%, about 98%, about 96%, about 94%, about 92%,
about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
about 76%, about 74%, about 72%, about 70%, about 65%, or about 60% (inclusive);
about 60% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%,
30 about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%,
about 74%, about 72%, about 70%, or about 65% (inclusive); about 65% to about
99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about
86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about

72%, or about 70% (inclusive); about 70% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, or about 72% (inclusive); about 72% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, or about 74% (inclusive); about 74% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, or about 76% (inclusive); about 76% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, or about 78% (inclusive); about 78% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, or about 80% (inclusive); about 80% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, or about 82% (inclusive); about 82% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, or about 84% (inclusive); about 84% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, or about 86% (inclusive); about 86% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, or about 88% (inclusive); about 88% to about 99%, about 98%, about 96%, about 94%, about 92%, or about 90% (inclusive); about 90% to about 99%, about 98%, about 96%, about 94%, or about 92% (inclusive); about 92% to about 99%, about 98%, about 96%, or about 94% (inclusive); about 94% to about 99%, about 98%, or about 96% (inclusive); or about 96% to about 99% or about 98% (inclusive)) reduction in the tumor burden in a subject (e.g., as compared to the tumor burden in the subject prior to treatment).

In some embodiments, the methods result in a decrease in the rate of progression of a cancer in the subject. For example, any of the methods described herein can result in at least about 1% to about 99% (e.g., about 1% to about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 2% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%,

about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%,
about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%,
about 10%, or about 5% (inclusive); about 3% to about 99%, about 98%, about 96%,
about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%,
5 about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%,
about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%,
about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 5% to
about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%,
about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%,
10 about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%,
about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, or about 10%
(inclusive); about 10% to about 99%, about 98%, about 96%, about 94%, about 92%,
about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%,
15 about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, or
about 15% (inclusive); about 15% to about 99%, about 98%, about 96%, about 94%,
about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%,
about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%,
about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, or
20 about 20% (inclusive); about 20% to about 99%, about 98%, about 96%, about 94%,
about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%,
about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%,
about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, or about 25%
(inclusive); about 25% to about 99%, about 98%, about 96%, about 94%, about 92%,
25 about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%,
about 50%, about 45%, about 40%, about 35%, or about 30% (inclusive); about 30%
to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%,
about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%,
30 about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%,
about 40%, or about 35% (inclusive); about 35% to about 99%, about 98%, about
96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about
82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about

65%, about 60%, about 55%, about 50%, about 45%, or about 40% (inclusive); about 40% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, or about 45% (inclusive); about 45% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, or about 50% (inclusive); about 50% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, or about 55% (inclusive); about 55% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, or about 60% (inclusive); about 60% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, or about 65% (inclusive); about 65% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, or about 70% (inclusive); about 70% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, or about 72% (inclusive); about 72% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, or about 74% (inclusive); about 74% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, or about 76% (inclusive); about 76% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, or about 78% (inclusive); about 78% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, or about 80% (inclusive); about 80% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, or about 82% (inclusive); about 82% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, or about 84%

(inclusive); about 84% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, or about 86% (inclusive); about 86% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, or about 88% (inclusive); about 88% to about 99%, about 98%, about 96%, about 94%, about 92%, or about 90% (inclusive); about 90% to about 99%, about 98%, about 96%, about 94%, or about 92% (inclusive); about 92% to about 99%, about 98%, about 96%, or about 94% (inclusive); about 94% to about 99%, about 98%, or about 96% (inclusive); or about 96% to about 99% or about 98% (inclusive)) reduction in the rate of progression of a cancer in a subject (e.g., as compared to the rate of progression of a cancer in the subject prior to treatment or in a control subject or a control population of subjects having the same cancer and administered no treatment or a different treatment).

In some embodiments of any of these methods, the methods result in an increase in the time of survival of a cancer in a subject. For example, any of the methods described herein can result in an about 1% to about 800% (e.g., about 1% to about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, about 150%, about 100%, about 80%, about 60%, about 40%, about 20%, about 10%, or about 5% (inclusive); about 5% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, about 150%, about 100%, about 80%, about 60%, about 40%, or about 20% (inclusive); about 20% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, about 150%, about 100%, about 80%, about 60%, or about 40% (inclusive); about 40% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, about 150%, about 100%, about 80%, or about 60% (inclusive); about 60% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, about 150%, about 100%, about 80% (inclusive); about 80% to about 800%, about 750%,

about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, about 150%, or about 100% (inclusive); about 100% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, about 200%, or about 150% (inclusive); about 150% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, about 250%, or about 200% (inclusive); about 200% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, about 300%, or about 250% (inclusive); about 250% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, about 350%, or about 300% (inclusive); about 300% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, about 400%, or about 350% (inclusive); about 350% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, about 450%, or about 400% (inclusive); about 400% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, about 500%, or about 450% (inclusive); about 450% to about 800%, about 750%, about 700%, about 650%, about 600%, about 550%, or about 500% (inclusive); about 500% to about 800%, about 750%, about 700%, about 650%, about 600%, or about 550% (inclusive); about 550% to about 800%, about 750%, about 700%, about 650%, or about 600% (inclusive); about 600% to about 800%, about 750%, about 700%, or about 650% (inclusive); about 650% to about 800%, about 750%, or about 700% (inclusive); about 700% to about 800% or about 750% (inclusive); or about 750% to about 800% (inclusive)) increase in the time of survival of a cancer in a subject (e.g., as compared to the time of survival for a control subject or a population of control subjects having the same cancer and receiving no treatment or a different treatment).

Also provided herein are methods of inducing cell death in a cancer cell in a subject in need thereof that include administering to the subject a therapeutically effective amount of any of the mammalian cells described herein.

Also provided herein are methods of decreasing the risk of developing a metastasis or an additional metastasis in a subject having a cancer that include administering to the subject a therapeutically effective amount of any of the

mammalian cells described herein. For example, any of the methods described herein can result in at least about 1% to about 99% (e.g., about 1% to about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 2% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 3% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5% (inclusive); about 5% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, or about 10% (inclusive); about 10% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, or about 15% (inclusive); about 15% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, or about 20% (inclusive); about 20% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, or about 25% (inclusive); about 25% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%,

about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, about 50%,
about 45%, about 40%, about 35%, or about 30% (inclusive); about 30% to about
99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, about
86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%, about
5 72%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about
40%, or about 35% (inclusive); about 35% to about 99%, about 98%, about 96%,
about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%,
about 80%, about 78%, about 76%, about 74%, about 72%, about 70%, about 65%,
about 60%, about 55%, about 50%, about 45%, or about 40% (inclusive); about 40%
10 to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%,
about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%,
about 72%, about 70%, about 65%, about 60%, about 55%, about 50%, or about 45%
(inclusive); about 45% to about 99%, about 98%, about 96%, about 94%, about 92%,
about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
15 about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, about 55%, or
about 50% (inclusive); about 50% to about 99%, about 98%, about 96%, about 94%,
about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%,
about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, about 60%, or
about 55% (inclusive); about 55% to about 99%, about 98%, about 96%, about 94%,
20 about 92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%,
about 78%, about 76%, about 74%, about 72%, about 70%, about 65%, or about 60%
(inclusive); about 60% to about 99%, about 98%, about 96%, about 94%, about 92%,
about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about 78%,
about 76%, about 74%, about 72%, about 70%, or about 65% (inclusive); about 65%
25 to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%,
about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, about 74%,
about 72%, or about 70% (inclusive); about 70% to about 99%, about 98%, about
96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about
82%, about 80%, about 78%, about 76%, about 74%, or about 72% (inclusive); about
30 72% to about 99%, about 98%, about 96%, about 94%, about 92%, about 90%, about
88%, about 86%, about 84%, about 82%, about 80%, about 78%, about 76%, or about
74% (inclusive); about 74% to about 99%, about 98%, about 96%, about 94%, about
92%, about 90%, about 88%, about 86%, about 84%, about 82%, about 80%, about

78%, or about 76% (inclusive); about 76% to about 99%, about 98%, about 96%,
about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about 82%,
about 80%, or about 78% (inclusive); about 78% to about 99%, about 98%, about
96%, about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, about
5 82%, or about 80% (inclusive); about 80% to about 99%, about 98%, about 96%,
about 94%, about 92%, about 90%, about 88%, about 86%, about 84%, or about 82%
(inclusive); about 82% to about 99%, about 98%, about 96%, about 94%, about 92%,
about 90%, about 88%, about 86%, or about 84% (inclusive); about 84% to about
99%, about 98%, about 96%, about 94%, about 92%, about 90%, about 88%, or about
10 86% (inclusive); about 86% to about 99%, about 98%, about 96%, about 94%, about
92%, about 90%, or about 88% (inclusive); about 88% to about 99%, about 98%,
about 96%, about 94%, about 92%, or about 90% (inclusive); about 90% to about
99%, about 98%, about 96%, about 94%, or about 92% (inclusive); about 92% to
about 99%, about 98%, about 96%, or about 94% (inclusive); about 94% to about
15 99%, about 98%, or about 96% (inclusive); or about 96% to about 99% or about 98%
(inclusive)) decrease in the risk of developing a metastasis or an additional metastasis
in the subject (e.g., as compared to the risk of developing a metastasis or an additional
metastasis in a control subject or a control population of subjects having the same
cancer and administered no treatment or a different treatment).

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EXAMPLES

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

Example 1. Modified IL7R α Proteins Express on the Surface of Primary T Cells

Primary T cells were stained with a fluorochrome conjugated antibody against an N-terminal His tag to detect the surface expression of the mutated IL7R α proteins. Briefly, up to 1×10^6 cells were harvested 72 hours post transduction with lentiviral vectors and incubated with anti-His (APC; 1:100) in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Flow cytometry was performed using BD LSRII Fortessa instruments and analyzed using FlowJo V10 software (Figure 3, data shown are from day 6 post-activation). Detection of GFP (FITC) by flow cytometry was used as a surrogate for expression, since GFP gene is encoded within the same transgene as the IL7R α mutant proteins and separated by a T2A self cleaving peptide sequence. Detection of GFP and surface detection of His tag was linear.

Example 2. Chimeric Transmembrane Proteins Express on the Surface of Primary T Cells

mbIL15-IL17R α -WT is a chimeric transmembrane protein having wildtype IL7R α transmembrane and intracellular domains, an IL15R α extracellular domain (including a sushi domain), a linker, and an IL15 polypeptide domain (See schematic of the construct shown in Figure 4). mbIL15-IL17R α -Ins_PPCL is a chimeric transmembrane protein having an wildtype IL7R α intracellular domain, an IL7R α transmembrane domain with an insertion of the sequence of PPCL between amino acid position 4 and 5 of mature wildtype IL7R α , an IL15R α extracellular domain (including a sushi domain), a linker, and an IL15 polypeptide domain.

Primary T cells were stained with a fluorochrome conjugated antibody against IL15R α to detect the surface expression of the chimeric mbIL15 proteins. Briefly, up to 1×10^6 cells were harvested 72 hours post transduction with lentiviral vectors and incubated with anti-IL15R α (APC; 1:100) in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Flow cytometry was performed using BD LSRII Fortessa instruments and analyzed using FlowJo V10 software (Figure 5, data shown are from day 6 post-activation). Detection of GFP (FITC) by flow cytometry was used as a surrogate for

expression, since GFP gene is encoded within the same transgene as the IL7R α mutants and chimeric mbIL15 proteins and separated by a T2A self cleaving peptide sequence. Detection of GFP and surface expression of IL15R α was linear.

Example 3. T cells Expressing IL7 Mutant Receptors Maintain High Expression of CD62L (Tscm and Tcm Populations) and Variable Expression of CD25

Primary human T cells were transduced with lentiviral vectors to express one of the specific IL7R α proteins or one of the specific chimeric membrane proteins shown on the x-axis of Figure 6A. Briefly, up to 1×10^6 cells were harvested at each time point and incubated with anti-Myc (PE; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), and anti-CD62L (BV605; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. The relative expression levels of CD45RO and CD62L were used to determine the differentiation status of the cells. Teff = effector T cells. Tem = effector memory T cells. Tcm = central memory T cells. Tscm = stem cell memory T cells. Figure 6A shows the percentage of different types of CD8⁺ T cells when the primary human T cells were transduced with the indicated proteins.

The expression of PD-1 (Figure 6B, left graph) or CD25 (Figure 6B, right graph) in primary human T cells expressing different IL7R α mutant proteins and chimeric transmembrane proteins were assessed at 15 days post-activation. Briefly, up to 1×10^6 cells were harvested at each time point and incubated with anti-Myc (PE; 1:100), anti-CD25 (BV421; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), anti-CD62L (BV605; 1:200), and anti-PD1 (PE-Cy7; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Untransduced (“UT”) control T-cells provide basal level of PD-1 and CD25 expression.

Example 4. IL7R α Transmembrane Domain Mutations Induce Phosphorylation of STAT5 in Primary T Cells

The levels of phosphorylated STAT5 in primary human T cells left untransduced (“UT”) or transduced with lentivirus encoding one of the specific IL7R α proteins or one of the specific chimeric membrane proteins showed in Figure 7A were assessed by Western blotting (top Western blot). Whole cell lysates were prepared from T-cells following 16-20 hours under serum/cytokine starved conditions 14 days

post activation. UT T cells stimulated with 100 ng/ml soluble IL2 for 15 min serve as a positive control (+ stim), while unstimulated UT cells (- stim) provide basal phospho/protein levels. A control protein (alpha tubulin) was also assessed by Western blotting (bottom Western blot).

The expression of various IL7R α mutant proteins and chimeric transmembrane proteins in primary human T cells was assessed at day 15 post-activation (Figure 7B). GFP data is shown as % expression (top bar graph) and mean fluorescence intensity (bottom bar graph). Detection of GFP expression by flow cytometry was used to track the relative numbers of cells expressing the various IL7R α mutants or chimeric transmembrane proteins over a 14-day culture period (cells transduced on day 1 post activation).

Example 5. IL7R α Transmembrane Domain Mutations Co-Infect Primary T-Cells with CD19 CAR

The expression of the CD19 CAR (1928z chimeric antigen receptor), EKV or Ins_CPT IL7R α mutant proteins, the mbIL15-17R α Ins_PPCL chimeric transmembrane protein, and the mbIL15 protein on the surface of primary T cells was assessed. Figures 8A and 8B show schematically the constructs and their co-expression on primary T cells.

Primary T cells were either stained with fluorochrome conjugated antibodies against the His and Myc tags present at the N-terminus of the IL7R α mutants and CAR, respectively, or stained with an anti-IL15R α antibody to detect the surface expression of the chimeric mbIL15 proteins. Briefly, up to 1×10^6 cells were harvested 72 hours post transduction with lentiviral vectors and incubated with anti-His (APC; 1:100), or anti-IL15R α (APC; 1:100) and anti-Myc (PE; 1:100) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Flow cytometry was performed using BD LSRII Fortessa instruments and analyzed using FlowJo V10 software (Figure 8C, data shown are from day 6 post-activation). GFP is expressed within the same transgene as the IL7R α mutants and chimeric mbIL15 proteins, detection of GFP (FITC) by flow cytometry can be used as a surrogate for expression. A double positive (Myc-PE⁺, FITC) population demonstrates co-expression of both proteins on primary human T-cells.

The number of cells expressing mbIL15-IL7R α _Ins_PPCL and mbIL15 decreased from day 6 to day 14 in culture (Figure 8D). In contrast, the number of cells expressing IL7R α _EKV, IL7R α _Ins_CPT, and the 1928z chimeric antigen receptor remained relatively constant over the same period. Detection of GFP expression and N-terminal Myc tag (Myc-PE; 1:100 in FACS Buffer for 30 min) by flow cytometry was used to track the relative numbers of cells expressing the various IL7R α mutants or chimeric transmembrane proteins and CD19 CAR, respectively, over a 14 day culture period (cells transduced on day 1 post activation).

CAR T-cells expressing various IL7R α mutants and chimeric transmembrane proteins maintain less differentiated memory phenotype at day 14-post activation (Figure 9). Briefly, up to 1×10^6 cells were harvested 14 days post activation and incubated with anti-Myc (FITC; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), and anti CD62L (BV605; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Flow cytometry was performed using BD LSRII Fortessa instruments and analyzed using FlowJo V10 software. T-cell memory subset populations (CD62L vs CD45RO) were gated on Myc+, CD8+ populations. Teff = effector T cells. Tem = effector memory T cells. Tcm = central memory T cells. Tscm = stem cell memory T cells.

The levels of phosphorylated STAT5 and STAT3 and total protein level of BCL-XL in primary human T cells left untransduced or transduced with lentiviral vectors encoding one of the IL7R α proteins shown or one of the chimeric transmembrane proteins shown (with and without CAR) was assessed. Whole cell lysates were prepared from T-cells following 16-20 hr under serum/cytokine starved conditions 14 days post activation. UT T cells stimulated with 100 ng/ml soluble IL2 for 15 min serve as a positive control (+ stim), while unstimulated-UT cells (- stim) provide basal phospho/protein levels.

Example 6. CAR-T Cells Expressing IL7R α Mutants Demonstrate Superior Expansion to Control CAR-T Cells Upon Serial Exposure to Antigen

The expansion of CAR T-cells expressing IL7R α mutants compared to control CAR upon serial exposure to antigen at a 1:1 ratio effector to target (E:T) ratio was assessed. At day 14 post activation, 5×10^5 CAR+ T-cells were plated against 5×10^5 Nalm6 B-cells on day 0 in each well of a GRex 24-well plate. Cells were plated in

triplicate repeats. Nalm6 B cells and fresh cell culture media were added back to culture plates on days 4, 7, 11, 14, and 17 at a 1:1 ratio based to the number of CAR⁺ cells in each well as calculated by flow cytometry. All cells expressing IL7R α mutants maintained 100% lysis of tumor cells throughout the 21 day assay relative to untransduced and tumor alone controls. Fold expansion of CAR⁺ T-cells was determined by flow cytometric analysis on days 4, 7, 11, 17, and 21. Briefly, up to 1×10^6 cells were harvested at each time point and incubated with anti-Myc (PE; 1:100), anti-CD3 (BV421; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), anti-CD62L (BV605; 1:200), and anti-PD1 (PE-Cy7; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Counting beads (50 μ l, 123count™ eBeads, Thermo Fisher Scientific) were added to each tube directly prior to flow cytometric analysis allowing for quantification of cell numbers in each condition. These numbers were used to track CAR⁺ expansion rates and to determine the number of Nalm6 target cells to add back to each well at a 1:1 E:T ratio. These data show that CAR T-cells expressing IL7R α mutants demonstrate superior expansion compared to control CAR upon serial exposure to antigen at a 1:1 ratio effector to target (E:T) ratio (Figures 11A and 11B).

Percent lysis of CD19⁺ Nalm6 B-cells (Figure 12A) and CD19⁺ K562 cells (Figure 12B) in a luciferase-based overnight killing assay was assessed. T-cells taken from the end of a 21-day serial killing assay (as described herein) and a Nalm6 or CD19⁺ K562 cell line engineered to express firefly-luciferase (fLuc⁺). 1×10^4 Nalm6 or K562 cells were plated per well, and T-cells were plated according to % CAR⁺ at various E:T ratios. Cell lysis was measured based on relative light unit (RLU) intensity of live Nalm6 B- or K562 cells, and normalized to Nalm6 K562 alone controls.

T-cells expressing IL7R α mutants demonstrate superior killing of CD19⁺ Nalm6 B-cells over control CD19 CAR at low E:T ratios following 22 days of serial antigen encounter (Figure 12A). As expected, donor and age matched (day 35 post activation) UT T-cells did not survive 21 day serial killing assay, therefore were not assayed here.

CAR T-cells expressing IL7R α _EKV demonstrated increased off-target cytotoxicity against CD19⁺ K562 at high E:T ratios (20:1 and 6:1), while CAR-T cells

expressing other cytokine receptor variants demonstrated comparable off-target cytotoxicity to control CAR (Figure 12B).

The memory type differentiation of CAR-T cells expressing IL7R α mutants at day 14 following serial exposure to antigen was assessed (Figure 13). Nalm6 B-cells were added to cell culture on days 0, 4, 7, 11, 14, 17, and 21. Teff = effector T cells. Tem = effector memory T cells. Tcm = central memory T cells. Tscm = stem cell memory T cells. Briefly, up to 1×10^6 cells were harvested at each time point and incubated with anti-Myc (PE; 1:100), anti-CD3 (BV421; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), anti-CD62L (BV605; 1:200), and anti-PD1 (PE-Cy7; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. These data show that CAR T-cells expressing IL7R α mutants maintained a less differentiated memory phenotype at day 14 following serial exposure to antigen.

Example 7. CAR-T cells Expressing IL7R α Mutants Demonstrate Superior Expansion to Control CAR-T Cells Upon Single Exposure to Antigen

Expansion of CAR T-cells expressing IL7R α mutants compared to control CAR upon a single exposure to target at a 1:3 E:T ratio was assessed (Figures 14A and 14B). At day 14 post activation, 5×10^5 CAR+ T-cells were plated against 1.5×10^6 Nalm6 B-cells on day 0 in each well of a GRex 24-well plate. Cells were plated in triplicate repeats. All cells maintained 100% lysis of tumor cells throughout the 21 day assay relative to untransduced and tumor alone controls. Fold expansion of CAR+ T-cells was determined by flow cytometric analysis on days 4, 7, 11, 17, and 21. Briefly, up to 1×10^6 cells were harvested at each time point and incubated with anti-Myc (PE; 1:100), anti-CD3 (BV421; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), anti-CD62L (BV605; 1:200), and anti-PD1 (PE-Cy7; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. Counting beads (50 μ l, 123count™ eBeads) were added to each tube directly prior to analysis allowing for quantification of cell numbers in each condition. These data show that CAR T-cells expressing IL7R α mutants demonstrate superior expansion to control CAR upon a single exposure to target.

The memory type differentiation of CAR-T cells expressing IL7R α mutants at day 14 following serial exposure to antigen was assessed (Figure 15). Nalm6 B-cells

were added to cell culture on day 0. Briefly, up to 1×10^6 cells were harvested at each time point and incubated with anti-Myc (PE; 1:100), anti-CD3 (BV421; 1:100), anti-CD4 (BV785; 1:200), anti-CD8 (PE; 1:200), anti-CD45RO (APC; 1:200), anti-CD62L (BV605; 1:200), and anti-PD1 (PE-Cy7; 1:200) antibodies in PBS + 2% FBS (FACS Buffer) for 30 minutes at 4°C. These data show that that CAR T-cells expressing IL7R α mutants maintained a less differentiated memory phenotype at day 14 following single exposure to antigen. Teff = effector T cells. Tem = effector memory T cells. Tcm = central memory T cells. Tscm = stem cell memory T cells.

EXAMPLE 8. CD19 CAR + IL7R α CPT, MCP or PPCL in vivo test with Nalm6 tumor.

CD19⁺ Acute Lymphoblastic Leukemia (ALL) cell line Nalm6 was purchased from ATCC and transduced with luciferase to allow for bioluminescent imaging. 0.5×10^6 Nalm6_luc cells were implanted into NOD-SCID IL2Rgamma^{null} (NSG) mice on the first day of the experiment. On day 6 (and every three to four days thereafter) animals were injected intraperitoneally with D-luciferin (150 mg/kg of 15 mg/mL substrate from Promega) and bioluminescence (BLI) was measured on an IVIS S5 Lumina (PerkinElmer, MA) imager. Images were analyzed using Living Image 4.3.1 (PerkinElmer, MA) to quantify whole body, fixed volume ROI, and total flux (photons/sec) was calculated and shown in Table 1 below. On day 6 of the experiment, animals were also divided into groups of six animals (with the exception of vehicle group for which there were only three animals) and dosed with non-transduced T cells or T cells carrying CD19 CAR alone, CD19 CAR + IL7R α CPT, CD19 CAR + IL7R α MCP, or CD19 CAR + IL7R α PPCL. All animals receiving CAR T cells received the stress test dose of 0.1×10^6 CAR T cells, and all animals received the same total number of T cells.

The protein and DNA sequences for each of the CD19 CAR, CD19 CAR + IL7R α CPT, CD19 CAR + IL7R α MCP, or CD19 CAR + IL7R α PPCL used in these experiments are shown below.

CD19 CAR DNA (SEQ ID NO: 100)

GAACAGAAGCTGATAAGTGAGGAGGACTTGgacatccagatgacccagaccaccagcagc
ctgagcgccagcctgggcgatagagtgacatcagctgcagagccagccaggacatcagcaagtacctaactggtatc

agcagaaacccgacggcaccgtgaagctgctgatctaccacaccagcagactgcacagcggcgtgccagcagatttc
tggcagcggctccggcaccgactacagcctgaccatctccaacctggaacaggaagatacgtactacttctgtcagca
aggcaacacctgcccacaccttcggcggaggcaccagctggaaatcacagcgggcggaggatctggcggaggcg
gaagtggcggagggggatctgaagtgaactgcaggaaagcgccctggcctggtggcccatctcagtctctgagcgt
gacctgtaccgtgtccggcgtgtccctgcctgactatggcgtgtcctggatcagacagccccagaaaggcctggaat
ggctgggagtgtctgggcagcgagacaacctactacaacagcgccctgaagtcccgctgaccatcatcaaggacaa
ctccaagagccaggtgttcctgaagatgaacagcctgcagaccgacgacaccgcatctactactgcgccaagcactact
actacggcggcagctacgccatggactactggggccagggcacaagcgtgaccgtgtctagcgggtccCTAGAC
AATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTG
TCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGT
GGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTAT
TATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTIONACA
TGAACATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTACCAGCCC
TATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCCTGAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCGCGTACCAGCAGGGCCAGAACCAGCTCT
ATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGCGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGC
CTACAGTGAGATTGGGATGAAAGGCGAGCGCCGAGGGGCAAGGGGCAC
GATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGC
CCTTCACATGCAGGCCCTGCCCCCTCGCT

CD19 CAR Protein (SEQ ID NO: 101)

EQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGT
VKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT
FGGGTKLEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVWGSETTYNSALKSRLTIKDNSKSQVFL
KMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSGLDNEKSNG
TIIHVKGKHLCPSPFPGPSKPFWVLVVGGVLACYSLLVTVAFIIFWVRSKRS
RLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSLRVKFSRSADAPAYQ
QQQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

CD19 CAR + IL7RA_CPT DNA (SEQ ID NO: 102)

GAACAGAAGCTGATAAGTGAGGAGGACTTGgacatccagatgacccagaccaccagcagc
ctgagcgccagcctgggcatagagtaccatcagctgcagagccagccaggacatcagcaagtacctaactggtatc
agcagaaacccgacggcaccgtgaagctgctgatctaccacaccagcagactgcacagcggcgtgccagcagatttc
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AATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTG
TCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGT
GGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTAT
TATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTIONACA
TGAACATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTACCAGCCC
TATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCCTGAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCT
ATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGCGTGGCCGGGACCCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGC
CTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCAC
GATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGC
CCTTCACATGCAGGCCCTGCCCCCTCGCCGGAAGAGAAGAGGCAAGCCCA
TCCCCAACCCACTGCTGGGCCTGGATAGCACCTCCGGAAGCGGAGAGGGC
AGAGGCTCTCTGCTGACCTGCGGCGACGTGGAAGAGAACCCAGGgCCCAT
GCTCCTGCTCGTGACTTCACTTCTTCTCTGTGAACTCCCACACCCCGCGTTT
TTGCTTATCCCTcatcatcaccatcaccacGGCGAGAGCGGCTACGCCCAGAACGGC
GACCTGGAGGACGCCGAGCTGGACGACTACAGCTTCAGCTGCTACAGCCA
GCTGGAGGTGAACGGCAGCCAGCACAGCCTGACCTGCGCCTTCGAGGACC
CCGACGTGAACATCACCAACCTGGAGTTCGAGATCTGCGGCGCCCTGGTG
GAGGTGAAGTGCCTGAACTTCAGGAAGCTGCAGGAGATCTACTTCATCGA
GACCAAGAAGTTCCTGCTGATCGGCAAGAGCAACATCTGCGTGAAGGTGG

GCGAGAAGAGCCTGACCTGCAAGAAGATCGACCTGACCACCATCGTGAA
GCCCCAGGCCCCCTTCGACCTGAGCGTGGTGTACAGGGAGGGCGCCAACG
ACTTCGTGGTGACCTTCAACACCAGCCACCTGCAGAAGAAGTACGTGAAG
GTGCTGATGCACGACGTGGCCTACAGGCAGGAGAAGGACGAGAACAAGT
GGACCCACGTGAACCTGAGCAGCACCAAGCTGACCCTGCTGCAGAGGAA
GCTGCAGCCCCGCCCATGTACGAGATCAAGGTGAGGAGCATCCCCGACC
ACTACTTCAAGGGCTTCTGGAGCGAGTGGAGCCCCAGCTACTACTTCAGG
ACCCCCGAGATCAACAACAGCAGCGGCAGATGGACCCCATCCTGCTGAC
CTGCCCCACCATCAGCATCCTGAGCTTCTTCAGCGTGGCCCTGCTGGTGAT
CCTGGCCTGCGTGCTGTGGAAGAAGAGGATCAAGCCCATCGTGTGGCCCA
GCCTGCCCCGACCACAAGAAGACCCTGGAGCACCTGTGTAAGAAGCCCAG
GAAGAACCTGAACGTGAGCTTCAACCCCGAGAGCTTCCTGGACTGCCAGA
TCCACAGGGTGGACGACATCCAGGCCAGGGACGAGGTGGAGGGCTTCCTG
CAGGACACCTTCCCCCAGCAGCTGGAGGAGAGCGAGAAGCAGAGGCTGG
GCGGCGACGTGCAGAGCCCCAACTGCCCCAGCGAGGACGTGGTGATCACC
CCCGAGAGCTTCGGCAGGGACAGCAGCCTGACCTGCCTGGCCGGCAACGT
GAGCGCCTGCGACGCCCCCATCCTGAGCAGCAGCAGGAGCCTGGACTGCA
GGGAGAGCGGCAAGAACGGCCCCCACGTGTACCAGGACCTGCTGCTGAG
CCTGGGCACCACCAACAGCACCCCTGCCACCCCCCTTCAGCCTGCAGAGCG
GCATCCTGACCCTGAACCCCGTGGCCCAGGGCCAGCCCATCCTGACCAGC
CTGGGCAGCAACCAGGAGGAGGCCTACGTGACCATGAGCAGCTTCTACCA
GAACCAG

CD19 CAR + IL7RA_CPT Protein (SEQ ID NO: 103)

EQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGT
VKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT
FGGGTKLEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFL
KMNSLQTDDEAIYYCAKHYYYGGSYAMDYWGQTSVTVSSGSLDNEKSNG
TIIHVKGKHLCPSPFLPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSKRS
RLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSLRVKFSRSADAPAYQ
QQQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRK

RRGKPIPNPLLGLDSTSGSGEGRGSLTTCGDVEENPGPMLLLVTSLLLCELPH
AFLIPH HHHHHHGESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAF
EDPDVNITNLEFEICGALVEVKCLNFRKLQEIFYFIETKKFLLIGKSNICVKVGEK
SLTCKKIDLT TIVKPEAPFDLSVVYREGANDFVVTFN TSHLQKKYVKVLMHD
VAYRQEKDENK WTHVNL SSTKL TLLQRKLQPAAMY EIKVRSIPDHYFKGFW
SEWSPSY YFRTPEINN SSGEMDPILLTCPTISILSFFSVALLVILACVLWKKRIKP
IVWPSLPD HKK TLEHLCKKPRKNLNV SFNPESFLDCQIHRVDDI QARDEVEGF
LQDTFPQQLE ESEKQRLGGDVQSPNCPS EDVVITPESFGRDSSLTCLAGNVSA
CDAPILSSSRSLDCRESGKN GPHVYQDLLLSL GTTNSTLPPPSLQSGILTLPV
AQGPILTSLG SNQEEAYVTMSSFYQNNQ

CD19CAR + IL7RaMCP DNA (SEQ ID NO: 104)

GAACAGAAGCTGATAAGTGAGGAGGACTTGgacatccagatgaccagaccaccagcagc
ctgagcgccagcctgggcgatagagtgaccatcagctgcagagccagccaggacatcagcaagtac tgaactggtatc
agcagaaacccgacggcaccgtgaagctgctgatctaccacaccagcagactgcacagcggcgtgccagcagatttc
tggcagcggctccggcaccgactacagcctgacctctccaacctggaacaggaagatatcgctacctactctgtcagca
aggcaacacctgccctacacctcggcggaggcaccaagctggaaatcacaggcggcggaggatctggcggaggcg
gaagtggcggagggggatctgaagtgaactgcaggaaagcgccctggcctggtggcccatctcagctctgagcgt
gacctgtaccgtgtccggcgtgtccctgcctgactatggcgtgtcctggatcagacagccccccagaaggcgctggaat
ggctgggagtgatctggggcagcgagacaacctactacaacagcgccctgaagtccggctgacctcatcaaggacaa
ctccaagagccaggtgttctctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactact
actacggcgagctacgccatggactactggggccagggcacaagcgtgaccgtgtctagcgggtccCTAGAC
AATGAGAAGAGCAATGGAACCAT TATCCATGTGAAAGGGAAACACCTTTG
TCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGT
GGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTAT
TATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGA CTACA
TGAACATGACTCCCCGCGCCCCGGGCCACCCGCAAGCATTACCAGCCC
TATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCCTGAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCGCGTACCAGCAGGGCCAGAACCAGCTCT
ATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGgCGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGC
CTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCAC

GATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGC
CCTTCACATGCAGGCCCTGCCCCCTCGCCGGAAGAGAAGAGGCAAGCCCA
TCCCCAACCCTACTGCTGGGCCTGGATAGCACCTCCGGAAGCGGAGAGGGC
AGAGGCTCTCTGCTGACCTGCGGCGACGTGGAAGAGAACCCAGGgCCCAT
GCTCCTGCTCGTGACTTCACTTCTTCTCTGTGAACTCCCACACCCCGCGTTT
TTGCTTATCCCTcatcatcaccatcaccacGGCGAGAGCGGCTACGCCCAGAACGGC
GACCTGGAGGACGCCGAGCTGGACGACTACAGCTTCAGCTGCTACAGCCA
GCTGGAGGTGAACGGCAGCCAGCACAGCCTGACCTGCGCCTTCGAGGACC
CCGACGTGAACATCACCAACCTGGAGTTCGAGATCTGCGGCGCCCTGGTG
GAGGTGAAGTGCTGAACTTCAGGAAGCTGCAGGAGATCTACTTCATCGA
GACCAAGAAGTTCTGCTGATCGGCAAGAGCAACATCTGCGTGAAGGTGG
GCGAGAAGAGCCTGACCTGCAAGAAGATCGACCTGACCACCATCGTGAA
GCCCCGAGGCCCCCTTCGACCTGAGCGTGGTGTACAGGGAGGGCGCCAACG
ACTTCGTGGTGACCTTCAACACCAGCCACCTGCAGAAGAAGTACGTGAAG
GTGCTGATGCACGACGTGGCCTACAGGCAGGAGAAGGACGAGAACAAGT
GGACCCACGTGAACCTGAGCAGCACCAAGCTGACCCTGCTGCAGAGGAA
GCTGCAGCCCCGCCGCCATGTACGAGATCAAGGTGAGGAGCATCCCCGACC
ACTACTTCAAGGGCTTCTGGAGCGAGTGGAGCCCCAGCTACTACTTCAGG
ACCCCCGAGATCAACAACAGCAGCGGCGAGATGGACCCCATCCTGCTGAT
GTGCCCCACCATCAGCATCCTGAGCTTCTTCAGCGTGGCCCTGCTGGTGAT
CCTGGCCTGCGTGCTGTGGAAGAAGAGGATCAAGCCCATCGTGTGGCCCA
GCCTGCCCCGACCACAAGAAGACCCTGGAGCACCTGTGTAAGAAGCCCAG
GAAGAACCTGAACGTGAGCTTCAACCCCGAGAGCTTCCTGGACTGCCAGA
TCCACAGGGTGGACGACATCCAGGCCAGGGACGAGGTGGAGGGCTTCCTG
CAGGACACCTTCCCCCAGCAGCTGGAGGAGAGCGAGAAGCAGAGGCTGG
GCGGCGACGTGCAGAGCCCCAACTGCCCCAGCGAGGACGTGGTGATCACC
CCCGAGAGCTTCGGCAGGGACAGCAGCCTGACCTGCCTGGCCGGCAACGT
GAGCGCCTGCGACGCCCCCATCCTGAGCAGCAGCAGGAGCCTGGACTGCA
GGGAGAGCGGCAAGAACGGCCCCCACGTGTACCAGGACCTGCTGCTGAG
CCTGGGCACCACCAACAGCACCCCTGCCACCCCCCTTCAGCCTGCAGAGCG
GCATCCTGACCCTGAACCCCGTGGCCCAGGGCCAGCCCATCCTGACCAGC
CTGGGCAGCAACCAGGAGGAGGCCTACGTGACCATGAGCAGCTTCTACCA
GAACCAG

CD19CAR + IL7RaMCP Protein (SEQ ID NO: 105)

EQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGT
VKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT
FGGGTKLEITGGGGSGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVWGETTYYN SALKSRLTIKDNSKSQVFL
KMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSGSLDNEKSNG
TIHVKGKHLCPSPFLPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSKRS
RLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSLRVKFSRSADAPAYQ
QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRK
RRGKPIPNPLLGLDSTSGSGEGRGSLLTCGDVEENPGPMLLLVTSLLLCELPH
AFLLIPIHHHHHGESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAF
EDPDVNITNLEFEICGALVEVKCLNFRKLQEIFYFIETKKFLLIGKSNICVKVGEK
SLTCKKIDLTIVKPEAPFDLSVVYREGANDFVVTFNTSHLQKKYVKVLMHD
VAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMY EIKVRSIPDHYFKGF
SEWSPSYFRTPEINNSSGEMDPILLMCPTISILSFFSVALLVILACVLWKKRIK
PIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIARDEVEG
FLQDTFPQQLESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNV
ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLNP
VAQQQPILTSLSNQEAYVTMSSFYQNNQ

CD19CAR + IL7RaPPCL DNA (SEQ ID NO: 106)

GAACAGAAGCTGATAAGTGAGGAGGACTTGgacatccagatgacccagaccaccagcagc
ctgagcgccagcctggcgatagagtaccatcagctgcagagccagccaggacatcagcaagtacctgaactggtatc
agcagaaaacccgacggcaccgtgaagctgctgatctaccacaccagcagactgcacagcggcggtgccagcagatttc
tggcagcggctccggcaccgactacagcctgaccatctccaacctggaacaggaagatcgctacctactctgtcagca
aggcaacaccctgccctacacctcggcgaggcaccaagctggaaatcacaggcggcgaggatctggcgaggcg
gaagtggcgagggggatctgaagtgaactgcaggaagcggccctggcctggtggccccatctcagctctctgagcgt
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ggctgggagtgtctgggcagcgagacaacctactacaacagcgcctgaagtcccgctgacctcatcaaggacaa
ctccaagagccaggtgttctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactact
actacggcgagcgtacgccatggactactggggccagggcacaagcgtgaccgtgtctagcgggtccCTAGAC
AATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTG

TCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGT
GGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTAT
TATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGA CTACA
TGAACATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTACCAGCCC
TATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCCTGAGAGTGAAGTTC
AGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCT
ATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGgCGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGC
CTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCAC
GATGGCCTTTACCAGGTCTCAGTACAGCCACCAAGGACACCTACGACGC
CCTTCACATGCAGGCCCTGCCCCCTCGCCGGAAGAGAAGAGGCAAGCCCA
TCCCCAACCCACTGCTGGGCCTGGATAGCACCTCCGGAAGCGGAGAGGGC
AGAGGCTCTCTGCTGACCTGCGGCGACGTGGAAGAGAACCCAGGgCCCAT
GCTCCTGCTCGTGACTTCACTTCTTCTCTGTGAACTCCCACACCCCGCGTTT
TTGCTTATCCCTcaccatcaccacGGCGAGAGCGGCTACGCCCAGAACGGC
GACCTGGAGGACGCCGAGCTGGACGACTACAGCTTCAGCTGCTACAGCCA
GCTGGAGGTGAACGGCAGCCAGCACAGCCTGACCTGCGCCTTCGAGGACC
CCGACGTGAACATCACCAACCTGGAGTTCGAGATCTGCGGCGCCCTGGTG
GAGGTGAAGTGCCTGAACTTCAGGAAGCTGCAGGAGATCTACTTCATCGA
GACCAAGAAGTTCTTGCTGATCGGCAAGAGCAACATCTGCGTGAAGGTGG
GCGAGAAGAGCCTGACCTGCAAGAAGATCGACCTGACCACCATCGTGAA
GCCCCGAGGCCCCCTTCGACCTGAGCGTGGTGTACAGGGAGGGCGCCAACG
ACTTCGTGGTGACCTTCAACACCAGCCACCTGCAGAAGAAGTACGTGAAG
GTGCTGATGCACGACGTGGCCTACAGGCAGGAGAAGGACGAGAACAAGT
GGACCCACGTGAACCTGAGCAGCACCAAGCTGACCCTGCTGCAGAGGAA
GCTGCAGCCCCGCCGCCATGTACGAGATCAAGGTGAGGAGCATCCCCGACC
ACTACTTCAAGGGCTTCTGGAGCGAGTGGAGCCCCAGCTACTACTTCAGG
ACCCCCGAGATCAACAACAGCAGCGGCGAGATGGACCCCATCCTGCTGCC
ACCCTGTTTAACCATCAGCATCCTGAGCTTCTTCAGCGTGGCCCTGCTGGT
GATCCTGGCCTGCGTGCTGTGGAAGAAGAGGATCAAGCCCATCGTGTGGC
CCAGCCTGCCCCGACCACAAGAAGACCCTGGAGCACCTGTGTAAGAAGCCC
AGGAAGAACCTGAACGTGAGCTTCAACCCCGAGAGCTTCCTGGACTGCCA

GATCCACAGGGTGGACGACATCCAGGCCAGGGACGAGGTGGAGGGCTTC
CTGCAGGACACCTTCCCCCAGCAGCTGGAGGAGAGCGAGAAGCAGAGGC
TGGGCGGCGACGTGCAGAGCCCCAACTGCCCCAGCGAGGACGTGGTGATC
ACCCCCGAGAGCTTCGGCAGGGACAGCAGCCTGACCTGCCTGGCCGGCAA
CGTGAGCGCCTGCGACGCCCCATCCTGAGCAGCAGCAGGAGCCTGGACT
GCAGGGAGAGCGGCAAGAACGGCCCCCACGTGTACCAGGACCTGCTGCT
GAGCCTGGGCACCACCAACAGCACCTGCCACCCCCCTTCAGCCTGCAGA
GCGGCATCCTGACCCTGAACCCCGTGGCCAGGGCCAGCCCATCCTGACC
AGCCTGGGCAGCAACCAGGAGGAGGCCTACGTGACCATGAGCAGCTTCTA
CCAGAACCAG

CD19CAR + IL7RaPPCL Protein (SEQ ID NO: 107)

EQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGT
VKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT
FGGGTKLEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVWGSETTYNSALKSRLTIKDNSKSQVFL
KMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSGSLDNEKSNG
TIIHVKGKHLCPSPFLPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS
RLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSLRVKFSRSADAPAYQ
QQQNQLYNELNLGRREEYDVLDKRRGRDPENGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRK
RRGKPIPNNLLGLDSTSGSGEGRGSLLTCGDVEENPGPMLLLVTSLLLCELPH
AFLIPHHHHHHGESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAF
EDPDVNITNLEFEICGALVEVKCLNFRKLQEIFYFIETKKFLLIGKSNICVKVGEK
SLTCKKIDLTIVKPEAPFDLSVVYREGANDFVVTFNSTHLQKKYVKVLMHD
VAYRQEKDENKWTHVNLSSTKLTLQKRLQPAAMYKVRSDHYFKGFW
SEWSPSYFRTPEINNSSGEMDPILLPPCLTISILSFFSVALLVILACVLWKKRIK
PIVWPSLPDHKKTLEHLCKKPRKNLNVSNPESFLDCQIHRVDDIQRDEVEG
FLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVS
ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPSLQSGILTLNP
VAQGQPILTSLSNQEAYVTMSSFYQNNQ

Tumor growth delay and the percentage of increase in life span was calculated as described below.

Tumor growth delay (TGD, or T-C) – TGD is a group endpoint. Tumor growth delay is expressed in units of days and is calculated from the median times it took the mice in a group to reach a specified tumor burden (time to evaluation size, TES). It was calculated as:

$$\text{TGD} = \text{median TES treated} - \text{median TES control}$$

Where control is UT T cells.

The % Increase in lifespan (ILS) - %ILS is a group endpoint. It was calculated as:

$$\%ILS = \left\{ \frac{[(\text{median Treated Lifespan}) - (\text{median Control Lifespan})]}{\text{median Control Lifespan}} \right\} * 100$$

The data from these experiments are shown in Figures 16-18. Non-transduced T-cells had no anti-cancer activity. At the stress test dose of 0.1×10^6 cells, CD19 CAR alone increased life span of animals by 68.8%, but no increase in tumor growth delay. Treatment with CD19 CAR + IL7RaCPT was most efficacious with 175% increase in life span relative to vehicle treatment, and tumor growth delay >29.2 days. Treatment with CD19 CAR + IL7RaMCP and CD19 CAR + IL7RaPPT were both efficacious and increased life span >175% each with tumor growth delay of >22.0 and >17.9 days respectively.

Table 1.

Days post inoculation	VEHICLE					
6	113000000	49300000	63300000			
9	2800000000	1120000000	1460000000			
12	1.29E+10	9630000000	10400000000			
15	3.57E+10	20300000000	31400000000			
22	1.48E+11					

26						
29						
36						
43						
Days post inoculation	NONTRANSDUCED					
6	64500000	54500000	112000000	62900000	79500000	82200000
9	1950000000	1340000000	2690000000	1750000000	2690000000	1710000000
12	8620000000	6800000000	22000000000	1.17E+10	1.27E+10	9370000000
15	3.72E+10	34200000000	54800000000	2.74E+10	3.66E+10	1.11E+10
22						
26						
29						
36						
43						
Days post inoculation	CD19 CAR					
6	84800000	61600000	68500000	110000000	54800000	77900000
9	1620000000	1320000000	1690000000	2450000000	1260000000	1370000000
12	8790000000	6760000000	10700000000	7700000000	8000000000	1.14E+10
15	3.44E+10	7770000000	28300000000	2.99E+10	2.25E+10	3.42E+10
22	3.14E+10	21900000000	33600000000	8.93E+10	3.12E+10	3.18E+10
26	1.67E+10	27300000000	28200000000	1.36E+11	4.9E+10	4.02E+10
29	1.21E+11	63500000000	83100000000	1.05E+11	8.4E+10	4.71E+10
36	1.72E+11		84000000000			
43						
Days post inoculation	CD19 CAR + IL7RaCPT					
6	68600000	88400000	107000000	59900000	76800000	55900000
9	2390000000	1510000000	1820000000	762000000	1740000000	1370000000
12	8220000000	8260000000	9080000000	5280000000	8400000000	5540000000
15	1.79E+10	16200000000	14200000000	1.14E+10	1.07E+10	1.56E+10
22	3620000000	3550000000	1740000000	1890000000	1360000000	3490000000
26	690000000	495000000	147000000	220000000	126000000	1350000000
29	28300000	38700000	6530000	5090000	14300000	40100000
36	3460000	1170000	1730000	1070000	938000	2640000
43	2550000	953000		873000	797000	3340000

Days post inoculation	CD19 CAR + IL7RaMCP					
6	69600000	58600000	90700000	74800000	100000000	55900000
9	1870000000	1950000000	1990000000	1110000000	2620000000	1010000000
12	1.03E+10	6350000000	11500000000	1.32E+10	1.38E+10	5740000000
15	1.69E+10	16100000000	25700000000	3.07E+10	1.86E+10	1.38E+10
22	1770000000	5900000000	24700000000	5390000000	8260000000	7520000000
26	115000000	1970000000	80200000000	5300000	182000000	9760000000
29	2340000	1260000000	90800000000	2130000	2900000	2.31E+10
36	2990000	2770000	1.33E+11	1890000	2230000	6.85E+10
43	1240000	1510000		978000	1330000	4.58E+10
Days post inoculation	CD19 CAR + IL7RaPPCL					
6	74000000	100000000	97400000	74700000	56700000	56200000
9	1890000000	1980000000	3170000000	1000000000	998000000	811000000
12	1.12E+10	11700000000	16100000000	6850000000	7990000000	9090000000
15	1.98E+10	23300000000	21200000000	1.3E+10	1.92E+10	2.52E+10
22	4300000000	18800000000	36000000000	1290000000	2610000000	1580000000
26	1440000000	14100000000	62400000000	3110000	446000000	8730000000
29	66500000	8210000000	75200000000	1570000	428000000	1530000000
36	2780000	47600000	1480000000	659000	1550000	2990000
43	1400000	4250000	150000000	1020000	1440000	2140000

EXAMPLE 9. In vitro serial killing experiment with GPC3 CAR + IL7RaCPT

An equal number of GPC3 CAR positive T cells and GPC3 CAR + IL7RaCPT positive T-cells were co-cultured with Hep3B target cells from American Type Culture Company (ATCC, Manassas, VA) (0.5×10^6 CAR T cells co-cultured with 0.5×10^6 Hep3B target cells). The IL7Ra CPT protein is the same as described in Example 8. Target cells were added two-times per week every three to four days (at a 1:1 CAR T cell to target Hep3B cell ratio) for 35 days and the number of CAR T cells was measured by counting beads and flow cytometry. The target cells were added until day 35 and the number of CAR T cells after day 35 were measured once a week. The CAR T cells numbers are shown in Table 2 below.

The protein and DNA sequences for the GPC3 CAR and the GPC3 CAR + IL7RaCPT are shown below.

GPC3 CAR DNA (SEQ ID NO: 108)

GATATCGTGATGACCCAGAGCCCCGACTCTTTAGCTGTGTCTTTAGGAGAG
AGGGCCACAATCAACTGCAAGAGCAGCCAGAGCCTCCTCTACAGCAGCA
ACCAGAAGAACTATTTAGCTTGGTACCAGCAAAAGCCCGGCCAGCCCCC
AAGCTGCTGATCTACTGGGCCAGCAGCAGAGAGAGCGGCGTGCCCGATAG
ATTCAGCGGAAGCGGCTCCGGCACAGATTTACCCCTCACCATTAGCTCTTT
ACAAGCTGAGGACGTGGCCGTGTACTACTGCCAGCAGTACTACAACCTACC
CTTTAACCTTCGGCCAAGGTACCAAGCTGGAGATCAAGGGCTCCACATCC
GGATCCGGCAAGCCCGGTAGCGGAGAAGGCAGCACAAAGGGAGAGGTGC
AGCTGGTGGAGAGCGGAGGCGGACTGGTCCAGCCCGGTGGATCTTTAAGG
CTGTCTTGTGCCGCCAGCGGCTTTACCTTTAACAAGAACGCTATGAACTGG
GTGAGGCAAGCTCCCGGTAAGGGTTTAGAGTGGGTGGGTTCGTATTCGTAA
TAAGACCAACAACCTACGCCACCTACTATGCCGACTCCGTGAAGGCTCGTT
TCACCATCTCTCGTGACGACAGCAAGAACAGCCTCTATTTACAGATGAAC
TCTTTAAAGACCGAGGACACCGCCGTGTACTATTGCGTGGCTGGCAACTC
CTTCGCCTACTGGGGCCAAGGCACTTTAGTGACCGTGAGCTCCgggtccACCA
CGACGCCAGCGCCGCGACCACCAACACCGGCGCCACCATCGCGTCGCAG
CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAgcggcggggggcgagTGCACA
CGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCC
GGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCCTTACTGCAAA
CGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACC
AGTACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAG
AAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGA
CGCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATC
TAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGgCGTGGCCGGGAC
CCTGAGATGGGGGGAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGT
ACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGG
GATGAAAGGCGAGCGCCGGAGGGCAAGGGGCACGATGGCCTTTACCAG
GGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGC
CCTGCCCCCTCGC

GPC3 CAR Protein (SEQ ID NO: 109)

DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKL
LIYWASSRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQYYNYPLTFG
QGTKLEIKGSTSGSGKPGSGEGSTKGEVQLVESGGGLVQPGGSLRLSCAASGF
TFNKNAMNWVRQAPGKGLEWVGRIRNKTNNYATYYADSVKARFTISRDDS
KNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQGLTVTVSSGSTTTPAPRPPT
PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSL
VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS
RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPR

GPC3CAR + IL7RaCPT (SEQ ID NO: 110)

GATATCGTGATGACCCAGAGCCCCGACTCTTTAGCTGTGTCTTTAGGAGAG
AGGGCCACAATCAACTGCAAGAGCAGCCAGAGCCTCCTCTACAGCAGCA
ACCAGAAGAACTATTTAGCTTGGTACCAGCAAAAGCCCGGCCAGCCCCC
AAGCTGCTGATCTACTGGGCCAGCAGCAGAGAGAGCGGCGTGCCCGATAG
ATTCAGCGGAAGCGGCTCCGGCACAGATTTACCCCTCACCATTAGCTCTTT
ACAAGCTGAGGACGTGGCCGTGTACTACTGCCAGCAGTACTACAACCTACC
CTTTAACCTTCGGCCAAGGTACCAAGCTGGAGATCAAGGGCTCCACATCC
GGATCCGGCAAGCCCGGTAGCGGAGAAGGCAGCACAAAGGGAGAGGTGC
AGCTGGTGGAGAGCGGAGGCGGACTGGTCCAGCCCGGTGGATCTTTAAGG
CTGTCTTGTGCCGCCAGCGGCTTTACCTTTAACAAGAACGCTATGAACTGG
GTGAGGCAAGCTCCCGGTAAGGGTTTAGAGTGGGTGGGTCTGATTTCGTAA
TAAGACCAACAACCTACGCCACCTACTATGCCGACTCCGTGAAGGCTCGTT
TCACCATCTCTCGTGACGACAGCAAGAACAGCCTCTATTTACAGATGAAC
TCTTTAAAGACCGAGGACACCGCCGTGTACTATTGCGTGGCTGGCAACTC
CTTCGCCTACTGGGGCCAAGGCACTTTAGTGACCGTGAGCTCCggtccACCA
CGACGCCAGCGCCGCGACCACCAACACCGGCGCCACCATCGCGTCGCAG
CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAgcggcggggggcgagTGACA
CGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGCC
GGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCTTTACTGCAAA
CGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACC

AGTACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAG
AAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGA
CGCCCCCGGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATC
TAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGgCGTGCCCGGAC
CCTGAGATGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGT
ACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGG
GATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAG
GGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGC
CCTGCCCCCTCGCggtcCGGAGAGGGCAGAGGCTCTCTGCTGACCTGCGGC
GACGTGGAAGAGAACCCAGGgCCCATGCTCCTGCTCGTGACTTCACTTCTT
CTCTGTGAACTCCCACACCCCGCGTTTTTGCTTATCCCTcatcatcaccatcaccacG
GCGAGAGCGGCTACGCCCAGAACGGCGACCTGGAGGACGCCGAGCTGGA
CGACTACAGCTTCAGCTGCTACAGCCAGCTGGAGGTGAACGGCAGCCAGC
ACAGCCTGACCTGCGCCTTCGAGGACCCCGACGTGAACATCACCAACCTG
GAGTTCGAGATCTGCGGCGCCCTGGTGGAGGTGAAGTGCCTGAACTTCAG
GAAGCTGCAGGAGATCTACTTCATCGAGACCAAGAAGTTCCTGCTGATCG
GCAAGAGCAACATCTGCGTGAAGGTGGGCGAGAAGAGCCTGACCTGCAA
GAAGATCGACCTGACCACCATCGTGAAGCCCGAGGCCCCCTTCGACCTGA
GCGTGGTGTACAGGGAGGGCGCCAACGACTTCGTGGTGACCTTCAACACC
AGCCACCTGCAGAAGAAGTACGTGAAGGTGCTGATGCACGACGTGGCCTA
CAGGCAGGAGAAGGACGAGAACAAGTGGACCCACGTGAACCTGAGCAGC
ACCAAGCTGACCCTGCTGCAGAGGAAGCTGCAGCCCGCCGCCATGTACGA
GATCAAGGTGAGGAGCATCCCCGACCACTACTTCAAGGGCTTCTGGAGCG
AGTGGAGCCCCAGCTACTACTTCAGGACCCCCGAGATCAACAACAGCAGC
GGCGAGATGGACCCCATCCTGCTGACCTGCCCCACCATCAGCATCCTGAG
CTTCTTCAGCGTGGCCCTGCTGGTGTATCCTGGCCTGCGTGCTGTGGAAGAA
GAGGATCAAGCCCATCGTGTGGCCAGCCTGCCCCGACCACAAGAAGACCC
TGGAGCACCTGTGTAAGAAGCCAGGAAGAACCTGAACGTGAGCTTCAAC
CCCGAGAGCTTCTGGAAGTGCAGATCCACAGGGTGGACGACATCCAGGC
CAGGGACGAGGTGGAGGGCTTCTGCAAGACACCTTCCCCCAGCAGCTGG
AGGAGAGCGAGAAGCAGAGGCTGGGCGGCGACGTGCAGAGCCCCAACTG
CCCCAGCGAGGACGTGGTGTATCCCCCGAGAGCTTCGGCAGGGACAGC
AGCCTGACCTGCCTGGCCGGCAACGTGAGCGCCTGCGACGCCCCCATCCT

GAGCAGCAGCAGGAGCCTGGACTGCAGGGAGAGCGGCAAGAACGGCCCC
 CACGTGTACCAGGACCTGCTGCTGAGCCTGGGCACCACCAACAGCACCT
 GCCACCCCCCTTCAGCCTGCAGAGCGGCATCCTGACCCTGAACCCGTGG
 CCCAGGGCCAGCCATCCTGACCAGCCTGGGCAGCAACCAGGAGGAGGC
 CTACGTGACCATGAGCAGCTTCTACCAGAACCAG

GPC3CAR + IL7RaCPT Protein (SEQ ID NO: 111)

DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGPPKL
 LIYWASSRESGVPDRFSGSGGTDFLTITSLQAEDVAVYYCQQYNYPLTFG
 QGTKLEIKGSTSGSGKPGSGEGSTKGEVQLVESGGGLVQPGSLRLSCAASGF
 TFNKNAMNWVRQAPGKGLEWVGRIRNKTNNYATYYADSVKARFTISRDDS
 KNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQGLTVTVSSGSTTTPAPRPPT
 PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSL
 VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS
 RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
 EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
 MQALPPRGSGEGRGSLLTCGDVEENPGPMLLLVTSLLLCELPHPAFLIPH
 HHHGESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNITN
 LEFEICGALVEVKCLNFRKLQEIFYIETKKFLLIGKSNICVKVGEKSLTCKKIDL
 TTIVKPEAPFDLSVVYREGANDFVVTFNSTHLQKKYVKVLMHADVAYRQEKD
 ENKWTHVNLSSSTKLTLQRKLQPAAMYEIKVRSIPDHYFKGFWSEWSPSYF
 RTPEINNSSGEMDPILLTCPTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDH
 KKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQQL
 EESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSR
 SLDCRESGKNGPHVYQDLLLSLGTNSTLPPFSLQSGILTLNPVAQQQPILTS
 LGSNQEEAYVTMSSFYQNNQ

The data from this experiment is shown in Figure 19. GPC3 CAR T cells peaked in their expansion on day 10 with a maximal 2.4-fold above the starting cell density. GPC3 CAR T cells that also express IL7R α CPT expanded up to 40-fold over baseline by day 28 and only stopped expanding after day 31 when targets were no longer added.

Table 2

Days of Co-culture	IL7RaCPT			GPC3 CAR			GPC3 CAR + IL7RaCPT		
	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05
0	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05	5.00E+05
3	6.53E+04	6.38E+04	6.43E+04	2.12E+04	1.90E+04	2.10E+04	1.33E+04	1.63E+04	1.71E+04
7	2.64E+05	2.89E+05	2.99E+05	6.16E+05	5.53E+05	5.25E+05	4.64E+05	4.88E+05	4.20E+05
10	2.09E+05	2.35E+05	2.73E+05	1.37E+06	1.19E+06	1.09E+06	1.08E+06	1.81E+06	1.40E+06
14	5.49E+04	6.72E+04	5.60E+04	1.29E+06	1.13E+06	8.15E+05	2.10E+06	2.06E+06	2.03E+06
17	1.58E+04	1.86E+04	2.05E+05	4.64E+05	3.65E+05	3.01E+05	3.51E+06	3.08E+06	2.81E+06
21	1.26E+04	9.37E+03	1.00E+04	6.30E+05	4.60E+05	3.99E+05	4.51E+06	3.94E+06	3.62E+06
24	8.21E+03	5.62E+03	4.18E+03	5.24E+05	2.30E+05	2.03E+05	9.75E+06	8.95E+06	7.39E+06
28	3.66E+04	3.41E+04	2.78E+04	1.68E+05	9.67E+04	1.26E+05	2.00E+07	2.28E+07	2.11E+07
35	4.44E+03	2.01E+03	9.94E+02	6.14E+04	4.68E+04	4.42E+04	1.24E+07	1.24E+07	1.22E+07
42	2.59E+03	1.97E+02	3.13E+02	1.40E+02	1.45E+02	9.93E+01	4.86E+05	5.42E+05	3.30E+05

OTHER EMBODIMENTS

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