POLYNUCLEOTIDE CONSTRUCT AND RELATED VIRAL VECTORS AND METHODS

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Application No. 63/422,920, filed November 4, 2022, U.S. Provisional Application No. 63/449,289, filed March 1, 2023, and U.S. Provisional Application No. 63/466,714, filed May 15, 2023, all entitled "POLYNUCLEOTIDE CONSTRUCT AND RELATED VIRAL VECTORS AND METHODS" the contents of which are incorporated by reference in their entirety.

Reference to An Electronic Sequence Listing

[0002] The contents of the electronic sequence listing (260132000940SEQLIST.xml; Size 246,342 bytes; and Date of Creation: October 31, 2023) is herein incorporated by reference in its entirety.

Field of the Invention

[0003] The present disclosure provides polycistronic constructs for coexpression of a synthetic cytokine receptor complex and a chimeric antigen receptor system, as well as vectors, such as viral vectors comprising same, cells comprising same, and methods of using same.

Background

[0004] Chimeric antigen receptor (CAR) T cell therapies have demonstrated limited efficacy against solid tumors, in part due to challenges overcoming solid tumor heterogeneity and CAR T cell exhaustion associated with the immunosuppressive tumor microenvironment (TME). In addition to this challenge, there also are challenges in methods of delivering CAR-expressing cells to subjects in a manner that can provide controllable persistence for therapeutic efficacy of the CAR in the treatment of various diseases, including cancer. Provided herein are embodiments that address such needs.

Summary

[0005] Provided herein is a polycistronic construct containing in 5' to 3' order (a) a first expression cassette including a nucleotide sequence encoding FRB, (b) a second expression cassette including a nucleotide sequence encoding a synthetic cytokine gamma chain polypeptide, (c) a third expression cassette including a nucleotide sequence encoding a synthetic cytokine beta chain polypeptide, and (d) a fourth expression cassette including a nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein each of the expression cassettes are separated by a cleavage site sequence.

[0005a] Disclosed herein is a polycistronic construct comprising in 5' to 3' order (a) a first nucleotide sequence encoding FKBP12-rapamycin binding (FRB), (b) a second nucleotide sequence encoding a synthetic cytokine gamma chain polypeptide, (c) a third nucleotide sequence encoding a synthetic cytokine beta chain polypeptide, and (d) a fourth nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein each of the nucleotide sequences are separated by a nucleotide sequence encoding a cleavage site sequence.

[0006] In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:3, 13, or 50. In some of any embodiments, the nucleotide sequence encoding the FRB includes the nucleotide sequence of SEQ ID Nos:3, 13, or 50. In some of any embodiments, the FRB includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some of any embodiments, the FRB includes the amino acid sequence of SEQ ID NOs:4, 14, or 51.

[0007] In some of any embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID No:15. In some of any embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide includes the nucleotide sequence of SEQ ID No:15.

[0008] In some of any embodiments, the synthetic cytokine gamma chain polypeptide includes interleukin 2 receptor subunit γ (IL2RG). In some embodiments, the IL2RG includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID No:16. In some of any embodiments, the IL2RG includes the amino acid sequence of SEQ ID No:16.

[0009] In some of any embodiments, the second expression cassette further includes a nucleotide sequence encoding FRB. In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:13. In some of any embodiments, the nucleotide sequence encoding the FRB includes the nucleotide sequence of SEQ ID NO:13. In some of any embodiments, the FRB includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14. In some of any embodiments, the FRB includes the amino acid sequence of SEQ ID NO: 14.

[0010] In some of any embodiments, the second expression cassette is codon optimized.

[0011] In some of any embodiments, the second expression cassette includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:11. In some of any embodiments, the second expression cassette includes the nucleotide sequence of SEQ ID NO:11. In some of any embodiments, the second expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:12. In some of any embodiments, the second expression cassette encodes an amino acid sequence including the sequence of SEQ ID NO:12.

[0012] In some of any embodiments, the second expression cassette further includes a nucleotide sequence encoding FKBP12. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:21 or 55. In some of any embodiments, the nucleotide sequence encoding the FKBP12 includes the nucleotide sequence of SEQ ID Nos:21 or 55. In some of any embodiments, the FKBP12 includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:22. In some of any embodiments, the FKBP12 includes the amino acid sequence of SEQ ID NO: 22.

[0013] In some of any embodiments, the second expression cassette includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some of any embodiments, the second expression cassette includes the nucleotide sequence of SEQ ID NOs: 53 or 56. In some of any embodiments, the second expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%,

97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some of any embodiments, the second expression cassette encodes an amino acid sequence including the sequence of SEQ ID NOs: 54, 57, or 128.

[0014] In some of any embodiments, the synthetic cytokine beta chain polypeptide includes interleukin 2 receptor subunit β (IL2RB). In some of any embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:23 or 61. In some of any embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide includes the nucleotide sequence of SEQ ID Nos:23 or 61. In some embodiments, the IL2RB includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID Nos:24 or 62. In some of any embodiments, the IL2RB includes the amino acid sequence of SEQ ID Nos:24 or 62.

[0015] In some of any embodiments, the third expression cassette further includes a nucleotide sequence encoding FKBP12. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 21. In some of any embodiments, the nucleotide sequence encoding the FKBP12 includes the nucleotide sequence of SEQ ID NO: 21. In some of any embodiments, the FKBP12 includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:22. In some of any embodiments, the FKBP12 includes the amino acid sequence of SEQ ID NO: 22.

[0016] In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 55. In some of any embodiments, the nucleotide sequence encoding the FKBP12 includes the nucleotide sequence of SEQ ID NO: 55.

[0017] In some of any embodiments, the third expression cassette is codon optimized.

[0018] In some of any embodiments, the third expression cassette further contains a nucleotide sequence encoding FRB. In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:13. In some of any embodiments, the nucleotide sequence encoding the FRB includes the nucleotide sequence of SEQ ID NO:13. In some of any embodiments, the FRB includes an

amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14. In some of any embodiments, the FRB includes the amino acid sequence of SEQ ID NO: 14.

[0019] In some of any embodiments, the third expression cassette includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 19. In some of any embodiments, the third expression cassette includes the nucleotide sequence of SEQ ID NO: 19. In some of any embodiments, the third expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 20. In some of any embodiments, the third expression cassette encodes an amino acid sequence including the sequence of SEQ ID NO: 20.

[0020] In some of any embodiments, the third expression cassette includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 59. In some of any embodiments, the third expression cassette includes the nucleotide sequence of SEQ ID NO: 59. In some of any embodiments, the third expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some of any embodiments, the third expression cassette encodes an amino acid sequence including the sequence of SEQ ID NOs: 60 or 129.

[0021] In some of any embodiments, the CAR includes an extracellular antigen binding domain, a transmembrane domain and an endodomain containing a costimulatory signaling domain and a primary activation signaling domain such as a CD3zeta signaling domain. In some embodiments, the extracellular antigen binding domain and transmembrane domain are separated by a spacer sequence, such as containing a hinge domain. In some embodiments, the extracellular antigen binding domain comprises an scFv.

[0022] In some of any embodiments, the CAR includes an scFv domain. In some embodiments, the scFv domain includes anti-fluorescein isothiocyanate (FITC) E2.

[0023] In some of any embodiments, the scFv domain includes a light chain variable domain (VL), a linker, and a heavy chain variable domain (VH). In some embodiments, the scFv VL includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%

identical to the nucleotide sequence of SEQ ID Nos:30 or 65. In some of any embodiments, the scFv VL includes the nucleotide sequence of SEQ ID Nos:30 or 65. In some of any embodiments, the scFv VL includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:31. In some of any embodiments, the scFv VL includes the amino acid sequence of SEQ ID NO:31.

[0024] In some of any embodiments, the scFv VH includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:34 or 67. In some of any embodiments, the scFv VH includes the nucleotide sequence of SEQ ID Nos:34 or 67. In some of any embodiments, the scFv VH includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:35. In some of any embodiments, the scFv VH includes the amino acid sequence of SEQ ID NO:35.

[0025] In some of any embodiments, the scFv linker includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:32 or 66. In some of any embodiments, the scFv linker includes the nucleotide sequence of SEQ ID Nos:32 or 66. In some of any embodiments, the scFv linker includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:33. In some of any embodiments, the scFv linker includes the amino acid sequence of SEQ ID NO:33.

[0026] In some of any embodiments, the scFv includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:28 or 64. In some of any embodiments, the scFv includes the nucleotide sequence of SEQ ID Nos:28 or 64. In some embodiments, the scFv includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:29. In some of any embodiments, the scFv includes the amino acid sequence of SEQ ID NO:29.

[0027] In some of any embodiments, the CAR includes a hinge domain. In some embodiments, the hinge domain includes a short hinge or a medium hinge domain.

[0028] In some of any embodiments, the hinge domain includes a CD8 or an IgG. In some embodiments, the CD8 hinge includes CD8α hinge.

[0029] In some embodiments, the CD8α hinge includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some of any embodiments, the CD8α hinge includes the nucleotide sequence of SEQ ID NO: 38. In some of any embodiments, the CD8α hinge includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some of any embodiments, the CD8α hinge includes the amino acid sequence of SEQ ID NOs: 39 or 115.

[0030] In some of any embodiments, the CAR contains a transmembrane domain. In some embodiments, the transmembrane domain includes a CD8 or a CD28. In some embodiments, the CD8 transmembrane domain includes CD8α transmembrane domain.

[0031] In some of any embodiments, the transmembrane domain includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:40. In some of any embodiments, the transmembrane domain includes the nucleotide sequence of SEQ ID NO:40. In some of any embodiments, the transmembrane domain includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:41. In some of any embodiments, the transmembrane domain includes the amino acid sequence of SEQ ID NO:41.

[0032] In some of any embodiments, the CAR includes an endodomain. In some embodiments, the endodomain includes a costimulatory molecule signaling domain.

[0033] In some of any embodiments, the endodomain includes a signaling domain of 4-1BB, CD3ζ, and/or CD28.

[0034] In some embodiments, the 4-1BB endodomain includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:42 or 69. In some of any embodiments, the 4-1BB endodomain includes the nucleotide sequence of SEQ ID Nos: 42 or 69. In some of any embodiments, the 4-1BB endodomain includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:43. In some of any embodiments, the 4-1BB endodomain includes the amino acid sequence of SEQ ID NO:43.

[0035] In some of any embodiments, the CD3ζ endodomain includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of

SEQ ID Nos: 46, 70, 100 or 118. In some of any embodiments, the CD3ζ endodomain includes the nucleotide sequence of SEQ ID Nos: 46, 70, 100 or 118. In some of any embodiments, the CD3ζ endodomain includes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:47. In some of any embodiments, the CD3ζ endodomain includes the amino acid sequence of SEQ ID NO:47.

[0036] In some of any embodiments, the fourth expression cassette includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos: 26, 63, 71, or 82. In some of any embodiments, the fourth expression cassette includes the nucleotide sequence of SEQ ID Nos: 26, 63, 71, or 82. In some of any embodiments, the fourth expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some of any embodiments, the fourth expression cassette encodes the amino acid sequence of SEQ ID NOs: 27, 72 or 127.

[0037] In some of any embodiments, each of the cleavage site sequences comprises a 2A cleavable linker sequence.

[0038] In some of any embodiments, each nucleotide encoding a 2A cleavable linker sequences is different.

[0039] In some of any embodiments, the 2A cleavable linker is independently a T2A, P2A, E2A or F2A cleavage site.

[0040] In some of any embodiments, the 2A cleavable linker is independently a P2A or a T2A.

[0041] In some of any embodiments, at least one 2A cleavable linker is a P2A and the nucleotide sequence encoding the P2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 17, 25, 52, or 58.

[0042] In some of any embodiments, the nucleotide sequence encoding the P2A cleavable linker is set forth in SEQ ID NOs: 17, 25, 52, or 58.

[0043] In some of any embodiments, the P2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:18.

[0044] In some of any embodiments, the P2A cleavable linker comprises the sequence set forth in SEQ ID NO:18.

[0045] In some of any embodiments, at least one 2A cleavable linker is a T2A and the nucleotide sequence encoding the T2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:9.

[0046] In some of any embodiments, the nucleotide sequence encoding the T2A cleavable linker is set forth in SEQ ID NO:9.

[0047] In some of any embodiments, the T2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:10.

[0048] In some of any embodiments, the T2A cleavable linker comprising the sequence set forth in SEQ ID NO:10.

[0049] In some of any embodiments, at least one of the cleavage site sequences comprises a furin cleavage site sequence.

[0050] In some of any embodiments, the furin cleavage site sequence is located between the first expression cassette and the second expression cassette.

[0051] In some of any embodiments, the nucleotide sequence encoding the furin cleavage site sequence comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:7.

[0052] In some of any embodiments, the nucleotide sequence encoding the furin cleavage site sequence comprises the sequence set forth in SEQ ID NO: 7. In some of any embodiments, the furin cleavage site sequence comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 8.

[0053] In some of any embodiments, the furin cleavage site sequence comprises the amino acid sequence of SEQ ID NO: 8.

[0054] In some of any embodiments, the cleavage site sequence comprises a furin cleavage site sequence and a T2A cleavage sequence (furinT2A).

[0055] In some of any embodiments, the nucleotide sequence encoding the cleavage site sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 5.

[0056] In some of any embodiments, the nucleotide sequence encoding the cleavage site sequence comprises the nucleotide sequence of SEQ ID NO: 5.

[0057] In some of any embodiments, the cleavage site sequence comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 6.

[0058] In some of any embodiments, the cleavage site sequence comprises the amino acid sequence of SEQ ID NO:6.

[0059] In some of any embodiments, the first expression cassette and second expression cassette are separated by a furinT2A, the second expression cassette and third expression cassette are separated by a P2A, and the third expression cassette and fourth expression cassette are separated by a P2A.

[0060] In some of any embodiments, the construct includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:1. In some of any embodiments, the construct includes the nucleotide sequence of SEQ ID NO:1.

[0061] In some of any embodiments, the construct encodes a polypeptide including an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:2. In some of any embodiments, the construct encodes a polypeptide including the amino acid sequence of SEQ ID NO:2.

[0062] In some of any embodiments, the construct includes a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:48. In some of any embodiments, the construct includes the nucleotide sequence of SEQ ID NO:48.

[0063] In some of any embodiments, the construct encodes a polypeptide including an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:49. In some of any embodiments, the construct encodes a polypeptide including the amino acid sequence of SEQ ID NO:49.

[0064] In some embodiments, provided herein is a viral vector containing any one of the polycistronic constructs disclosed herein. Disclosed herein is a viral vector comprising the polycistronic construct as defined herein. In some embodiments, the viral vector is a lentiviral vector.

[0065] In some of any embodiments, the viral vector further contains one or more surface T cell activating agents. In some embodiments, the one or more surface T cell activating agents include CD58, anti-CD3, or CD80.

[0066] In some embodiments, provided herein is a cell containing any one of the viral vectors disclosed herein. Disclosed herein is a cell comprising the polycistronic construct as defined herein. In some embodiments, the cell includes a stem cell or a progenitor cell. In some embodiments, the stem cell includes an induced pluripotent stem cell (iPSC).

[0067] In some of any embodiments, the progenitor cell includes a peripheral blood mononuclear cell (PBMC). In some of any embodiments, the cell includes a T cell. In some of any embodiments, the cell includes a cytotoxic innate lymphocyte (CIL) cell. In some of any embodiments, the cell includes a natural killer (NK) cell.

[0068] In some embodiments, provided herein is a method of transducing a cell including contacting a target cell with any of the polycistronic constructs disclosed herein. Disclosed herein is a method of transducing a cell comprising contacting a target cell with any one of the viral vectors disclosed herein. In some of any embodiments, the target cell includes a stem cell. In some embodiments, the stem cell includes an induced pluripotent stem cell (iPSC).

[0069] In some of any embodiments, the target cell includes a progenitor cell. In some embodiments, the progenitor cell includes a peripheral blood mononuclear cell (PBMC).

[0070] In some of any embodiments, the target cell includes a T cell. In some embodiments, the T cell includes a CD4+ or CD8+ T cell.

[0071] In some of any embodiments, the method further includes contacting the target cell with a (i) a guide RNA (gRNA) targeting a target site in an endogenous gene, and (ii) an RNA-guided endonuclease, thereby inserting the nucleotide sequence into the endogenous gene.

[0072] In some embodiments, provided herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell. Disclosed herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell comprising contacting the target cell with any one of the viral vectors disclosed herein. In some embodiments, the target cell includes a stem cell. In some embodiments, the stem cell includes an induced pluripotent stem cell (iPSC).

[0073] In some embodiments, the target cell includes a progenitor cell. In some embodiments, the progenitor cell includes a peripheral blood mononuclear cell (PBMC).

[0074] In some embodiments, the target cell includes a T cell. In some embodiments, the T cell includes a CD4+ or CD8+ T cell.

[0075] In some of any embodiments, the method is performed *ex vivo* or *in vitro*.

[0076] In some of any embodiments, the method is performed *in vivo*.

[0077] In some embodiments, provided herein is a method of transducing a T cell, the method comprising contacting the T cell with a viral vector comprising one or more T cell activating agents and any one of the polycistronic constructs disclosed herein, wherein the one or more T cell activating agents bind a receptor on the T cell. Disclosed herein is a method of transducing a T cell, comprising contacting the T cell with a viral vector as defined herein. In some embodiments provided herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a T cell, the method comprising contacting the T cell with a viral vector comprising one or more T cell activating agents and any one of the polycistronic constructs disclosed herein, wherein the one or more T cell activating agents bind a receptor on the T cell. Disclosed herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a T cell, comprising contacting the T cell with a viral vector as defined herein. In some embodiments, provided herein is a method of delivering a payload to a T cell, the method comprising contacting the T cell with a viral vector comprising one or more T cell activating agents and any one of the polycistronic constructs disclosed herein, wherein the one or more T cell activating agents bind a receptor on the T cell. Disclosed herein is a method of delivering a payload to a T cell, comprising contacting the T cell with a viral vector as defined herein. In some of any embodiments, the T cell comprises a a CD4+ or CD8+ T cell. In some of any embodiments, the method is performed *ex vivo* or *in vitro.* In some of any embodiments, the method is performed *in vivo.* In some of any embodiments, the one or more T cell activating agents comprise CD58, anti-CD3, or CD80. In some of any embodiments, the viral vector comprises a lentiviral vector.

[0078] Disclosed herein is a cell produced by any one of the methods disclosed herein.

[0079] In some embodiments, provided herein is a method of administering to a subject any one of the cells disclosed herein. In some embodiments, the chimeric antigen receptor is able to be targeted to an antigen associated with a disease or condition in the subject and the subject has a

disease or condition that is able to be treated by the chimeric antigen receptor. In some embodiments, the disease or condition is a cancer. Disclosed herein is the use of a cell as defined herein in the manufacture of a medicament for treating a disease or condition in a subject. Disclosed herein is a cell as defined herein for use in a method of treating a subject having a disease or condition.

[0080] In some embodiments, provided herein is a method of administering to a subject any one of the viral vectors disclosed herein. In some of any embodiments, the method treats a disease or condition in the subject. In some of any embodiment, the the disease or condition is treatable by the chimeric antigen receptor (CAR) encoded by the polycistronic construct. In some of any embodiments, the CAR is an anti-FITC CAR and the CAR is targeted to a cell of the disease or condition by administering a bifunctional ligand comprising FITC and a ligand that specifically binds a molecule expressed on the cell of the disease or condition. In some of any embodiments, the disease or condition is a cancer. In some of any embodiments, the cancer is a solid tumor. In some of any embodiments, the cell is a cancer cell. Disclosed herein is the use of a viral vector as defined herein in the manufacture of a medicament for treating a disease or condition in a subject. Disclosed herein is a viral vector as defined herein for use in a method of treating a subject having a disease or condition.

[0081] In some of any embodiments, the chimeric antigen receptor is able to be targeted to an antigen associated with a disease or condition in the subject and the subject has a disease or condition that is able to be treated by the chimeric antigen receptor. In some of any embodiments, the disease or condition is a cancer.

[0082] In some or any embodiments, the CAR is a CAR that targets a ligand that is able to bind to a an antigen on the surface of a cell associated with a disease or condition. In some of any embodiments, the CAR of any of the provided embodiments is an anti-FITC CAR directed against FITC and the ligand is a bifunctional ligand composed of FITC and a binding molecule that is able to bind to a surface molecule or receptor on the target cell. In some of any embodiments, the method further including administering the bifunctional ligand to tag a cancer cell in the subject, wherein the bifunctional ligand specifically binds a molecule expressed on a tumor. In some of any embodiments, the bifunctional ligand is FITC-folate. In some embodiments, the cancer is an osteosarcoma. In some of any embodiments, the bifunctional ligand comprises a fluorescein

isothiocyanate (FITC) moiety and the chimeric antigen receptor (CAR) encoded by the polycistronic construct is an anti-FITC CAR.

[0083] In some of any embodiments, the method further including administering a non-physiological ligand to the subject. In some of any embodiments, the non-physiological ligand is able to bind to the synthetic cytokine receptor and induce gamma cytokine signaling in the cell. In some of any embodiments, the nonphysiological ligand. In some of any embodiments, the non-physiological ligand binds to a synthetic cytokine receptor composed of the synthetic gamma chain polypeptide and the synthetic cytokine beta chain polypeptide encoded by the polycistronic construct. In some of any embodiment, the non-physiological ligand includes rapamycin or a rapamycin analog. In some of any embodiments, binding of the non-physiological ligand to the synthetic cytokine receptor stimulates an intracellular cytokine signal in cells transduced to express the synthetic cytokine receptor. In some of any embodiments, binding of the non-physiological ligand to the synthetic cytokine receptor promotes proliferation of cells transduced to express the synthetic cytokine receptor.

Brief Description of the Drawings

[0084] **FIG. 1A** shows lentivirus particles surface-engineered to engage and activate T cells to deliver a payload containing free FKBP12-rapamycin binding (FRB), rapamycin activated cytokine receptor (RACR), and a chimeric antigen receptor (CAR) that binds a tumor tag (TagCAR). Rapamycin, FRB and RACR drive expansion of transduced TagCAR T cells while also inhibiting tumor proliferation and immune responses against the lentivirus particles.

[0085] **FIG. 1B** shows how the TagCAR T system targets tumor cells. Bispecific tumor tags comprise a universal tag antigen on one end and a swappable ligand on the other end that engages a tumor- or tumor microenvironment-associated antigen. Once T cells express TagCAR, the TagCAR can bind the universal tag (e.g., FITC-Folate).

[0086] **FIGS. 2A-2B** show 8 polycistronic constructs. The polynucleotide of the TagCAR encodes a CAR with the following components in N-terminal to C-terminal order: an scFv (e.g. anti-FITC E2), a hinge (spacer), a transmembrane domain, and an endodomain with a costimulatory signaling domain and a CD3zeta signaling domain (Z). The constructs differed in the hinge (spacer) domain, either IgG4 hinge (IgG4H) or CD8alpha hinge (CD8H); transmembrane domain,

either CD28 TM or CD8 TM; and costimulatory domain, either 41BB costimulatory domain or CD28 costimulatory domain. The constructs also differed in the placement of the TagCAR, being present either as the front or end of the construct transgene sequence. The individual polynucleotide components of the construct were separated by a 2A cleavage site sequence.

[0087] **FIGS. 3A-3B** show day 3 activation and day 7 transduction of CD8+ and CD4+T cell populations cultured with the polynucleotide constructs in **FIGS. 2A-2B** at a multiplicity of infection (MOI) of 2 or 10.

[0088] **FIG. 3C** shows FRB expression in T cells when the FRB is placed first or second in the transgenes as depicted in **FIGS. 2A-2B**.

[0089] **FIG. 4** shows the percentage of TagCAR+ T cells generated when transduced with Construct D.2 or Construct C.2, and cultured in IL-2 alone, rapamycin alone, or both.

[0090] **FIGS. 5A-5B** show a tumor cell killing assay with PBMCs transduced with the constructs disclosed in **FIGS. 2A-2B** and treated with IL-2 only or IL-2 and rapamycin (rapa).

[0091] **FIG. 6A** shows CD19 chimeric antigen receptor (CAR) and TagCAR expression in PBMCs transduced with a lentivirus encoding Construct V or Construct C.2 polynucleotide.

[0092] **FIG. 6B** shows the number of CD19-CAR+ (left panel) or FITC-Folate+ (right panel) PBMCs across 11 days. PBMCs were either transduced with lentivirus encoding Construct V or Construct C.2 polynucleotide.

[0093] **FIG. 7A** shows the orientation of polynucleotide constructs from N- to C-terminal. In Construct C.2, the FRB is encoded next to IL2Rβ. In the Construct C.2U, the FRB is encoded next to IL2Rγ.

[0094] **FIG. 7B** shows the percentage of CD25 T cells 3 days after transfection with lentivirus encoding Construct V or Construct C.2 polynucleotide, in 5 donors.

[0095] **FIG. 7C** shows the percentage of TagCAR T cells on day 7. PBMCs were transduced with a lentivirus encoding Construct C.2 or Construct C.2U polynucleotide and stained on day 7.

[0096] **FIG. 7D** shows TagCAR MFI in T cells on day 7. PBMCs were transduced with a lentivirus encoding Construct C.2 or Construct C.2U polynucleotide.

[0097] **FIG. 7E** shows an immunoblot depicting expression of FRB:IL2Rβ and FRB:IL2Rγ in T cells. PBMCs were transduced with a lentivirus encoding Construct C.2 or Construct C.2U

polynucleotide. A rabbit pAb against FRB was used to detect FRB. Cells were collected on day 8 for western blot.

[0098] FIG. 7F shows an immunoblot depicting expression of FKBP12:IL2Rβ and FKBP12:IL2Rγ in T cells. PBMCs transduced with a lentivirus encoding Construct C.2 or Construct C.2U polynucleotide. A mouse mAb against FKBP12 was used to detect FKBP12. Cells were collected on day 8 for western blot.

[0099] FIG. 8A show total Tag-CAR+ T cells transduced with Construct C.2U or Construct C.2 across 14 days in 1 donor. Tag-CAR+ T cells were treated with either IL-2 (250 U/mL) or rapamycin (10nM).

[0100] FIG. 8B show total Tag-CAR+ T cells transduced with Construct C.2U or Construct C.2 across 12 days in 1 donor. Tag-CAR+ T cells were treated with either IL-2 (250 U/mL), rapamycin (10nM), rapamycin and IL-2, or AP21967 (50nM).

[0101] FIG. 9A shows day 3 activation and day 7 transduction of CD8+ and CD4+T cell populations cultured with Construct C.2 at a multiplicity of infection (MOI) of 2 or 10.

[0102] FIG. 9B shows representative flow cytometry plots of TagCAR expressing CD8+ T cells transduced with Construct C.2 on day 7 post-transduction.

[0103] FIG. 10A shows a timeline of an *in vitro* assay method for measuring T cell activation and TagCAR T cell abundance. On day 0, PBMCs are transduced with the lentivirus particles. On day 3, IL-2 or IL-2 and rapamycin are added to measure T cell activation. On days 7, 11 and 14, TagCAR T cell abundance was measured.

[0104] FIG. 10B shows enrichment (left) and expansion (right) of TagCAR T cells across 2 weeks. Enrichment was measured by flow cytometry. Expansion was measured by flow cytometry with counting beads.

[0105] FIG. 11A shows a graph depicting tumor cell growth after incubating breast carcinoma cells (MDA-MB-231 or MDA) with rapamycin, TagCAR T cells and FITC-Folate, or TagCAR T cells, FITC-Folate and rapamycin. Breast carcinoma cells that have not been incubated with TagCAR T cells, FITC-folate or rapamycin are shown by arrow. Tumor cells were reintroduced (i.e., tumor cell re-challenge) at 72 hours, 144 hours, and 216 hours.

[0106] FIG. 11B shows a graph depicting T cell proliferation after incubating breast carcinoma cells (MDA-MB-231 or MDA) with rapamycin, TagCAR T cells and FITC-Folate, or TagCAR T

cells, FITC-Folate and rapamycin (rapa). Breast carcinoma cells that have not been incubated TagCAR T cells, FITC-folate or rapamycin are shown by arrow. Tumor cells were reintroduced (i.e., tumor cell re-challenge) at 72 hours, 144 hours, and 216 hours.

[0107] FIG. 12A shows a timeline of an *in vivo* mouse model of breast cancer. Fourteen days before the experiment began, NSG MHCI/II^{DKO} mice were injected with FRα+ MDA-MB-231 cells. On the first day of the experiment (D0), mice were infused with ex vivo generated TagCAR T cells. Mice were injected with FITC-Folate subcutaneously two times per week for four weeks. Blood was collected weekly for flow cytometry.

[0108] FIG. 12B shows a graph depicting tumor volume in mice across 4 weeks. Mice were injected with: (i) 10e6 T cells that have not been transduced with lentivirus particles (Mock T cells) with FITC-Folate; (ii) 10e6 TagCAR T cells without FITC-Folate; (iii) 5e6 TagCAR T cells with FITC-Folate; and (iv) 10e6 TagCAR T cells with FITC-Folate. Tumor volume was measured using calipers.

[0109] FIG. 13A shows a timeline of an *in vivo* mouse model of breast cancer. Fourteen days before the experiment began, NSG MHCI/II^{DKO} mice were injected with FRα+ MDA-MB-231 cells. On the first day of the experiment (D0), mice were humanized with PBMCs and administered lentivirus particles. Mice were injected with FITC-Folate subcutaneously two times per week for 7 weeks. Blood was collected weekly for flow cytometry. Tumor volume was measured using calipers.

[0110] FIG. 13B shows circulating TagCAR T cell detection by flow cytometry. The left panel depicts the percentage of TagCAR+ per CD3+ T cells on day 7. The right panel depicts the total number of CD3+/TagCAR+ T cells per μL of blood on day 7. Mice were injected with: (i) no vector and FITC-Folate; (ii) 100e6 transfecting units (TU) of TagCAR vector and FITC-Folate; (iii) 25e6 TU TagCAR vector and FITC-Folate; and (iv) 100e6 TU TagCAR vector and FITC-Folate.

[0111] FIG. 13C shows a graph depicting tumor volume in mice across 7 weeks. Tumor volume was measured using calipers.

[0112] FIG. 14A shows a graph depicting tumor volume in mice across 25 days. Mice were injected with: (i) FITC-Folate alone; (ii) 5.0e6 transfecting units (TU) of TagCAR vector without FITC-Folate; (iii) 0.2e6 TU of TagCAR vector with FITC-Folate; (iv) 1.0e6 TU TagCAR vector

with FITC-Folate; and (v) 5.0e6 TU of TagCAR vector with FITC-Folate. Tumor volume was measured using calipers.

[0113] **FIG. 14B** shows circulating CD3 TagCAR T cells per μL of blood as detected by flow cytometry. Mice were injected with: (i) FITC-Folate alone (triangle); (ii) 5.0e6 TU TagCAR vector alone; (iii) 0.2e6 TU TagCAR vector with FITC-Folate; (iv) 1.0e6 TU TagCAR vector with FITC-Folate; and (v) 5.0e6 TU TagCAR vector with FITC-Folate.

Detailed Description

[0114] The disclosure relates generally to a polynucleotide construct comprising a contiguous polynucleotide sequence encoding at least two synthetic receptors and methods for uses thereof. In some embodiments, the polynucleotide construct is a polycistronic construct encoding a synthetic cytokine receptor, a synthetic chimeric antigen receptor (CAR), and a freely diffusible FRB, in which the cytokine receptor is responsive to rapamycin binding. Advantageously, FRB reduces the inhibitory effects of rapamycin on mTOR in cells engineered to express the polycistronic constructs provided herein. Expression of the freely diffusible FRB can promote consistent activation and proliferation of engineered cells.

[0115] The disclosure also provides that the 5' to 3' order of the polycistronic construct is important for the expression of polypeptides encoded by the construct. In some embodiments, the 5' to 3' order of the polynucleotide constructs improve expression of encoded polypeptides. In some embodiments, a polycistronic construct provided herein comprises a nucleotide encoding FRB at the 5' end, which improves FRB expression and correlates with increased protection against rapamycin-mediated immunosuppression. Moreover, it was surprisingly found that the CAR expression – even when positioned at the 3' end of the construct – was sufficiently high to mediate antigen-directed killing by cells in which the polynucleotide construct was expressed.

[0116] In some aspects, provided herein is a polycistronic construct comprising four expression cassettes separate by cleavage site sequences. In some embodiments, the four expression cassettes comprise, in 5' to 3' order, a first expression a first expression cassette comprising a nucleotide sequence encoding FRB, a second expression cassette comprising a nucleotide sequence encoding a synthetic cytokine gamma chain polypeptide, a third expression cassette comprising a nucleotide

sequence encoding a synthetic cytokine beta chain polypeptide, and a fourth expression cassette comprising a nucleotide sequence encoding a CAR.

[0117] In some aspects, provided herein is a viral vector comprising any one of the polycistronic constructs disclosed herein.

[0118] In some aspects, provided herein is a cell comprising any of the viral vectors disclosed herein.

[0119] In some aspects, provided herein is a method of transducing a cell comprising contacting a target cell with any one of the viral vectors disclosed herein.

[0120] In some aspects, provided herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell comprising contacting the target cell with any one of the viral vectors disclosed herein.

[0121] In some aspects, provided herein is a cell produced by any of the methods disclosed herein.

[0122] In some aspects, provided herein is a method of administering to a subject any of the cells disclosed herein. In some aspects, provided herein is a method of administering to a subject any of the viral vectors disclosed herein.

[0123] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0124] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. POLYCISTRONIC CONSTRUCTS

[0125] Provided herein are polycistronic constructs encoding one or more separate proteins. In some embodiments, the polycistronic constructs comprise one, two, three, or four expression cassettes each encoding a separate protein. In some embodiments, the polycistronic constructs

comprise four expression cassettes each encoding a separate protein. In some embodiments, the expression cassettes are separated by cleavable linkers.

[0126] In some embodiments, the polycistronic constructs provided herein comprise a nucleotide sequence encoding an FRB. In some embodiments, the polycistronic constructs provided herein comprise a nucleotide sequence encoding a chimeric antigen receptor (CAR). In some embodiments, the polycistronic constructs provided herein comprise a nucleotide sequence encoding a synthetic cytokine polypeptide. In some embodiments, the synthetic cytokine polypeptide comprises a synthetic cytokine gamma chain polypeptide and a synthetic cytokine beta chain polypeptide. In some embodiments, the synthetic cytokine gamma chain comprises interleukin 2 receptor subunit γ (IL2RG). In some embodiments, the synthetic cytokine gamma chain further comprises FRB. In some embodiments, the synthetic cytokine beta chain comprises interleukin 2 receptor subunit β (IL2RB). In some embodiments, the synthetic cytokine gamma chain comprises further FKBP12. In other embodiments, the synthetic cytokine gamma chain comprises interleukin 2 receptor subunit γ (IL2RG). In some embodiments, the synthetic cytokine gamma chain further comprises FKBP12. In some embodiments, the synthetic cytokine beta chain comprises interleukin 2 receptor subunit β (IL2RB). In some embodiments, the synthetic cytokine beta chain further comprises FRB.

[0127] In some embodiments, the polycistronic construct provided herein comprises nucleotide sequences encoding an FRB, a synthetic cytokine polypeptide, and a CAR.

[0128] In some embodiments, the polycistronic construct comprises in 5' to 3' order a nucleotide sequence encoding FRB, a nucleotide sequence encoding a synthetic cytokine polypeptide, and a nucleotide sequence encoding a CAR. In some embodiments, the nucleotide sequence encoding the synthetic cytokine polypeptide comprises in 5' to 3' order a first nucleotide sequence encoding FRB:IL2RG and a second nucleotide sequence encoding FKBP12:IL2RB. In some embodiments, the nucleotide sequence encoding the synthetic cytokine polypeptide comprises in 5' to 3' order a first nucleotide sequence encoding FKBP12:IL2RG and a second nucleotide sequence encoding FRB:IL2RB.

[0129] In one aspect, provided herein is a polycistronic construct comprising in 5' to 3' order (a) a first expression cassette comprising a nucleotide sequence encoding FRB, (b) a second expression cassette comprising a nucleotide sequence encoding a synthetic cytokine gamma chain

polypeptide, (c) a third expression cassette comprising a nucleotide sequence encoding a synthetic cytokine beta chain polypeptide, and (d) a fourth expression cassette comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein each of the expression cassettes are separated by a nucleotide sequence encoding a cleavage site sequence.

A. Cytosolic FRB

[0130] In some embodiments, an expression cassette of the polycistronic construct encodes an FRB domain. The FRB domain is an approximately 270 base pair (bp) domain derived from the mTOR protein kinase. It may be expressed in the cytosol as a freely diffusible soluble protein.

[0131] In some embodiments, the first expression cassette in the polycistronic construct comprises a nucleotide sequence encoding an FRB. In some embodiments, when the FRB is expressed, it is a freely diffusible soluble protein.

[0132] In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 80% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 85% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 90% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 95% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 96% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 97% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 98% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 99% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB is at least 100% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50. In some embodiments, the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID Nos: 3, 13, or

50. In some embodiments, the nucleotide sequence encoding the FRB consists the nucleotide sequence of SEQ ID Nos: 3, 13, or 50.

[0133] In some embodiments, the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51. In some embodiments, the FRB comprises the amino acid sequence of SEQ ID NOs: 4, 14, or 51. In some embodiments, the FRB consists of the amino acid sequence of SEQ ID NOs: 4, 14, or 51.

[0134] In some embodiments, synthetic cytokine receptor complex comprises a cytosolic polypeptide that binds to the ligand or a complex comprising the ligand.

[0135] Advantageously, the cytosolic FRB confers resistance to the immunosuppressive effect of the non-physiological ligand (*e.g.*, rapamycin or rapalog).

B. Synthetic Cytokine Receptor

[0136] In some embodiments, an expression cassette of the polycistronic construct encodes a synthetic cytokine receptor. The synthetic cytokine receptors of the present disclosure comprise a synthetic gamma chain and a synthetic beta chain, each comprising a dimerization domain. The

dimerization domains controllable dimerize in the present of a non-physiological ligand, thereby activating signaling the synthetic cytokine receptor.

[0137] The synthetic cytokine receptor can include transmembrane receptor proteins that include the synthetic gamma chain polypeptide and the synthetic beta chain polypeptide, such as provided as a first transmembrane receptor and a second transmembrane receptor. The synthetic gamma chain polypeptide comprises a first dimerization domain, a first transmembrane domain, and an interleukin-2 receptor subunit gamma (IL-2RG) intracellular domain. The dimerization domain may be extracellular (N-terminal to the transmembrane domain) or intracellular (C-terminal to the transmembrane domain and N- or C-terminal to the IL-2G intracellular domain). The synthetic beta chain polypeptide comprises a second dimerization domain, a second transmembrane domain, and an intracellular domain selected from an interleukin-2 receptor subunit beta (IL-2RB) intracellular domain, an interleukin-7 receptor subunit beta (IL-7RB) intracellular domain, or an interleukin-21 receptor subunit beta (IL-21RB) intracellular domain. The dimerization domain may be extracellular (N-terminal to the transmembrane domain) or intracellular (C-terminal to the transmembrane domain and N- or C-terminal to the IL-2RB or IL-7RB intracellular domain).

[0138] In some embodiments, the polycistronic construct provided herein comprises one or more nucleotide sequences encoding a synthetic cytokine receptor. In some embodiments, the one or more nucleotide sequences correspond to one or more expression cassettes. In some embodiments, the polynucleotide construct provided herein comprises one expression cassette encoding IL2RG chain of the synthetic cytokine receptor and a second expression cassette encoding IL2RB chain of the synthetic cytokine receptor.

[0139] In some embodiments, the synthetic gamma chain polypeptide is encoded by a nucleic acid sequence that encodes a signal peptide. In some embodiments, the synthetic beta chain polypeptide is encoded by a nucleic acid sequence that encodes a signal peptide. A skilled artisan is readily familiar with signal peptides that can provide a signal to transport a nascent protein in the cells. Any of a variety of signal peptides can be employed.

1. Intracellular Domain

[0140] In some embodiments, the intracellular signaling domain of the first transmembrane receptor protein comprises an interleukin-2 receptor subunit gamma (IL2Rg) domain.

[0141] In some embodiments, the synthetic cytokine receptor comprises a first transmembrane receptor protein comprising an IL-2RG intracellular domain, a first dimerization domain, a second transmembrane receptor protein comprising an IL-2RB intracellular domain, and a second dimerization domain.

[0142] In some embodiments, the synthetic beta chain comprises an interleukin-2 receptor subunit beta (IL2RB) intracellular domain. IL2RB is also known as IL15RB or CD122. Thus, when referred to herein, IL2RB can also mean IL15RB. That is, the terms are used interchangeably in the present disclosure.

[0143] In some embodiments, the synthetic cytokine receptor comprises a first transmembrane receptor protein comprising an IL-2RG intracellular domain, a first dimerization domain, a second transmembrane receptor protein comprising an IL-7RB intracellular domain, and a second dimerization domain.

[0144] In some embodiments, the synthetic beta chain comprises an interleukin-7 receptor subunit beta (IL7RB) intracellular domain.

[0145] In some embodiments, the synthetic cytokine receptor comprises a first transmembrane receptor protein comprising an IL-2RG intracellular domain, a first dimerization domain, a second transmembrane receptor protein comprising an IL-21RB intracellular domain, and a second dimerization domain.

[0146] In some embodiments, the synthetic beta chain comprises an interleukin-21 receptor subunit beta (IL21RB) intracellular domain.

2. Dimerization Domain

[0147] The dimerization domains may be heterodimerization domains, including but not limited to FK506-Binding Protein of size 12 kD (FKBP) and a FKBP12-rapamycin binding (FRB) domain, which are known in the art to dimerize in the presence of rapamycin or a rapalog.

[0148] Alternatively, the first dimerization domain and the second dimerization domain may be a FK506-Binding Protein of size 12 kD (FKBP) and a calcineurin domain, which are known in the art to dimerize in the presence of FK506 or an analogue thereof.

[0149] In some embodiments the dimerization domains are homodimerization domains selected from:

i) FK506-Binding Protein of size 12 kD (FKBP);

ii) ii) cyclophiliA (CypA); or

iii) iii) gyrase B (CyrB);

with the corresponding non-physiological ligands being, respectively.

i) FK1012, AP1510, AP1903, or AP20187;

ii) ii) cyclosporin-A (CsA); or

iii) iii) coumermycin or analogs thereof.

[0150] In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are a FKBP domain and a cyclophilin domain.

[0151] In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are a FKBP domain and a bacterial dihydrofolate reductase (DHFR) domain.

[0152] In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are a calcineurin domain and a cyclophilin domain.

[0153] In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

3. Transmembrane Domain

[0154] The transmembrane domain is the sequence of the synthetic cytokine receptor that spans the membrane. The transmembrane domain may comprise a hydrophobic alpha helix. In some embodiments, the transmembrane domain is derived from a human protein.

[0155] In some embodiments, the TM domain and the intracellular signaling domain are from the same cytokine receptor. In some embodiments, the synthetic gamma chain polypeptide contains an IL-2RG TM domain and an IL-2RG intracellular domain. In some embodiments, the synthetic beta chain polypeptide contains an IL-2RB TM domain and an IL-2RB intracellular domain. In some embodiments, the synthetic beta chain polypeptide contains an IL-7RB TM domain and an IL-7RB intracellular domain. In some embodiments, the synthetic beta chain polypeptide contains an IL-21RB TM domain and an IL-21RB intracellular domain.

[0156] In some embodiments, one or more additional contiguous amino acids of the ectodomain directly adjacent to the TM domain of the cytokine receptor also can be included as part of the polypeptide sequence of a chain of the synthetic cytokine receptor. In some embodiments, 1-20

contiguous amino acids of the ectodomain adjacent to the TM domain of the cytokine receptor is included as part of the polypeptide sequence of a chain of the synthetic cytokine receptor. The portion of the ectodomain may be a contiguous sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids directly adjacent (e.g. N-terminal to) the TM sequence.

[0157] In some embodiments, the synthetic cytokine receptor is able to be bound by the non-physiological ligand rapamycin or a rapamycin analog. In some embodiments, the synthetic cytokine receptor is responsive to the non-physiological ligand rapamycin or a rapamycin analog, in which binding of the non-physiological ligand to the dimerization domains of the synthetic cytokine receptor induces cytokine receptor-mediated signaling in the cell, such as via the JAK/STAT pathway.

4. Exemplary Synthetic Cytokine Receptors

[0158] The synthetic cytokine receptors of the present disclosure comprise a synthetic gamma chain and a synthetic beta chain, each comprising a dimerization domain. The dimerization domains controllable dimerize in the present of a non-physiological ligand, thereby activating signaling the synthetic cytokine receptor.

[0159] The synthetic gamma chain polypeptide comprises a first dimerization domain, a first transmembrane domain, and an interleukin-2 receptor subunit gamma (IL-2RG) intracellular domain. The dimerization domain may be extracellular (N-terminal to the transmembrane domain) or intracellular (C-terminal to the transmembrane domain and N- or C-terminal to the IL-2G intracellular domain. In some embodiments, the synthetic gamma chain polypeptide comprises an FRB:IL2RG fusion protein. In some embodiments, the synthetic gamma chain polypeptide comprises an FKBP12:IL2RG fusion protein.

[0160] The synthetic beta chain polypeptide comprises a second dimerization domain, a second transmembrane domain, and an intracellular domain selected from an interleukin-2 receptor subunit beta (IL-2RB) intracellular domain, an interleukin-7 receptor subunit beta (IL-7RB) intracellular domain, or an interleukin-21 receptor subunit beta (IL-21RB) intracellular domain. The dimerization domain may be extracellular (N-terminal to the transmembrane domain) or intracellular (C-terminal to the transmembrane domain and N- or C-terminal to the IL-2RB or IL-7RB intracellular domain). In some embodiments, the synthetic beta chain polypeptide comprises

an FKBP12:IL2RB fusion protein. In some embodiments, the synthetic beta chain polypeptide comprises an FRB:IL2RB fusion protein.

[0161] In some embodiments, the second expression cassette comprises a nucleotide sequence encoding a synthetic cytokine gamma chain polypeptide, wherein the synthetic cytokine gamma chain polypeptide is an FRB:IL2RG fusion protein. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 80% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 85% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 90% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 95% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 96% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 97% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 98% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 99% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 100% identical to the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide comprises the nucleotide sequence of SEQ ID NO:15. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide consists of the nucleotide sequence of SEQ ID NO:15.

[0162] In some embodiments, the synthetic cytokine gamma chain polypeptide comprises interleukin 2 receptor subunit γ (IL2RG). In some embodiments, the IL2RG comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid

sequence at least 80% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG comprises the amino acid sequence of SEQ ID NO:16. In some embodiments, the IL2RG consists of the amino acid sequence of SEQ ID NO:16.

[0163] In some embodiments, the second expression cassette further comprises a nucleotide sequence encoding FRB. In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 80% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 85% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 90% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 95% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 96% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 97% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 98% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 99%

identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 100% identical to the nucleotide sequence of SEQ ID NO:13.

[0164] In some embodiments, the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB consists of the nucleotide sequence of SEQ ID NO:13.

[0165] In some embodiments, the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the FRB consists of the amino acid sequence of SEQ ID NO: 14.

[0166] In some embodiments, the second expression cassette is codon optimized.

[0167] In some embodiments, the second expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID

NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:11.

[0168] In some embodiments, the second expression cassette comprises the nucleotide sequence of SEQ ID NO:11. In some embodiments, the second expression cassette consists of the nucleotide sequence of SEQ ID NO:11.

[0169] In some embodiments, the second expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:12. In some embodiments, the

second expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NO:12. In some embodiments, the second expression cassette encodes an amino acid sequence consists of the sequence of SEQ ID NO:12.

[0170] In some embodiments, the second expression cassette comprises a nucleotide encoding a synthetic cytokine gamma chain polypeptide, wherein the synthetic cytokine gamma chain polypeptide is an FKBP12:IL2RG fusion protein. In some embodiments, the second expression cassette comprises a nucleotide sequence encoding FKBP12. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 85% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 90% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 95% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 96% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 97% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 98% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 99% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 100% identical to the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NOs:21 or 55. In some embodiments, the nucleotide sequence encoding the FKBP12 consists of the nucleotide sequence of SEQ ID NOs:21 or 55.

[0171] In some embodiments, the FKBP12 comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID

NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:22. In some embodiments, the FKBP12 comprises the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 consists of the amino acid sequence of SEQ ID NO: 22.

[0172] In some embodiments, the second expression cassette comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the second expression cassette comprises a nucleotide sequence comprising the nucleotide sequence of SEQ ID NOs: 53 or 56. In some embodiments, the

second expression cassette consists of a nucleotide sequence comprising the nucleotide sequence of SEQ ID NOs: 53 or 56

[0173] In some embodiments, the second expression cassette encodes an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128.In some embodiments, the second expression cassette encodes an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128.In some embodiments, the second expression cassette encodes an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NOs: 54, 57, or 128. In some embodiments, the second expression cassette encodes an amino acid sequence consisting of the amino acid sequence of SEQ ID NOs: 54, 57, or 128.

[0174] In some embodiments, the third expression cassette comprises a nucleotide encoding a synthetic cytokine beta chain polypeptide, wherein the synthetic cytokine beta chain polypeptide is an FKBP12:IL2RB fusion protein. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 85% identical to the nucleotide sequence of SEQ ID

NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 90% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 95% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 96% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 97% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 98% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 99% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 100% identical to the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide comprises the nucleotide sequence of SEQ ID NOs:23 or 61. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide consists of the nucleotide sequence of SEQ ID NOs:23 or 61.

[0175] In some embodiments, the synthetic cytokine beta chain polypeptide comprises interleukin 2 receptor subunit β (IL2RB).

[0176] In some embodiments, the IL2RB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 98% identical to the

amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB comprises the amino acid sequence of SEQ ID NOs:24 or 62. In some embodiments, the IL2RB consists of the amino acid sequence of SEQ ID NOs:24 or 62.

[0177] In some embodiments, the third expression cassette further comprises a nucleotide sequence encoding FKBP12.

[0178] In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 85% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 90% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 95% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 96% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 97% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 98% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 99% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 100% identical to the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NO: 21. In some embodiments, the nucleotide sequence encoding the FKBP12 consists of the nucleotide sequence of SEQ ID NO: 21.

[0179] In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide

sequence encoding the FKBP12 is at least 85% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 90% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 95% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 96% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 97% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 98% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 99% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 100% identical to the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NO: 55. In some embodiments, the nucleotide sequence encoding the FKBP12 consists of the nucleotide sequence of SEQ ID NO: 55.

[0180] In some embodiments, the FKBP12 comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO :22. In some embodiments, the FKBP12 comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 comprises the

amino acid sequence of SEQ ID NO: 22. In some embodiments, the FKBP12 consists of the amino acid sequence of SEQ ID NO: 22.

[0181] In some embodiments, the third expression cassette is codon optimized.

[0182] In some embodiments, the third expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette comprises the nucleotide sequence of SEQ ID NO: 19. In some embodiments, the third expression cassette consists of the nucleotide sequence of SEQ ID NO: 19.

[0183] In some embodiments, the third expression cassette comprises a nucleotide encoding a synthetic cytokine beta chain polypeptide, wherein the synthetic cytokine beta chain polypeptide is an FRB:IL2RB fusion protein. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least

95% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette comprises the nucleotide sequence of SEQ ID NO: 59. In some embodiments, the third expression cassette consists of the nucleotide sequence of SEQ ID NO: 59.

[0184] In some embodiments, the third expression cassette encodes an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NOs: 60 or 129. In some embodiments, the third expression cassette encodes an amino acid sequence consisting of the sequence of SEQ ID NOs: 60 or 129.

[0185] In some embodiments, the third expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NO:60. In some embodiments, the third expression cassette encodes an amino acid sequence consisting the sequence of SEQ ID NO:60.

[0186] In some embodiments, the third expression cassette further comprises a nucleotide sequence encoding FRB. In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 80% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 85% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 90% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 95% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 96% identical to the nucleotide

sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 97% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 98% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 99% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB is at least 100% identical to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the FRB consists of the nucleotide sequence of SEQ ID NO:13.

[0187] In some embodiments, the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:14. In some embodiments, the FRB comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the FRB consists of the amino acid sequence of SEQ ID NO: 14.

C. Chimeric Antigen Receptor

[0188] In some embodiments, an expression cassette of the polycistronic construct encodes a chimeric antigen receptor.

I. CAR Constructs and Encoding Nucleotides

[0189] In some embodiments, the CAR construct contains an extracellular binding portion, a transmembrane domain and an intracellular signaling domain. In some embodiments, the intracellular signaling domain contains a costimulatory signaling domain and/or an activation signaling domain. In some embodiments, the CAR construct contains an extracellular binding portion, a transmembrane domain and an intracellular signaling domain comprising a costimulatory signaling domain. In some embodiments, the CAR construct contains an extracellular binding portion, a transmembrane domain and an intracellular signaling domain comprising an activation signaling domain. In some embodiments, the CAR construct contains an extracellular binding portion, a transmembrane domain and an intracellular signaling domain comprising a costimulatory signaling domain and an activation signaling domain.

[0190] In any embodiments described herein, the binding portion of the CAR can be, for example, a single chain fragment variable region (scFv) of an antibody, a Fab, Fv, Fc, or (Fab')2 fragment, and the like.

[0191] In some embodiments, a costimulatory signaling domain serves to enhance the proliferation and survival of the lymphocytes upon binding of the CAR to a targeted moiety. The identity of the costimulatory signaling domain is limited only in that it has the ability to enhance cellular proliferation and survival activation upon binding of the targeted moiety by the CAR. Suitable costimulatory signaling domains include, but are not limited to: CD28 (see, e.g., Alvarez-Vallina, L. et al., *Eur J Immunol*. 1996. 26(10):2304-9); CD137 (4-1BB), a member of the tumor necrosis factor (TNF) receptor family (see, e.g., Imai, C. et al., *Leukemia*. 2004. 18:676–84); and CD134 (OX40), a member of the TNFR-superfamily of receptors (see, e.g., Latza, U. et al., *Eur. J. Immunol*. 1994. 24:677). A skilled artisan will understand that sequence variants of these costimulatory signaling domains can be used, where the variants have the same or similar activity as the domain on which they are modeled. In various embodiments, such variants have at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to the amino acid sequence of the domain from which they are derived.

[0192] In some embodiments of the invention, the CAR constructs comprise two costimulatory signaling domains. While the particular combinations include all possible variations of the four

noted domains, specific examples include: 1) CD28+CD137 (4-1BB) and 2) CD28+CD134 (OX40).

[0193] In some embodiments, the activation signaling domain serves to activate cells upon binding of the CAR to a targeted moiety. The identity of the activation signaling domain is limited only in that it has the ability to induce activation of the selected cell upon binding of the targeted moiety by the CAR. Suitable activation signaling domains include the CD3ζ chain and Fc receptor γ. In some embodiments, the signaling domain is a signaling domain of NKG2C or NKp44. The skilled artisan will understand that sequence variants of these noted activation signaling domains can be used without adversely impacting the invention, where the variants have the same or similar activity as the domain on which they are modeled. Such variants may have at least about 80%, at least about 90%, at least about 95%. at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to the amino acid sequence of the domain from which they are derived.

[0194] In some embodiments, the CARs may include additional elements, such a signal peptide to ensure proper export of the fusion protein to the cells surface, a transmembrane domain to ensure the fusion protein is maintained as an integral membrane protein, and a hinge domain that imparts flexibility to the recognition region and allows strong binding to the targeted moiety.

[0195] In some embodiments, nucleotide sequence that encodes a CAR comprising an extracellular domain, optionally a hinge domain, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a costimulatory domain and an activation signaling domain. In some embodiments, the costimulatory and activation signaling domains are a single domain, for example a single intracellular domain that provides both costimulation and activation signals to a cell. In other embodiments, the intracellular signaling domain comprises either a costimulatory domain or an activation signaling domain. In some embodiments, the CAR comprises an extracellular domain, a CD8a hinge, a CD8a transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain. In some embodiments, a nucleotide sequence encodes an extracellular domain, an CD28 hinge domain, a CD28 transmembrane domain, a CD28 co-stimulatory domain, and a CD3zeta signaling domain. In some embodiments, the nucleotide sequence encodes an extracellular domain, an IgG4 hinge domain, a CD28 transmembrane domain, a 4-1BB co-stimulatory domain, and a CD3zeta signaling

domain. In some embodiments, the nucleotide sequence encodes a CAR comprising an extracellular domain, a CD8a hinge, a CD28 transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain.

[0196] Illustrative CAR constructs suitable for the provided polycistronic constructs are provided below:

(1) scFv-$CD8_{TM}$-4-$1BB_{IC}$-CD3ζs (see, e.g., Liu E, Tong Y, Dotti G, et al., *Leukemia*. 2018; 32: 520-531);

(2) scFv-$CD28_{TM+IC}$-CD3ζs (see, e.g., Han J, Chu J, Keung CW et al., *Sci Rep*. 2015; 5: 11483; Kruschinski A, Moosmann A, Poschke I et al., *Proc Natl Acad Sci U S A.* 2008; 105: 17481-17486; and Chu J, Deng Y, Benson DM et al., *Leukemia*. 2014; 28: 917-927);

(3) scFv-$DAP12_{TM+IC}$ (see, e.g., Muller N, Michen S, Tietze S et al., *J Immunother.* 2015; 38: 197-210);

(4) scFv-$CD8_{TM}$-$2B4_{IC}$-CD3ζs (see, e.g., Xu Y, Liu Q, Zhong M et al., *J Hematol Oncol.* 2019; 12: 49);

(5) scFv-$2B4_{TM+IC}$-CD3ζs (see, e.g., Altvater B, Landmeier S, Pscherer S et al., Clin Cancer Res. 2009; 15: 4857-4866);

(6) scFv-$CD28_{TM+IC}$-4-$1BB_{IC}$-CD3ζs (see, e.g., Kloss S, Oberschmidt O, Morgan M et al., *Hum Gene Ther*. 2017; 28: 897-913);

(7) scFv-$CD16_{TM}$-$2B4_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(8) scFv-$NKp44_{TM}$-$DAP10_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(9) scFv-$NKp46_{TM}$-$2B4_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(10) scFv-$NKG2D_{TM}$-$2B4_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(11) scFv-$NKG2D_{TM}$-4-$1BB_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(12) scFv-$NKG2D_{TM}$-$2B4_{IC}$-$DAP12_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(13) scFv-NKG2D$_{TM}$-2B4$_{IC}$-DAP10$_{IC}$-CD3ζs (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192);

(14) scFv-NKG2D$_{TM}$-4-1BB$_{IC}$-2B4$_{IC}$-CD3ζS (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192); and

(15) scFv-NKG2D$_{TM}$-CD3ζS (see, e.g., Li Y, Hermanson DL, Moriarity BS Kaufman DS, *Cell Stem Cell*. 2018; 23: 181-192).

a. CAR Extracellular Domain

[0197] In some embodiments, the binding portion of the CAR can be directed to any antigen that is desired to be targeted, such as due to its overexpression on cells or association with a disease or conditions like cancer.

[0198] In some embodiments, the binding portion of the CAR is specific to a tumor antigen. The selection of the antigen binding domain will depend on the particular type of cancer to be treated. Tumor antigens are well known in the art and include, for example, a glioma-associated antigen, carcinoembryonic antigen (CEA), EGFRvIII, IL-11Ra, IL-13Ra, EGFR, FAP, B7H3, Kit, CA LX, CS-1, MUC1, BCMA, bcr-abl, HER2, β-human chorionic gonadotropin, alphafetoprotein (AFP), ALK, CD19, CD123, cyclin B1, lectin-reactive AFP, Fos-related antigen 1, ADRB3, thyroglobulin, EphA2, RAGE-1, RU1, RU2, SSX2, AKAP-4, LCK, OY-TES1, PAXS, SART3, CLL-1, fucosyl GM1, GloboH, MN-CA IX, EPCAM, EVT6-AML, TGS5, human telomerase reverse transcriptase, plysialic acid, PLAC1, RU1, RU2 (AS), intestinal carboxyl esterase, lewisY, sLe, LY6K, mut hsp70-2, M-CSF, MYCN, RhoC, TRP-2, CYPIBI, BORIS, prostase, prostate-specific antigen (PSA), PAX3, PAP, NY-ESO-1, LAGE-la, LMP2, NCAM, p53, p53 mutant, Ras mutant, gplOO, prostein, OR51E2, PANX3, PSMA, PSCA, Her2/neu, hTERT, HMWMAA, HAVCR1, VEGFR2, PDGFR-beta, survivin and telomerase, legumain, HPV E6, E7, sperm protein 17, SSEA-4, tyrosinase, TARP, WT1, prostate-carcinoma tumor antigen-1 (PCTA-1), ML-IAP, MAGE, MAGE-A1, MAD-CT-1, MAD-CT-2, MelanA/MART 1, XAGE1, ELF2M, ERG (TMPRSS2 ETS fusion gene), NA17, neutrophil elastase, sarcoma translocation breakpoints, NY-BR-1, ephnnB2, CD20, CD22, CD24, CD30, CD33, CD38, CD44v6, CD97, CD171, CD179a, androgen receptor, FAP, insulin growth factor (IGF)-I, IGFII, IGF-I receptor, GD2, o-acetyl-GD2, GD3, GM3, GPRCSD, GPR20, CXORF61, folate receptor (FRa), folate receptor beta, ROR1, Flt3,

TAG72, TN Ag, Tie 2, TEM1, TEM7R, CLDN6, TSHR, UPK2, and mesothelin. Non-limiting examples of tumor antigens include the following: Differentiation antigens such as tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, pi 5; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER-2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, IL13Ra2, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\P1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG1 6, TA-90\Mac-2 binding protein\cyclophilm C-associated protein, TAAL6, TAG72, TLP, TPS, GPC3, MUC16, LMP1, EBMA-1, BARF-1, CS1, CD319, HER1, B7H6, L1CAM, IL6, and MET. In some embodiments, the CAR comprises binding domains that target two or more antigens as disclosed herein, in any combination. For example: CD19 and CD3, BCMA and CD3, GPRC5D and CD3, FCRL5 and CD3, CD38 and CD3, CD19 and CD20, CD19 and CD22, BCMA and GPRC5D, or CD20 and CD22. In some embodiments, the CAR comprises binding domains that target two or more antigens on the same target protein, for example two epitopes in BCMA.

[0199] A skilled artisan is readily familiar with CARs against diverse tumor antigens. Any one of such CARs can be employed as the CAR. Numerous CARs have been incorporated into products approved by the FDA and include, but are not limited to, anti-CD19 and anti-BCMA CAR T cells such as tisagenlecleucel (Kymriah), axicabtagene ciloleucel (Yescarta), brexucabtagene autoleucel (Tecartus), lisocabtagene maraleucel (Breyanzi), idecabtagene vicleucel (Abecma), or ciltacabtagene autoleucel (Carvykti). It is within the level of a skilled artisan to generate similar constructs for specific targeting of a desired tumor antigen.

[0200] In some embodiments, the binding portion of the CAR can be directed to a universal antigen to target a wide variety of tumors without the need to prepare separate CAR constructs. The targeted moiety recognized by the CAR may also remain constant. In some embodiments, a ligand

may be administered to the subject to allow interaction with target cells and interaction with the binding portion of the CAR. It is only the ligand portion of the small conjugate molecule that needs to be altered to allow the system to target cancer cells of different identity. Exemplary CAR systems are described in the section below.

[0201] In some embodiments, the CAR is an anti-CD19 CAR and the extracellular binding domain of the CD19 CAR is specific to CD19, for example, human CD19. In some embodiments, the extracellular domain of the CD19 CAR comprises an scFv derived from the FMC63 monoclonal antibody (FMC63), which comprises the heavy chain variable region (VH) and the light chain variable region (VL) of FMC63 connected by a linker. FMC63 and the derived scFv have been described in Nicholson et al., Mol. Immun. 34(16-17):1157-1165 (1997) and PCT Application Publication No. WO2018/213337, the entire contents of each of which are incorporated by reference herein. An exemplary anti-CD19 CAR is shown in **Table 1** with its different portions including the extracellular domain.

[0202] In some embodiments, the CAR is an anti-CD20 CAR and the extracellular binding domain of the CD20 CAR is specific to CD20, for example, human CD20. In some embodiments, the extracellular binding domain of the CD20 CAR is derived from an antibody specific to CD20, including, for example, Leu16, IF5, 1.5.3, rituximab, obinutuzumab, ibritumomab, ofatumumab, tositumumab, odronextamab, veltuzumab, ublituximab, and ocrelizumab. In any of these embodiments, the extracellular binding domain of the CD20 CAR can comprise or consist of the VH, the VL, and/or one or more CDRs of any of the antibodies. An exemplary anti-CD20 CAR is shown in **Table 2** and **Table 3** with its different portions including its extracellular domain.

1) Universal CARs

[0203] Conventionally, CARs are generated by fusing a polynucleotide encoding a VL, VH, or scFv to the 5′ end of a polynucleotide encoding transmembrane and intracellular domains, and transducing cells with that polynucleotide as well as with the corresponding VH or VL, if needed. Numerous variations on CARs well known in the art and the disclosure contemplates using any of the known variations. Additionally, VL/VH pairs and scFvs for innumerable haptens are known in the art or can be generated by conventional methods routinely. Accordingly, the present disclosure contemplates using any known hapten-binding domain.

[0204] In some embodiments, the CAR is an anti-FITC CAR and the ligand is composed of a fluorescein or fluorescein isothiocyanate (FITC) moiety conjugated to an agent that binds to a desired target cell (such as a cancer cell). Exemplary ligands are described below. In some embodiments, the ligand is FITC-folate.

[0205] An exemplary anti-FITC CAR is shown in **Table 4** and its different portions.

[0206] In some embodiments, the CAR comprises an scFv domain. In some embodiments, the scFv domain comprises anti-fluorescein isothiocyanate (FITC) E2. In some embodiments, the scFv domain comprises a light chain variable domain (VL), a linker, and a heavy chain variable domain (VH).

[0207] In some embodiments, the scFv VL comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL comprises the nucleotide sequence of SEQ ID NOs:30 or 65. In some embodiments, the scFv VL consists of the nucleotide sequence of SEQ ID NOs:30 or 65.

[0208] In some embodiments, the scFv VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 80%

identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:31.In some embodiments, the scFv VL comprises the amino acid sequence of SEQ ID NO:31. In some embodiments, the scFv VL consists the amino acid sequence of SEQ ID NO:31.

[0209] In some embodiments, the scFv VH comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises a nucleotide sequence at least 100% identical to the

nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH comprises the nucleotide sequence of SEQ ID NOs:34 or 67. In some embodiments, the scFv VH consists of the nucleotide sequence of SEQ ID NOs:34 or 67.

[0210] In some embodiments, the scFv VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH comprises the amino acid sequence of SEQ ID NO:35. In some embodiments, the scFv VH consists the amino acid sequence of SEQ ID NO:35.

[0211] In some embodiments, the scFv linker comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 96%

identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker comprises the nucleotide sequence of SEQ ID NOs:32 or 66. In some embodiments, the scFv linker consists the nucleotide sequence of SEQ ID NOs:32 or 66.

[0212] In some embodiments, the scFv linker comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:33.In some embodiments, the scFv linker comprises the amino acid sequence of SEQ ID NO:33. In some embodiments, the scFv linker consists the amino acid sequence of SEQ ID NO:33.

[0213] In some embodiments, the scFv comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 80% identical to the

nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv comprises the nucleotide sequence of SEQ ID NOs:28 or 64. In some embodiments, the scFv consists of the nucleotide sequence of SEQ ID NOs:28 or 64.

[0214] In some embodiments, the scFv comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv

comprises the amino acid sequence of SEQ ID NO:29. In some embodiments, the scFv consists of the amino acid sequence of SEQ ID NO:29.

[0215] Various methods to target CARs and CAR-expressing cells have been described in the art, including, for example in US 2020/0123224, the disclosure of which is incorporated by reference herein. For example, a fluorescein or fluorescein isothiocyanate (FITC) moiety may be conjugated to an agent that binds to a desired target cell (such as a cancer cell), and thereby a CAR expressing an anti-fluorescein/FITC chimeric antigen receptor may be selectively targeted to the target cell labeled by the conjugate. In variations, other haptens recognized by CARs may be used in place of fluorescein/FITC. The CAR may be generated using various scFv sequences known in the art, or scFv sequences generated by conventional and routine methods. Further illustrative scFv sequences for fluorescein/FITC and for other haptens are provided in, for example, WO 2021/076788, the disclosure of which is incorporated by reference herein.

[0216] In one embodiment, the disclosure provides an illustration of this conjugate molecule/CAR system.

[0217] In some embodiments, the CAR system of the disclosure utilizes conjugate molecules as the bridge between CAR-expressing cells and targeted cancer cells. The conjugate molecules are conjugates comprising a hapten and a cell-targeting moiety, such as any suitable tumor cell-specific ligand. Illustrative haptens that can be recognized and bound by CARs, include small molecular weight organic molecules such as DNP (2,4-dinitrophenol), TNP (2,4,6-trinitrophenol), biotin, and digoxigenin, along with fluorescein and derivatives thereof, including FITC (fluorescein isothiocyanate), NHS-fluorescein, and pentafluorophenyl ester (PFP) and tetrafluorophenyl ester (TFP) derivatives, a knottin, a centyrin, and a DARPin. Suitable cell-targeting moiety that may themselves act as a hapten for a CAR include knottins (see Kolmar H. et al., *The FEBS Journal*. 2008. 275(11):26684-90), centyrins, and DARPins (see Reichert, J.M. *MAbs* 2009. 1(3):190–209).

[0218] In some embodiments, the cell-targeting moiety is DUPA (DUPA-(99m) Tc), a ligand bound by PSMA-positive human prostate cancer cells with nanomolar affinity (K_D = 14 nM; see Kularatne, S.A. et al., *Mol Pharm*. 2009. 6(3):780-9). In one embodiment, a DUPA derivative can be the ligand of the small molecule ligand linked to a targeting moiety, and DUPA derivatives are described in WO 2015/057852, incorporated herein by reference.

[0219] In some embodiments, the cell-targeting moiety is CCK2R ligand, a ligand bound by CCK2R-positive cancer cells (e.g., cancers of the thyroid, lung, pancreas, ovary, brain, stomach, gastrointestinal stroma, and colon; see Wayua. C. et al., *Molecular Pharmaceutics*. 2013. ePublication).

[0220] In some embodiments, the cell-targeting moiety is folate, folic acid, or an analogue thereof, a ligand bound by the folate receptor on cells of cancers that include cancers of the ovary, cervix, endometrium, lung, kidney, brain, breast, colon, and head and neck cancers; see Sega, E.I. et al., *Cancer Metastasis Rev*. 2008. 27(4):655-64).

[0221] In some embodiments, the cell-targeting moiety is an NK-1R ligand. Receptors for NK-1R the ligand are found, for example, on cancers of the colon and pancreas. In some embodiments, the NK-1R ligand may be synthesized according to the method disclosed in Int'l Patent Appl. No. PCT/US2015/044229, incorporated herein by reference.

[0222] In some embodiments, the cell-targeting moiety may be a peptide ligand, for example, the ligand may be a peptide ligand that is the endogenous ligand for the NK1 receptor. In some embodiments, the small conjugate molecule ligand may be a regulatory peptide that belongs to the family of tachykinins which target tachykinin receptors. Such regulatory peptides include Substance P (SP), neurokinin A (substance K), and neurokinin B (neuromedin K), (see Hennig et al., *International Journal of Cancer:* 61, 786-792).

[0223] In some embodiments, the cell-targeting moiety is a CAIX ligand. Receptors for the CAIX ligand found, for example, on renal, ovarian, vulvar, and breast cancers. The CAIX ligand may also be referred to herein as CA9.

[0224] In some embodiments, the cell-targeting moiety is a ligand of gamma glutamyl transpeptidase. The transpeptidase is overexpressed, for example, in ovarian cancer, colon cancer, liver cancer, astrocytic gliomas, melanomas, and leukemias.

[0225] In some embodiments, the cell-targeting moiety is a CCK2R ligand. Receptors for the CCK2R ligand found on cancers of the thyroid, lung, pancreas, ovary, brain, stomach, gastrointestinal stroma, and colon, among others.

[0226] In some embodiments, the cell-targeting moiety is a PSMA ligand.

[0227] In some embodiments, the cell-targeting moiety is a FAP ligand.

[0228] In one embodiment, the cell-targeting moiety may have a mass of less than about 10,000 Daltons, less than about 9000 Daltons, less than about 8,000 Daltons, less than about 7000 Daltons, less than about 6000 Daltons, less than about 5000 Daltons, less than about 4500 Daltons, less than about 4000 Daltons, less than about 3500 Daltons, less than about 3000 Daltons, less than about 2500 Daltons, less than about 2000 Daltons, less than about 1500 Daltons, less than about 1000 Daltons, or less than about 500 Daltons. In another embodiment, the small molecule ligand may have a mass of about 1 to about 10,000 Daltons, about 1 to about 9000 Daltons, about 1 to about 8,000 Daltons, about 1 to about 7000 Daltons, about 1 to about 6000 Daltons, about 1 to about 5000 Daltons, about 1 to about 4500 Daltons, about 1 to about 4000 Daltons, about 1 to about 3500 Daltons, about 1 to about 3000 Daltons, about 1 to about 2500 Daltons, about 1 to about 2000 Daltons, about 1 to about 1500 Daltons, about 1 to about 1000 Daltons, or about 1 to about 500 Daltons.

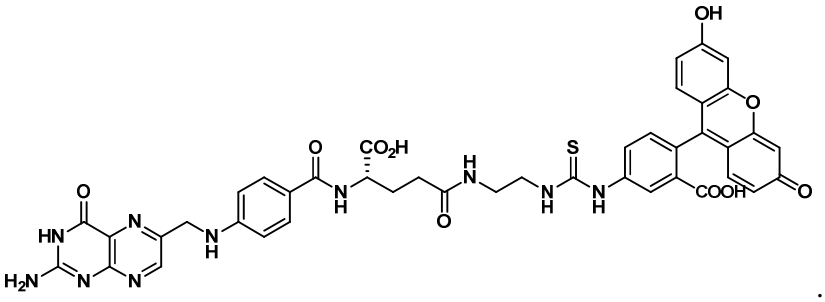
[0229] In one illustrative embodiment, the linkage in a conjugate described herein can be a direct linkage (*e.g.*, a reaction between the isothiocyanate group of FITC and a free amine group of a small molecule ligand) or the linkage can be through an intermediary linker. In one embodiment, if present, an intermediary linker can be any biocompatible linker known in the art, such as a divalent linker. In one illustrative embodiment, the divalent linker can comprise about 1 to about 30 carbon atoms. In another illustrative embodiment, the divalent linker can comprise about 2 to about 20 carbon atoms. In other embodiments, lower molecular weight divalent linkers (i.e., those having an approximate molecular weight of about 30 to about 300 Da) are employed. In another embodiment, linkers lengths that are suitable include, but are not limited to, linkers having 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, or more atoms.

[0230] In some embodiments, the hapten and the cell-targeting moiety can be directly conjugated through such means as reaction between the isothiocyanate group of FITC and free amine group of small ligands (*e.g.*, folate, DUPA, and CCK2R ligand). However, the use of a linking domain to connect the two molecules may be helpful as it can provide flexibility and stability. Examples of suitable linking domains include: 1) polyethylene glycol (PEG); 2) polyproline; 3) hydrophilic amino acids; 4) sugars; 5) unnatural peptidoglycans; 6) polyvinylpyrrolidone; 7) Pluronic F-127. Linker lengths that are suitable include, but are not limited

to, linkers having 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, or more atoms.

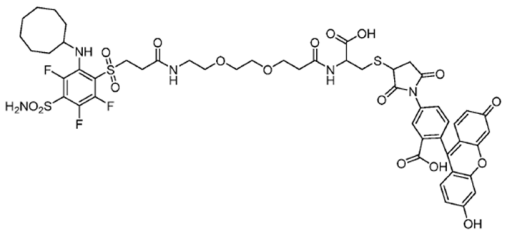
[0231] In some embodiments, the linker may be a divalent linker that may include one or more spacers.

[0232] An illustrative conjugate of the disclosure is FITC-Folate



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An illustrative conjugate of the disclosure is FITC-CA9



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[0233] Illustrative conjugates of the disclosure include the following molecules: FITC-$(PEG)_{12}$-Folate, FITC-$(PEG)_{20}$-Folate, FITC-$(PEG)_{108}$-Folate, FITC-DUPA, FITC-$(PEG)_{12}$-DUPA, FITC-CCK2R ligand, FITC-$(PEG)_{12}$-CCK2R ligand, FITC-$(PEG)_{11}$-NK1R ligand and FITC-$(PEG)_2$-CA9.

[0234] While the affinity at which the ligands and cancer cell receptors bind can vary, and in some cases low affinity binding may be preferable (such as about 1 μM), the binding affinity of the ligands and cancer cell receptors will generally be at least about 100 μM, 1 nM, 10 nM, or 100 nM, preferably at least about 1 pM or 10 pM, even more preferably at least about 100 pM.

[0235] Examples of conjugates and methods of making them are provided in U.S. patent applications US 2017/0290900, US 2019/0091308, and US 2020/0023009, all of which are incorporated herein by reference.

b. Spacer (e.g, hinge domain)

[0236] In some embodiments, the CAR comprises a hinge domain. In some embodiments, the hinge domain comprises a short hinge or a medium hinge domain. In some embodiments, the hinge domain comprises a CD8 or an IgG. In some embodiments, the CD8 hinge comprises CD8α hinge. In some embodiments, the IgG hinge comprises an IgG4 hinge. In some embodiments, the IgG4 hinge is modified. In some embodiments, the IgG hinge comprises an IgG1 hinge. In some embodiments, the hinge domain comprises a PD1 hinge. In some embodiments, the hinge domain comprises a CD28 hinge.

[0237] In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID Nos:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs:38 or 114. In some embodiments, the CD8α hinge comprises the nucleotide sequence of SEQ ID NOs: 38 or 114. In some embodiments, the CD8α hinge consists of the nucleotide sequence of SEQ ID NOs: 38 or 114.

[0238] In some embodiments, the CD8α hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID Nos:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least

80% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID Nos:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID Nos:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID Nos:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some embodiments, the CD8α hinge comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs:39 or 115. In some embodiments, the CD8α hinge comprises the amino acid sequence of SEQ ID Nos: 39 or 115. In some embodiments, the CD8α hinge consists of the amino acid sequence of SEQ ID NOs: 39 or 115.

[0239] In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID

NO:123. In some embodiments, the CD8 hinge comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge comprises the nucleotide sequence of SEQ ID NO:123. In some embodiments, the CD8 hinge consists of the nucleotide sequence of SEQ ID NO:123.

[0240] In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge comprises the amino acid sequence of SEQ ID NO:119. In some embodiments, the modified IgG4 hinge consists of the amino acid sequence of SEQ ID NO:119.

[0241] In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID

NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge comprises the amino acid sequence of SEQ ID NO:120. In some embodiments, the modified IgG4 hinge consists of the amino acid sequence of SEQ ID NO:120.

[0242] In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge comprises the amino acid sequence of SEQ ID NO:122. In some embodiments, the IgG1 hinge consists of the amino acid sequence of SEQ ID NO:122.

[0243] In some embodiments, the PD1 hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge comprises the amino acid sequence of SEQ ID NO:121. In some embodiments, the PD1 hinge consists of the amino acid sequence of SEQ ID NO:121.

[0244] In some embodiments, the CD28 hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 98%

identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge comprises the amino acid sequence of SEQ ID NO:124. In some embodiments, the CD28 hinge consists of the amino acid sequence of SEQ ID NO:124.

c. Transmembrane Domain

[0245] In some embodiments, the CAR comprises a transmembrane domain. In some embodiments, the transmembrane domain comprises a CD8 or a CD28. In some embodiments, the transmembrane domain comprises a CD8 domain. In some embodiments, the transmembrane domain comprises a CD28 domain. In some embodiments, the CD8 transmembrane domain comprises CD8α transmembrane domain.

[0246] In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:40. In some embodiments, the

transmembrane domain comprises the nucleotide sequence of SEQ ID NO:40. In some embodiments, the transmembrane domain consists of the nucleotide sequence of SEQ ID NO:40.

[0247] In some embodiments, the transmembrane domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO:41. In some embodiments, the transmembrane domain consists of the amino acid sequence of SEQ ID NO:41.

d. Intracellular domain (i.e. endodomain)

[0248] In some embodiments, the CAR comprises an endodomain. In some embodiments, the endodomain comprises a costimulatory molecule. In some embodiments, the endodomain comprises 4-1BB, CD3ζ, and/or CD28. In some embodiments, the endodomain comprises 4-1BB. In some embodiments, the endodomain comprises CD3ζ. In some embodiments, the endodomain comprises CD28.

[0249] In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ

ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs:42 or 69. In some embodiments, the 4-1BB endodomain comprises the nucleotide sequence of SEQ ID NOs: 42 or 69. In some embodiments, the 4-1BB endodomain consists the nucleotide sequence of SEQ ID NOs: 42 or 69.

[0250] In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 98% identical to the amino acid sequence of

SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain comprises the amino acid sequence of SEQ ID NO:43. In some embodiments, the 4-1BB endodomain consists of the amino acid sequence of SEQ ID NO:43.

[0251] In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain comprises the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118. In some embodiments, the CD3ζ endodomain consists the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118.

[0252] In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least

80% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain comprises the amino acid sequence of SEQ ID NO:47. In some embodiments, the CD3ζ endodomain consists of the amino acid sequence of SEQ ID NO:47.

2. Exemplary CAR Polynucleotides

[0253] In some embodiments the CAR is an anti-CD19 CAR, and in these embodiments, the polycistronic vector comprises a fourth expression cassette that contains a nucleotide sequence encoding a CD19 CAR. In some embodiments, the CD19 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD19, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and an intracellular activation signaling domain. In some embodiments, the fourth expression cassette encodes an anti-CD19 CAR with features set forth in **Table 1**.

Table 1. Exemplary sequences of anti-CD19 CAR and components

	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Full Anti-CD19 CAR		104	105
Extracellular binding domain	CD19 scFv	106	107
	CD19 scFv VL	108	109
	linker	110	111

Table 1. Exemplary sequences of anti-CD19 CAR and components			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
	CD19 scFv VH	112	113
Hinge and transmembrane domain	CD8 hinge	114	115
	CD28 transmembrane	116	117
Costimulatory signaling domain	4-1BB	99	43
Activation signaling domain	CD3 zeta	118	47

[0254] In some embodiments the CAR is an anti-CD20 CAR, and in these embodiments, the polycistronic vector comprises a fourth expression cassette that contains a nucleotide sequence encoding a CD20 CAR. In some embodiments, the CD20 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD20, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and an intracellular activation signaling domain. In some embodiments, the fourth expression cassette encodes an anti-CD20 CAR with features set forth in **Table 2** (anti-CD20 CAR with flag) or **Table 3** (anti-CD20 CAR without flag).

Table 2. Exemplary sequences of anti-CD20 CAR and components (with flag)			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Full Anti-CD20 CAR		83	84
Extracellular binding domain	CD20 scFv	87	88
	CD20 scFv VL	89	90
	218 linker	91	92
	CD20 scFv VH	93	94
Flag domain	Flag	95	96
Hinge and transmembrane domain	CD8 hinge and transmembrane	97	98
Costimulatory signaling domain	4-1BB	99	43

Table 2. Exemplary sequences of anti-CD20 CAR and components (with flag)			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Activation signaling domain	CD3 zeta	100	47

Table 3. Exemplary sequences of anti-CD20 CAR and components (without flag)			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Full Anti-CD20 CAR		85	86
Extracellular binding domain	CD20 scFv	87	88
	CD20 scFv VL	89	90
	218 linker	91	92
	CD20 scFv VH	93	94
Hinge and transmembrane domain	CD8 hinge and transmembrane	97	98
Costimulatory signaling domain	4-1BB	99	43
Activation signaling domain	CD3 zeta	100	47

[0255] In some embodiments, the fourth expression cassette encodes a CAR with features set forth in **Table 4**. In some embodiments, the CAR is an anti-FITC CAR.

Table 4. Exemplary sequences of anti-FITC CAR and components			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Full Anti-FITC Tag CAR		26, 63, 82	27, 127
Extracellular binding domain	E2 scFv	28, 64	29
	E2 scFv VL	30, 65	31
	linker	32, 66	33
	E2 scFv VH	34, 67	35

Table 4. Exemplary sequences of anti-FITC CAR and components			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Hinge and transmembrane domain	CD8 hinge and transmembrane	36	37
	CD8 hinge	38	39
	CD8 transmembrane	40	41
Costimulatory signaling domain	4-1BB	42	43
Activation signaling domain	CD3 zeta	46	47

[0256] In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71,

or 82. In some embodiments, the fourth expression cassette comprises the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82. In some embodiments, the fourth expression cassette consists of the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82.In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 80% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 85% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 90% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 95% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 96% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 97% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 98% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 99% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence at least 100% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NOs: 27, 72 or 127. In some embodiments, the fourth expression cassette encodes an amino acid sequence consisting of the sequence of SEQ ID NOs: 27, 72 or 127.

[0257] In further embodiments, an illustrative nucleotide sequence encoding a CAR may comprise SEQ ID NO: 71, and an illustrative CAR amino acid sequence may comprise SEQ ID NO:72.

[0258] An illustrative nucleotide insert may comprise SEQ ID NO:73.

[0259] In some embodiments, the CAR may be encoded by a nucleic acid sequence that encodes a signal peptide to signal transport of the CAR in the cell. It is understood that typically the signal peptide is removed from the protein.

[0260] An illustrative CAR amino acid sequence without a signal peptide may comprise SEQ ID NO:74:

[0261] An illustrative CAR amino acid sequence signal peptide may comprise SEQ ID NO:75. In various embodiments, CAR-expressing cells comprising the nucleic acid of SEQ ID NO:71 or 73 are provided. In some embodiments, a chimeric antigen receptor polypeptide comprising SEQ ID NO:72 is contemplated. In some embodiments, a chimeric antigen receptor polypeptide comprising SEQ ID NO:74 is contemplated. In some embodiments, a vector is contemplated comprising SEQ ID NO: 71 or 73. In some embodiments, a lentiviral vector is contemplated comprising SEQ ID NO: 71 or 73. In some embodiments, SEQ ID NO: 72 can comprise or consist of human or humanized amino acid sequences. In some embodiments, SEQ ID NO: 74 can comprise or consist of human or humanized amino acid sequences.

[0262] In some embodiments, variant nucleic acid sequences or amino acid sequences having at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73 or SEQ ID NO: 74 are contemplated.

[0263] While the affinity at which the CARs, expressed by the lymphocytes, bind to the targeted moiety can vary, and in some cases low affinity binding may be preferable (such as about 50 nM), the binding affinity of the CARs to the targeted ligand will generally be at least about 100 nM, 1 pM, or 10 pM, preferably at least about 100 pM, 1 fM or 10 fM, even more preferably at least about 100 fM.

D. Cleavable Linkers

[0264] As provided herein, expression cassettes of the polycistronic constructs can be separated by linkers. In some embodiments, the linkers comprise sites for cleavage, making them cleavable linkers.

[0265] Cleavage sites can be used in the design of polycistronic constructs to achieve co-expression of multiple genes. In some embodiments, the cleavage sites comprise self-cleaving sites.

In some embodiments, the self-cleaving site comprises a 2A site. 2A peptides are a class of 18-22 amino acid-long peptides that can induce ribosomal skipping during translation resulting in loss of a peptide bond between glycine and proline residues, which allows a proteolytic enzyme to recognize the 2A site. The most commonly used 2A peptides in molecular biology include T2A, P2A, E2A, and F2A.

[0266] In some embodiments, the polycistronic constructs provided herein comprise one or more cleavable linkers. In some embodiments, the one or more cleavable linkers separating the expression cassettes are the same. In some embodiments, the cleavable linkers separating the expression cassettes are different. In some embodiments, the one or more cleavable linkers separating the expression cassettes comprise one or more cleavage sites. In some embodiments, the one or more cleavage sites are the same. In some embodiments, the one or more cleavage sites are different.

[0267] In some embodiments, in addition to a 2A site, the cleavable linker may also comprise another cleavage site. In some embodiments, the additional cleavage site comprises a furin site. There are three known furin sites, including FC1, FC2 and FC3.

[0268] In some embodiments, the polycistronic construct provided herein comprises a T2A, P2A, E2A, or F2A cleavage site in the cleavable linker. In some embodiments, the polycistronic construct comprises a T2A cleavage site in a cleavable linker. In some embodiments, the polycistronic construct comprises a P2A cleavage site in a cleavable linker. In some embodiments, the polycistronic construct comprises a furin cleavage site in a cleavable linker. In some embodiments, the polycistronic construct comprises a T2A cleavage site and a furin cleavage site in a cleavable linker.

[0269] In some embodiments, the polycistronic construct provided herein comprises at least one, at least two or at least three 2A cleavable linker sequences. In some embodiments, the polycistronic construct herein comprises a T2A cleavage site and a P2A, E2A or F2A cleavage site. In some embodiments, the polycistronic construct herein comprises a P2A cleavage site and a T2A, E2A or F2A cleavage site. In some embodiments, the polycistronic construct herein comprises a E2A cleavage site and a P2A, T2A or F2A cleavage site. In some embodiments, the polycistronic construct herein comprises a F2A cleavage site and a P2A, E2A or T2A cleavage site.

[0270] In some embodiments, the polycistronic construct provided herein comprises a 2A cleavable linker sequence. In some embodiments, each nucleotide sequence encoding the 2A cleavable linker sequences is different. In some embodiments, the 2A cleavable linker is independently a T2A, P2A, E2A or F2A cleavage site. In some embodiments, the 2A cleavable linker is independently a P2A or a T2A.

[0271] In some embodiments, the 2A cleavable linker is a P2A and the nucleotide sequence encoding the P2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 17, 25, 52, or 58. In some embodiments, the nucleotide sequence encoding the P2A cleavable linker is set forth in SEQ ID NOs: 17, 25, 52, or 58. In some embodiments, the P2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:18. In some embodiments, the P2A cleavable linker comprises the sequence set forth in SEQ ID NO:18.

[0272] In some embodiments, at least one 2A cleavable linker is a T2A and the nucleotide sequence encoding the T2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:9. In some embodiments, the nucleotide sequence encoding the T2A cleavable linker is set forth in SEQ ID NO:9. In some embodiments, the T2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:10. In some embodiments, the T2A cleavable linker comprising the sequence set forth in SEQ ID NO:10.

[0273] In some embodiments, at least one of the cleavage site sequences comprises a furin cleavage site sequence. In some embodiments, the furin cleavage site sequence is located between the first expression cassette and the second expression cassette. In some embodiments, the nucleotide sequence encoding the furin cleavage site sequence comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:7. In some embodiments, the nucleotide sequence encoding the furin cleavage site sequence comprises the sequence set forth in SEQ ID NO: 7. In some embodiments, the furin cleavage site sequence comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 8. In some embodiments, the furin cleavage site sequence comprises the amino acid sequence of SEQ ID NO: 8.

[0274] In some embodiments, the cleavage site sequence comprises a furin cleavage site sequence and a T2A cleavage sequence (furinT2A). In some embodiments, the nucleotide sequence encoding the cleavage site sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 5. In some embodiments, the nucleotide sequence encoding the cleavage site sequence comprises the nucleotide sequence of SEQ ID NO: 5.

[0275] In some embodiments, the cleavage site sequence comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the cleavage site sequence comprises the amino acid sequence of SEQ ID NO:6.

[0276] In some embodiments, the first expression cassette and second expression cassette are separated by a furinT2A, the second expression cassette and third expression cassette are separated by a P2A, and the third expression cassette and fourth expression cassette are separated by a P2A.

E. Exemplary Polycistronic Constructs

[0277] In some embodiments the polycistronic constructs provided herein comprise the features set forth in **Table 5**, **Table 6** or **Table 7**.

Table 5. Exemplary sequences of RACR construct

	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Construct C2.U Full Vector		1	
Construct C2 .U		125	2
FRB		3	4
RACR	FRB-IL2Rγ	11	12
	FRB	13	14
	IL2Rγ	15	16
	FKBP12-IL2Rβ	19	20
	FKBP12	21	22
	IL2Rβ	23	24
Universal anti-FITC CAR		26, 63, 82	27, 127

[0278] In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:2. In some

embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2. In some embodiments, the construct encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2.

[0279] In some embodiments, the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct

comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct comprises the nucleotide sequence of SEQ ID NO:125. In some embodiments, the construct consists of the nucleotide sequence of SEQ ID NO:125.

[0280] In some embodiments, the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct comprises the nucleotide sequence of SEQ ID NO:1. In some embodiments, the construct consists of the nucleotide sequence of SEQ ID NO:1.

Table 6. Exemplary sequences of RACR construct

	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Construct C2 Full Vector		48	
Construct C2		126	49
FRB		50	51
RACR	FKBP12-IL2Rγ	53	54
	FKBP12	55	22
	IL2Rγ	56	57
	FRB-IL2Rβ	59	60
	FRB	13	14
	IL2Rβ	61	62
Universal anti-FITC CAR		26, 63, 82	27, 127

[0281] In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 48. In some embodiments, the construct encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO: 48.

[0282] In some embodiments, the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the

construct comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct comprises the nucleotide sequence of SEQ ID NO:126. In some embodiments, the construct consists of the nucleotide sequence of SEQ ID NO:126.

[0283] In some embodiments, the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct comprises the nucleotide sequence of SEQ ID NO:48. In some embodiments, the construct consists of the nucleotide sequence of SEQ ID NO:48.

Table 7. Exemplary sequences of RACR construct

	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Construct C3.U Full Vector		101	

Table 7. Exemplary sequences of RACR construct			
	Component	**Nucleotide (SEQ ID NO)**	**Amino Acid (SEQ ID NO)**
Construct C3.U		102	103
FRB		3	4
RACR	FRB-IL2Rγ	11	12
	FRB	13	14
	IL2Rγ	15	16
	FKBP12-IL2Rβ	19	20
	FKBP12	21	22
	IL2Rβ	23	24
anti-CD19 CAR		104	105

[0284] In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 96% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 97% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 98% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:103. In some embodiments, the construct encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:103.

[0285] In some embodiments, the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct comprises the nucleotide sequence of SEQ ID NO:102. In some embodiments, the construct consists of the nucleotide sequence of SEQ ID NO:102.

[0286] In some embodiments, the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 80% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 90% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 96% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 97% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 98% identical to the nucleotide sequence of SEQ ID

NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 99% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct comprises the nucleotide sequence of SEQ ID NO:101. In some embodiments, the construct consists of the nucleotide sequence of SEQ ID NO:101.

II. NUCLEIC ACID VECTORS

[0287] In some embodiments, the polycistronic constructs can be inserted into a nucleic acid vector. As used herein, the term "nucleic acid vector" is intended to mean any nucleic acid that functions to carry, harbor or express a nucleic acid of interest. Nucleic acid vectors can have specialized functions such as expression, packaging, pseudotyping, transduction or sequencing, for example. Nucleic acid vectors also can have, for example, manipulatory functions such as a cloning or shuttle vector. The structure of the vector can include any desired form that is feasible to make and desirable for a particular use. Such forms include, for example, circular forms such as plasmids and phagemids, as well as linear or branched forms. A nucleic acid vector can be composed of, for example, DNA or RNA, as well as contain partially or fully, nucleotide derivatives, analogs and mimetics. Such nucleic acid vectors can be obtained from natural sources, produced recombinantly or chemically synthesized.

[0288] Non-limiting examples of vector systems of the present disclosure include a retrovirus, a lentivirus, a foamy virus, and a Sleeping Beauty transposon.

A. Retroviral Vectors

[0289] Retroviruses include lentiviruses, gamma-retroviruses, and alpha-retroviruses, each of which may be used to deliver polynucleotides to cells using methods known in the art. Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes gag, pol, and env, contain other genes with regulatory or structural function. The higher complexity enables the virus to modulate its life cycle, as in the course of latent infection. Some examples of lentivirus include the Human Immunodeficiency Viruses (HIV-1 and HIV-2) and the Simian Immunodeficiency Virus (SIV). Retroviral vectors have been generated by multiply attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted, making the vector biologically safe.

[0290] Illustrative lentiviral vectors include those described in Naldini et al. (1996) Science 272:263-7; Zufferey et al. (1998) J. Virol. 72:9873-9880; Dull et al. (1998) J. Virol. 72:8463-8471; U.S. Pat. No. 6,013,516; and U.S. Pat. No. 5,994,136, which are each incorporated herein by reference in their entireties. In general, these vectors are configured to carry the essential sequences for selection of cells containing the vector, for incorporating foreign nucleic acid into a lentiviral particle, and for transfer of the nucleic acid into a target cell.

[0291] A commonly used lentiviral vector system is the so-called third-generation system. Third-generation lentiviral vector systems include four plasmids. The "transfer plasmid" encodes the polynucleotide sequence that is delivered by the lentiviral vector system to the target cell. The transfer plasmid generally has one or more transgene sequences of interest flanked by long terminal repeat (LTR) sequences, which facilitate integration of the transfer plasmid sequences into the host genome. For safety reasons, transfer plasmids are generally designed to make the resulting vector replication incompetent. For example, the transfer plasmid lacks gene elements necessary for generation of infective particles in the host cell. In addition, the transfer plasmid may be designed with a deletion of the 3′ LTR, rendering the virus "self-inactivating" (SIN). See Dull et al. (1998) J. Virol. 72:8463-71; Miyoshi et al. (1998) J. Virol. 72:8150-57. The viral particle may also comprise a 3′ untranslated region (UTR) and a 5′ UTR. The UTRs comprise retroviral regulatory elements that support packaging, reverse transcription and integration of a proviral genome into a cell following contact of the cell by the retroviral particle.

[0292] Third-generation systems also generally include two "packaging plasmids" and an "envelope plasmid." The "envelope plasmid" generally encodes an Env gene operatively linked to a promoter. In an illustrative third-generation system, the Env gene is VSV-G and the promoter is the CMV promoter. The third-generation system uses two packaging plasmids, one encoding gag and pol and the other encoding rev as a further safety feature; an improvement over the single packaging plasmid of so-called second-generation systems. Although safer, the third-generation system can be more cumbersome to use and result in lower viral titers due to the addition of an additional plasmid. Illustrative packing plasmids include, without limitation, pMD2.G, pRSV-rev, pMDLG-pRRE, and pRRL-GOI.

[0293] Many retroviral vector systems rely on the use of a "packaging cell line." In general, the packaging cell line is a cell line whose cells are capable of producing infectious retroviral particles

when the transfer plasmid, packaging plasmid(s), and envelope plasmid are introduced into the cells. Various methods of introducing the plasmids into the cells may be used, including transfection or electroporation. In some cases, a packaging cell line is adapted for high-efficiency packaging of a retroviral vector system into retroviral particles.

[0294] As used herein, the terms "retroviral vector" or "lentiviral vector" is intended to mean a nucleic acid that encodes a retroviral or lentiviral cis nucleic acid sequence required for genome packaging and one or more polynucleotide sequence to be delivered into the target cell. Retroviral particles and lentiviral particles generally include an RNA genome (derived from the transfer plasmid), a lipid-bilayer envelope in which the Env protein is embedded, and other accessory proteins including integrase, protease, and matrix protein. As used herein, the terms "retroviral particle" and "lentiviral particle" refers a viral particle that includes an envelope, has one or more characteristics of a lentivirus, and is capable of invading a target host cell. Such characteristics include, for example, infecting non-dividing host cells, transducing non-dividing host cells, infecting or transducing host immune cells, containing a retroviral or lentiviral virion including one or more of the gag structural polypeptides, containing a retroviral or lentiviral envelope including one or more of the env encoded glycoproteins, containing a genome including one or more retrovirus or lentivirus cis-acting sequences functioning in replication, proviral integration or transcription, containing a genome encoding a retroviral or lentiviral protease, reverse transcriptase or integrase, or containing a genome encoding regulatory activities such as Tat or Rev. The transfer plasmids may comprise a cPPT sequence, as described in U.S. Patent No. 8,093,042.

[0295] The efficiency of the system is an important concern in vector engineering. The efficiency of a retroviral or lentiviral vector system may be assessed in various ways known in the art, including measurement of vector copy number (VCN) or vector genomes (vg) such as by quantitative polymerase chain reaction (qPCR), or titer of the virus in infectious units per milliliter (IU/mL). For example, the titer may be assessed using a functional assay performed on the cultured tumor cell line HT1080 as described in Humbert et al. Development of third-generation Cocal Envelope Producer Cell Lines for Robust Retroviral Gene Transfer into Hematopoietic Stem Cells and T-cells. Molecular Therapy 24:1237–1246 (2016). When titer is assessed on a cultured cell line that is continually dividing, no stimulation is required and hence the measured titer is not influenced by surface engineering of the retroviral particle. Other methods for assessing the efficiency of

retroviral vector systems are provided in Gaererts et al. Comparison of retroviral vector titration methods. BMC Biotechnol. 6:34 (2006).

[0296] In some embodiments, the retroviral particles and/or lentiviral particles of the disclosure comprise a polynucleotide comprising a sequence encoding a receptor that specifically binds to the gating adaptor. In some embodiments, a sequence encoding a receptor that specifically binds to the gating adaptor is operatively linked to a promoter. Illustrative promoters include, without limitation, a cytomegalovirus (CMV) promoter, a CAG promoter, an SV40 promoter, an SV40/CD43 promoter, and a MND promoter.

[0297] In some embodiments, the retroviral particles comprise transduction enhancers. In some embodiments, the retroviral particles comprise tagging proteins.

[0298] In some embodiments, each of the retroviral particles comprises a polynucleotide comprising, in 5′ to 3′ order: (i) a 5′ long terminal repeat (LTR) or untranslated region (UTR), (ii) a promoter, (iii) a sequence encoding a receptor that specifically binds to a ligand, and (iv) a 3′ LTR or UTR.

[0299] In some embodiments, the retroviral particles comprise a cell surface receptor that binds to a surface marker on a target host cell, allowing host cell transduction. In some embodiments, the cell surface receptor is a T cell surface receptor. The viral vector may comprise a heterologous viral envelope glycoprotein giving a pseudotyped viral vector. For example, the viral envelope glycoprotein may be derived from RD114 or one of its variants, VSV-G, Gibbon-ape leukaemia virus (GALV), or is the Amphotropic envelope, Measles envelope or baboon retroviral envelope glycoprotein. In some embodiments, the cell-surface receptor is a VSV G protein from the Cocal strain or a functional variant thereof.

[0300] Various fusion glycoproteins can be used to pseudotype lentiviral vectors. While the most commonly used example is the envelope glycoprotein from vesicular stomatitis virus (VSVG), many other viral proteins have also been used for pseudotyping of lentiviral vectors. See Joglekar et al. Human Gene Therapy Methods 28:291-301 (2017). The present disclosure contemplates substitution of various fusion glycoproteins. Notably, some fusion glycoproteins result in higher vector efficiency.

[0301] In some embodiments, pseudotyping a fusion glycoprotein or functional variant thereof facilitates targeted transduction of specific cell types, including, but not limited to, innate lymphoid

cells, cytotoxic innate lymphoid cells, or NK cells. In some embodiments, the fusion glycoprotein or functional variant thereof is/are full-length polypeptide(s), functional fragment(s), homolog(s), or functional variant(s) of Human immunodeficiency virus (HIV) gp160, Murine leukemia virus (MLV) gp70, Gibbon ape leukemia virus (GALV) gp70, Feline leukemia virus (RD114) gp70, Amphotropic retrovirus (Ampho) gp70, 10A1 MLV (10A1) gp70, Ecotropic retrovirus (Eco) gp70, Baboon ape leukemia virus (BaEV) gp70, Measles virus (MV) H and F, Nipah virus (NiV) H and F, Rabies virus (RabV) G, Mokola virus (MOKV) G, Ebola Zaire virus (EboZ) G, Lymphocytic choriomeningitis virus (LCMV) GP1 and GP2, Baculovirus GP64, Chikungunya virus (CHIKV) E1 and E2, Ross River virus (RRV) E1 and E2, Semliki Forest virus (SFV) E1 and E2, Sindbis virus (SV) E1 and E2, Venezualan equine encephalitis virus (VEEV) E1 and E2, Western equine encephalitis virus (WEEV) E1 and E2, Influenza A, B, C, or D HA, Fowl Plague Virus (FPV) HA, Vesicular stomatitis virus VSV-G, or Chandipura virus and Piry virus CNV-G and PRV-G.

[0302] In some embodiments, the fusion glycoprotein or functional variant thereof is a full-length polypeptide, functional fragment, homolog, or functional variant of the G protein of Vesicular Stomatitis Alagoas Virus (VSAV), Carajas Vesiculovirus (CJSV), Chandipura Vesiculovirus (CHPV), Cocal Vesiculovirus (COCV), Vesicular Stomatitis Indiana Virus (VSIV), Isfahan Vesiculovirus (ISFV), Maraba Vesiculovirus (MARAV), Vesicular Stomatitis New Jersey virus (VSNJV), Bas-Congo Virus (BASV). In some embodiments, the fusion glycoprotein or functional variant thereof is the Cocal virus G protein.

[0303] In some embodiments, the fusion glycoprotein or functional variant thereof is a full-length polypeptide, functional fragment, homolog, or functional variant of the G protein of Vesicular Stomatitis Alagoas Virus (VSAV), Carajas Vesiculovirus (CJSV), Chandipura Vesiculovirus (CHPV), Cocal Vesiculovirus (COCV), Vesicular Stomatitis Indiana Virus (VSIV), Isfahan Vesiculovirus (ISFV), Maraba Vesiculovirus (MARAV), Vesicular Stomatitis New Jersey virus (VSNJV), Bas-Congo Virus (BASV). In some embodiments, the fusion glycoprotein or functional variant thereof is the Cocal virus G protein. The disclosure further provides various retroviral vectors, including but not limited to gamma-retroviral vectors, alpha-retroviral vectors, and lentiviral vectors. In some embodiments, the vector may be a viral vector, a retroviral vector, a lentiviral vector, a gamma-retroviral vector. In some embodiments, the viral vector comprises a

VSV G-protein or functional variant thereof. In some embodiments, the viral vector comprises a Cocal G-protein or functional variant thereof.

[0304] In some embodiments, provided herein is a viral vector comprising any of the polycistronic constructs provided herein. In some embodiments, the viral vector is a lentiviral vector. In some embodiments, the viral vector further comprises one or more surface T cell activating agents. In some embodiments, the one or more surface T cell activating agents comprise CD58, anti-CD3, or CD80.

III. VIRUS PARTICLES

[0305] In some embodiments, provided herein is a virus particle encapsulating the polycistronic constructs disclosed herein. In some embodiments, any of the polycistronic constructs can be provided as a payload in the generation of a viral particle. Also provided herein are viral particles, such as lentiviral vectors, incorporating any of the provided polycistronic constructs for delivery of components of the rapamycin activated cytokine receptor (RACR) system, including FRB, synthetic cytokine receptor and CAR, to a target cell. In further embodiments, the virus particles can be engineered to express one or more surface T cell activating agents. In some embodiments, the one or more surface T cell activating agents comprise a T cell surface receptor. In some embodiments, the T cell surface receptor comprises CD58, anti-CD3, or CD80.

[0306] As it is well known in the art, a viral particle is a tool that allows or facilitates the transfer of an entity from one environment to another. In accordance with the disclosure, and by way of example, some viral particles used in recombinant DNA techniques allow entities, such as a segment of DNA, to be transferred into a host cell. Examples of vectors used in recombinant DNA techniques include but are not limited to, plasmids, chromosomes, artificial chromosomes or viruses. The term "expression vector" means a construct capable of in vivo or in vitro/ex vivo expression.

A. Retroviral Particles

[0307] In some embodiments, the virus particle comprises a retroviral particle. In some embodiments, the disclosure provides a method for preparing a viral formulation. In some embodiments, the virus is a retrovirus. A large number of different retroviruses have been identified. Examples of retrovirus include but are not limited to: murine leukemia virus (MLV),

human immunodeficiency virus (HIV), human T-cell leukemia virus (HTLV), mouse mammary tumor virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV). A detailed list of retroviruses may be found in Coffin et al., 1997, "Retroviruses", Cold Spring Harbor Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763.

[0308] Retroviruses include lentiviruses, gamma-retroviruses, and alpha-retroviruses, each of which may be used to deliver polynucleotides to cells using methods known in the art. Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes gag, pol, and env, contain other genes with regulatory or structural function. The higher complexity enables the virus to modulate its life cycle, as in the course of latent infection. Some examples of lentivirus include the Human Immunodeficiency Viruses (HIV-1 and HIV-2) and the Simian Immunodeficiency Virus (SIV). Retroviral vectors have been generated by multiply attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted, making the vector biologically safe.

[0309] A lentiviral vector of the disclosure may be derived from or may be derivable from any suitable lentivirus. A recombinant retroviral vector particle is capable of transducing a recipient cell with a nucleotide of interest (NOI). Once within the cell, the RNA genome from the vector particle is reverse transcribed into DNA and integrated into the DNA of the recipient cell. In some embodiments of the disclosure, at least part of one or more protein coding regions essential for replication may be removed from the virus. This makes the viral vector replication defective. Portions of the viral genome may also be replaced by an NOI in order to generate a vector comprising an NOI which is capable of transducing a target non-dividing host cell and/or integrating its genome into a host genome.

[0310] Illustrative lentiviral vectors include those described in Naldini et al. (1996) Science 272:263-7; Zufferey et al. (1998) J. Virol. 72:9873-9880; Dull et al. (1998) J. Virol. 72:8463-8471; U.S. Pat. No. 6,013,516; and U.S. Pat. No. 5,994,136, which are each incorporated herein by reference in their entireties. In general, these vectors are configured to carry the essential sequences for selection of cells containing the vector, for incorporating foreign nucleic acid into a lentiviral particle, and for transfer of the nucleic acid into a target cell.

[0311] A commonly used lentiviral vector system is the so-called third-generation system. Third-generation lentiviral vector systems include four plasmids. The "transfer plasmid" encodes the polynucleotide sequence that is delivered by the lentiviral vector system to the target cell. The transfer plasmid generally has one or more transgene sequences of interest flanked by long terminal repeat (LTR) sequences, which facilitate integration of the transfer plasmid sequences into the host genome. For safety reasons, transfer plasmids are generally designed to make the resulting vector replication incompetent. For example, the transfer plasmid lacks gene elements necessary for generation of infective particles in the host cell. In addition, the transfer plasmid may be designed with a deletion of the 3′ LTR, rendering the virus "self-inactivating" (SIN). See Dull et al. (1998) J. Virol. 72:8463-71; Miyoshi et al. (1998) J. Virol. 72:8150-57. The viral particle may also comprise a 3′ untranslated region (UTR) and a 5′ UTR. The UTRs comprise retroviral regulatory elements that support packaging, reverse transcription and integration of a proviral genome into a cell following contact of the cell by the retroviral particle.

[0312] Third-generation systems also generally include two "packaging plasmids" and an "envelope plasmid." The "envelope plasmid" generally encodes an Env gene operatively linked to a promoter. In an illustrative third-generation system, the Env gene is VSV-G and the promoter is the CMV promoter. The third-generation system uses two packaging plasmids, one encoding gag and pol and the other encoding rev as a further safety feature; an improvement over the single packaging plasmid of so-called second-generation systems. Although safer, the third-generation system can be more cumbersome to use and result in lower viral titers due to the addition of an additional plasmid. Illustrative packing plasmids include, without limitation, pMD2.G, pRSV-rev, pMDLG-pRRE, and pRRL-GOI.

[0313] Many retroviral vector systems rely on the use of a "packaging cell line." In general, the packaging cell line is a cell line whose cells are capable of producing infectious retroviral particles when the transfer plasmid, packaging plasmid(s), and envelope plasmid are introduced into the cells. Various methods of introducing the plasmids into the cells may be used, including transfection or electroporation. In some cases, a packaging cell line is adapted for high-efficiency packaging of a retroviral vector system into retroviral particles.

[0314] As used herein, the terms "retroviral vector" or "lentiviral vector" is intended to mean a nucleic acid that encodes a retroviral or lentiviral cis nucleic acid sequence required for genome

packaging and one or more polynucleotide sequence to be delivered into the target cell. Retroviral particles and lentiviral particles generally include an RNA genome (derived from the transfer plasmid), a lipid-bilayer envelope in which the Env protein is embedded, and other accessory proteins including integrase, protease, and matrix protein. As used herein, the terms "retroviral particle" and "lentiviral particle" refers a viral particle that includes an envelope, has one or more characteristics of a lentivirus, and is capable of invading a target host cell. Such characteristics include, for example, infecting non-dividing host cells, transducing non-dividing host cells, infecting or transducing host immune cells, containing a retroviral or lentiviral virion including one or more of the gag structural polypeptides, containing a retroviral or lentiviral envelope including one or more of the env encoded glycoproteins, containing a genome including one or more retrovirus or lentivirus cis-acting sequences functioning in replication, proviral integration or transcription, containing a genome encoding a retroviral or lentiviral protease, reverse transcriptase or integrase, or containing a genome encoding regulatory activities such as Tat or Rev. The transfer plasmids may comprise a cPPT sequence, as described in U.S. Patent No. 8,093,042.

[0315] The efficiency of the system is an important concern in vector engineering. The efficiency of a retroviral or lentiviral vector system may be assessed in various ways known in the art, including measurement of vector copy number (VCN) or vector genomes (vg) such as by quantitative polymerase chain reaction (qPCR), or titer of the virus in infectious units per milliliter (IU/mL). For example, the titer may be assessed using a functional assay performed on the cultured tumor cell line HT1080 as described in Humbert et al. Development of third-generation Cocal Envelope Producer Cell Lines for Robust Retroviral Gene Transfer into Hematopoietic Stem Cells and T-cells. Molecular Therapy 24:1237–1246 (2016). When titer is assessed on a cultured cell line that is continually dividing, no stimulation is required and hence the measured titer is not influenced by surface engineering of the retroviral particle. Other methods for assessing the efficiency of retroviral vector systems are provided in Gaererts et al. Comparison of retroviral vector titration methods. BMC Biotechnol. 6:34 (2006).

[0316] In some embodiments, the retroviral particles and/or lentiviral particles of the disclosure comprise a polynucleotide comprising a sequence encoding a receptor that specifically binds to the gating adaptor. In some embodiments, a sequence encoding a receptor that specifically binds to the gating adaptor is operatively linked to a promoter. Illustrative promoters include, without limitation,

a cytomegalovirus (CMV) promoter, a CAG promoter, an SV40 promoter, an SV40/CD43 promoter, and a MND promoter.

[0317] In some embodiments, the retroviral particles comprise transduction enhancers. In some embodiments, the retroviral particles comprise tagging proteins.

[0318] In some embodiments, each of the retroviral particles comprises a polynucleotide comprising, in 5′ to 3′ order: (i) a 5′ long terminal repeat (LTR) or untranslated region (UTR), (ii) a promoter, (iii) a sequence encoding a receptor that specifically binds to a ligand, and (iv) a 3′ LTR or UTR.

[0319] In some embodiments, the retroviral particles comprise a cell surface receptor that binds to a surface marker on a target host cell, allowing host cell transduction. In some embodiments, the cell surface receptor is a T cell surface receptor. The viral vector may comprise a heterologous viral envelope glycoprotein giving a pseudotyped viral vector. For example, the viral envelope glycoprotein may be derived from RD114 or one of its variants, VSV-G, Gibbon-ape leukaemia virus (GALV), or is the Amphotropic envelope, Measles envelope or baboon retroviral envelope glycoprotein. In some embodiments, the cell-surface receptor is a VSV G protein from the Cocal strain or a functional variant thereof.

[0320] In some embodiments, the viral envelope comprises a viral envelope protein. In some embodiments, a viral envelope protein is a VSV-G envelope protein, a measles virus envelope protein, a nipha virus envelope protein, or a cocal virus G protein. In some embodiments, the viral particle comprises a modified VSV G protein that lacks LDLR binding affinity. In some embodiments, these mutations comprise mutations at positions 47 (for example, K47Q) and/or 354 (for example, R354A).

[0321] In some embodiments, the viral envelope protein is a protein from the Cocal strain (Cocal glycoprotein). In some embodiments, the protein is a Cocal envelope protein containing a mutation at position 354 (R354). In some embodiments, the protein is a Cocal envelope protein containing a mutation at position 47 (K47). In some embodiments, the protein is a Cocal envelope variant containing a R354Q mutation. In some embodiments, the protein is a Cocal envelope variant containing a K47Q mutation. In some embodiments, this variant may be referred to as "blinded" Cocal envelope. Illustrative Cocal envelope variants are provided in, e.g., US 2020/0216502 A1, which is incorporated herein by reference in its entirety.

[0322] Various fusion glycoproteins can be used to pseudotype lentiviral vectors. While the most commonly used example is the envelope glycoprotein from vesicular stomatitis virus (VSVG), many other viral proteins have also been used for pseudotyping of lentiviral vectors. See Joglekar et al. Human Gene Therapy Methods 28:291-301 (2017). The present disclosure contemplates substitution of various fusion glycoproteins. Notably, some fusion glycoproteins result in higher vector efficiency.

[0323] In some embodiments, pseudotyping a fusion glycoprotein or functional variant thereof facilitates targeted transduction of specific cell types, including, but not limited to, innate lymphoid cells or NK-cells. In some embodiments, the fusion glycoprotein or functional variant thereof is/are full-length polypeptide(s), functional fragment(s), homolog(s), or functional variant(s) of Human immunodeficiency virus (HIV) gp160, Murine leukemia virus (MLV) gp70, Gibbon ape leukemia virus (GALV) gp70, Feline leukemia virus (RD114) gp70, Amphotropic retrovirus (Ampho) gp70, 10A1 MLV (10A1) gp70, Ecotropic retrovirus (Eco) gp70, Baboon ape leukemia virus (BaEV) gp70, Measles virus (MV) H and F, Nipah virus (NiV) H and F, Rabies virus (RabV) G, Mokola virus (MOKV) G, Ebola Zaire virus (EboZ) G, Lymphocytic choriomeningitis virus (LCMV) GP1 and GP2, Baculovirus GP64, Chikungunya virus (CHIKV) E1 and E2, Ross River virus (RRV) E1 and E2, Semliki Forest virus (SFV) E1 and E2, Sindbis virus (SV) E1 and E2, Venezualan equine encephalitis virus (VEEV) E1 and E2, Western equine encephalitis virus (WEEV) E1 and E2, Influenza A, B, C, or D HA, Fowl Plague Virus (FPV) HA, Vesicular stomatitis virus VSV-G, or Chandipura virus and Piry virus CNV-G and PRV-G.

[0324] In some embodiments, the fusion glycoprotein or functional variant thereof is a full-length polypeptide, functional fragment, homolog, or functional variant of the G protein of Vesicular Stomatitis Alagoas Virus (VSAV), Carajas Vesiculovirus (CJSV), Chandipura Vesiculovirus (CHPV), Cocal Vesiculovirus (COCV), Vesicular Stomatitis Indiana Virus (VSIV), Isfahan Vesiculovirus (ISFV), Maraba Vesiculovirus (MARAV), Vesicular Stomatitis New Jersey virus (VSNJV), Bas-Congo Virus (BASV). In some embodiments, the fusion glycoprotein or functional variant thereof is the Cocal virus G protein.

[0325] In some embodiments, the fusion glycoprotein or functional variant thereof is a full-length polypeptide, functional fragment, homolog, or functional variant of the G protein of Vesicular Stomatitis Alagoas Virus (VSAV), Carajas Vesiculovirus (CJSV), Chandipura

Vesiculovirus (CHPV), Cocal Vesiculovirus (COCV), Vesicular Stomatitis Indiana Virus (VSIV), Isfahan Vesiculovirus (ISFV), Maraba Vesiculovirus (MARAV), Vesicular Stomatitis New Jersey virus (VSNJV), Bas-Congo Virus (BASV). In some embodiments, the fusion glycoprotein or functional variant thereof is the Cocal virus G protein.

[0326] The disclosure further provides various retroviral vectors, including but not limited to gamma-retroviral vectors, alpha-retroviral vectors, and lentiviral vectors. In some embodiments, the vector may be a viral vector, a retroviral vector, a lentiviral vector, a gamma-retroviral vector. In some embodiments, the viral vector comprises a VSV G-protein or functional variant thereof. In some embodiments, the viral vector comprises a Cocal G-protein or functional variant thereof.

B. Engineered Viral Envelope

[0327] In some embodiments, the virus particles encapsulating nucleotide vectors provided herein can comprise engineered viral envelopes. In some embodiments, the viral envelope comprises a transduction enhancer. In some embodiments, the viral envelope comprises at an immune cell-activating protein. In some embodiments, the viral envelope comprises a co-stimulation molecule. In some embodiments, the viral envelope comprises an immune cell-activating protein, and a co-stimulation molecule.

[0328] In some embodiments, the viral envelope comprises one or more transduction enhancers. In some embodiments, the transduction enhancers include T cell activation receptors, NK cell activation receptors, and/or co-stimulatory molecules. In some embodiments, one or more transduction enhancers comprise one or more of anti-CD3scFv, CD86, CD80, and/or CD58. In some embodiments, the transduction enhancers comprise at least an anti-CD3 scFv, and CD58. In some embodiments, the transduction enhancers comprise at least an anti-CD3 scFv, and CD80. In some embodiments, the transduction enhancers comprise at least an anti-CD3 scFv, and CD86. In some embodiments, the transduction enhancers comprise at least an anti-CD3 scFv, a CD80, and CD58. In some embodiments, the transduction enhancers comprise at least an anti-CD3 scFv, a CD86, and CD58.

[0329] In some embodiments, the viral particle comprises a cell surface receptor that binds to a ligand on a target host cell, allowing host cell transduction. In some embodiments, the cell surface receptor is a T cell surface receptor. In some embodiments, the viral particle comprises a

heterologous viral envelope glycoprotein yielding a pseudotyped viral particle. For example, the viral envelope glycoprotein may be derived from RD114 or one of its variants, VSV-G, Gibbon-ape leukemia virus (GALV), or is the Amphotropic envelope, Measles envelope or baboon retroviral envelope glycoprotein. In some embodiments, the viral envelope glycoprotein is a VSV G protein from the Cocal strain (Cocal glycoprotein) or a functional variant thereof.

[0330] In some embodiments, the viral envelope comprises more than one polypeptide on the surface. In some embodiments, the more than one polypeptide binds to a target immune cells and replicates an immunological synapse. In some embodiments, the viral envelope comprises an immune cell-activating protein, a co-stimulatory molecule, and an adhesion molecule, wherein the immune cell-activating protein, co-stimulatory molecule, and adhesion molecule each bind a target immune cell.

1. Immune Cell Activating Agents

[0331] In some embodiments, the transduction enhancer comprises a mitogenic stimulus, which is incorporated into a retroviral or lentiviral capsid, such that the virus both activates and transduces T cells. This removes the need to add vector and mitogen. In some embodiments, the transduction enhancer comprises a mitogenic transmembrane protein and/or one or more costimulatory molecules, which get(s) incorporated into the retrovirus when it buds from the producer/packaging cell membrane. In some embodiments, the transduction enhancers are expressed as separate cell surface molecules on the producer cell rather than being part of the viral envelope glycoprotein.

[0332] In some embodiments, the viral vector described herein comprises a mitogenic transduction enhancer in the viral envelope. In some embodiments, the mitogenic transduction enhancer is derived from the host cell during retroviral vector production. In some embodiments, the mitogenic transduction enhancer is made by the packaging cell and expressed at the cell surface. When the nascent retroviral vector buds from the host cell membrane, the mitogenic transduction enhancer may be incorporated in the viral envelope as part of the packaging cell-derived lipid bilayer. In some embodiments, the mitogenic enhancer is an antibody or fragment thereof. In some embodiments, the mitogenic enhancer is a single domain antibody, for example, a camelid antibody. In some embodiments, the mitogenic enhancer is an scFv. In some embodiments, the mitogenic enhancer is a nanobody.

[0333] In some embodiments, the transduction enhancer is host-cell derived. The term "host-cell derived" indicates that the mitogenic transduction enhancer is derived from the host cell as described above and is not produced as a fusion or chimera from one of the viral genes, such as gag, which encodes the main structural proteins; or env, which encodes the envelope protein.

[0334] Envelope proteins are formed by two subunits, the transmembrane (TM) that anchors the protein into the lipid membrane and the surface (SU) which binds to the cellular receptors. In some embodiments, the packaging-cell derived mitogenic transduction enhancer of the present invention does not comprise the surface envelope subunit (SU).

[0335] In some embodiments, the mitogenic transduction enhancer has the structure: M-S-TM, in which M is a mitogenic domain; S is an optional spacer domain and TM is a transmembrane domain.

[0336] The mitogenic domain is the part of the mitogenic transduction enhancer which causes T-cell activation. It may bind or otherwise interact, directly or indirectly, with a T cell, leading to T cell activation. In some embodiments, the mitogenic domain binds a T cell surface antigen, such as CD3, CD28, CD134 and CD137.

[0337] CD3 is a T-cell co-receptor. It is a protein complex composed of four distinct chains. In mammals, the complex contains a CD3y chain, a CD35 chain, and two CD3e chains. These chains associate with the T-cell receptor (TCR) and the z-chain to generate an activation signal in T lymphocytes. The TCR, z-chain, and CD3 molecules together comprise the TCR complex. In some embodiments, the mitogenic domain binds to a CD3 e chain.

[0338] In some embodiments, the mitogenic domain comprises all or part of an antibody or other molecule which specifically binds a T-cell surface antigen. In some embodiments, the antibody activates the TCR or CD28. In some embodiments, the antibody binds the TCR, CD3 or CD28. Examples of such antibodies include: OKT3, 15E8 and TGN1412. Other suitable antibodies include:

[0339] Anti-CD28: CD28.2, 10F3

[0340] Anti-CD3/TCR: UCHT1 , YTH12.5, TR66

[0341] In some embodiments, the mitogenic domain comprises the binding domain from OKT3, 15E8, TGN1412, CD28.2, 10F3, UCHT1, YTH12.5 or TR66.

[0342] In some embodiments, the mitogenic domain comprises all or part of a co-stimulatory molecule such as OX40L and 41BBL. For example, the mitogenic domain may comprise the binding domain from OX40L or 41BBL.

[0343] OKT3, also known as Muromonab-CD3 is a monoclonal antibody targeted at the CD3e chain. It is clinically used to reduce acute rejection in patients with organ transplants. It was the first monoclonal antibody to be approved for clinical use in humans.

[0344] In some embodiments, the viral envelope comprises an immune cell-activating protein. In some embodiments, the immune cell-activating protein specifically binds a receptor on an immune cell. In some embodiments, the immune cell-activating protein provides signal one for T cell activation.

[0345] In some embodiments, the immune cell-activating protein specifically binds CD2, CD3, CD28H, LFA-1, DNAM-1, CD27, ICOS, LIGHT, GITR, CD30, SLAM, Ly-9, CD84, Ly108, NKG2D, NKp46, NKp44, NKp30, CD244, or NKp80. In some embodiments, the immune cell-activating protein specifically binds CD3γ, CD3δ, or CD3ε. In some embodiments, the immune cell-activating protein specifically binds CD3γ, CD3δ, CD3ε, CD9, CD5, CD22, CD33, CD37, CD64, CD45, CD28H, LFA-1, DNAM-1, CD27, ICOS, LIGHT, GITR, CD30, SLAM, Ly-9, CD84, Ly108, CD16, CD56, NKG2D, NKp46, NKp44, NKp30, CD244, NKp80, TCRα chain, TCRβ chain, TCRγ chain, or TCRδ chain. In some embodiments, the immune cell-activating protein specifically binds CD3γ, CD3δ, or CD3ε. In some embodiments, the immune cell-activating protein specifically binds CD3.

[0346] In some embodiments, the immune cell-activating protein is an antibody or antigen binding fragment thereof that specifically binds a receptor on an immune cell. In some embodiments, the immune cell-activating protein is an antibody or antigen binding fragment thereof that specifically binds CD28, CD2, CD3, CD28H, LFA-1, OX40, 4-1BB, CD40L, DNAM-1, CD27, ICOS, LIGHT, GITR, CD30, SLAM, Ly-9, CD84, Ly108, NKG2D, NKp46, NKp44, NKp30, CD244, or NKp80. In some embodiments, the immune cell-activating protein is an antibody or antigen binding fragment thereof that specifically binds CD28, CD2, CD3γ, CD3δ, CD3ε, CD4, CD8, CD9, CD5, CD22, CD33, CD37, CD64, CD45, CD28H, LFA-1, OX40, 4-1BB, CD40L, DNAM-1, CD27, ICOS, LIGHT, GITR, CD30, SLAM, Ly-9, CD84, Ly108, CD16, CD56, NKG2D, NKp46, NKp44, NKp30, CD244, NKp80, TCRα chain, TCRβ chain, TCRγ chain, or

TCRδ chain. some embodiments, the immune cell-activating protein is an antibody or antigen binding fragment thereof that specifically binds CD3γ, CD3δ, or CD3ε. In some embodiments, immune cell-activating protein is an antibody or antigen binding fragment thereof that specifically binds CD3.

[0347] Antibodies targeting the polypeptides described herein are known to those of skill in the art. Methods for generating antibodies are known to those of skill in the art.

[0348] In some embodiments, the viral envelope comprises an anti-CD3ε antibody, or antigen-binding fragment thereof. In some embodiments, the anti-CD3ε antibody, or antigen-binding fragment thereof is coupled to a transmembrane domain. An illustrative anti-CD3ε antibody is OKT3. OKT3, also known as Muromonab-CD3, is a monoclonal antibody targeted at the CD3ε chain.

[0349] In some embodiments, the viral envelope comprises a single chain Fv fragment (scFv) of an anti-CD3 antibody.

2. Co-Stimulatory Molecules

[0350] In some embodiments, the viral envelope comprises at least one co-stimulatory molecule. In some embodiments, the co-stimulatory molecule specifically binds a receptor on an immune cell. In some embodiments, the co-stimulatory provides signal two for cell activation.

[0351] As used herein, the term "costimulatory molecule" refers to a molecule capable of generating a costimulatory signal to T cells. Lymphocytes, such as T cells and natural killer (NK) cells, typically require several signals and interactions with antigen presenting cells (APCs) for optimal priming to gain full effector functions. For T cells these include signaling through the T cell receptor (TCR), costimulatory molecules (such as CD28 and CD2), cytokines, as well as various adhesion molecules necessary to allow sufficient time for proper synapse formation and signal transduction. NK cells require similar types of stimulation but may rely on different activating receptors, such as NKG2D, NKp46, and DNAM-1. For T cells, proper costimulation, in addition to TCR stimulation, is especially important for effective priming and many studies have shown that TCR stimulation alone can lead to functional anergy and unresponsiveness. Costimulatory signals augment T and NK cell function by enhancing cell metabolism, cytokine production, differentiation, and long-term persistence. Costimulation is an important factor for cell proliferation, differentiation

and survival. In some embodiments, costimulatory molecules include, but are not limited to, CD45, CD2, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD28, CD37, CD64, CD80, CD86, CD134, CD137, and CD154. In some embodiments, the costimulatory molecule includes, but is not limited to, binding agents, such as scFvs, antibodies, single-domain antibodies, antibody fragments, nanobodies that bind to any of the costimulatory molecules described herein. In some embodiments, these binding agents may include anti-CD28, anti-CD2, anti-CD45, anti-CD4, anti-CD5, anti-CD8, anti-CD9, anti-CD16, anti-CD22, anti-CD33, anti-CD37, anti-CD64, anti-CD80, anti-CD86, anti-CD137, anti-CD154, anti-CD28H, anti-LFA-1, anti-OX40, anti-4-1BB, anti-CD40L, anti-DNAM-1, anti-CD27, anti-ICOS, anti-LIGHT, anti-GITR, anti-CD30, anti-SLAM, anti-Ly-9, anti-CD84, anti-Ly108, anti-NKG2D, anti-NKp46, anti-NKp44, anti-NKp30, anti-CD244, anti-NKp80, anti-TCRα chain, anti-TCRβ chain, anti-TCRγ chain, and anti-TCRδ chain agents.

[0352] In some embodiments, the co-stimulation molecule is a ligand for CD28. CD28 is one of the proteins expressed on T cells that provide co-stimulatory signals required for T cell activation and survival. T cell stimulation through CD28 in addition to the T-cell receptor (TCR) can provide a potent signal for the production of various interleukins (IL-6 in particular). In some embodiments, the co-stimulation molecule is an antibody, or fragment thereof, that binds to CD28. Examples of such antibodies include: 15E8 and TGN1412. Other suitable antibodies include: CD28.2 and 10F3.

[0353] In some embodiments, the co-stimulation molecule is CD86. CD86, also known as B7-2, is a ligand for CD28. In some embodiments, the ligand for CD28 is CD86. In some embodiments, the co-stimulation molecule is CD80. CD80 is an additional ligand for CD28. In some embodiments, the ligand for CD28 is CD80. In some embodiments, the ligand for CD28 is an anti-CD28 antibody or an anti-CD28 scFv. In some embodiments, the anti-CD28 antibody or an anti-CD28 scFv is coupled to a transmembrane domain for display on the surface of the viral envelope.

[0354] In some embodiments, the co-stimulation molecule is a CD86 polypeptide comprising the amino acid sequence of SEQ ID NO: 76. In some embodiments, the co-stimulation molecule is a CD86 polypeptide comprising an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 76.

[0355] In some embodiments, the co-stimulation molecule is a CD80 polypeptide comprising the amino acid sequence of SEQ ID NO: 77. In some embodiments, the co-stimulation molecule is

a CD80 polypeptide comprising an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 77.

[0356] In some embodiments, the CD86 polypeptide is encoded by the nucleotide sequence of SEQ ID NO: 78. In some embodiments, the CD86 polypeptide is encoded by a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 78.

[0357] In some embodiments, the CD80 polypeptide is encoded by the nucleotide sequence of SEQ ID NO: 79. In some embodiments, the CD80 polypeptide is encoded by a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 79.

[0358] CD134, also known as OX40, is a member of the TNFR-superfamily of receptors which is expressed on activated T cells. OX40 may promote cell division and survival. OX40 is a secondary costimulatory molecule, expressed after 24 to 72 hours following activation; its ligand, OX40L, is also not expressed on resting antigen presenting cells, but is following their activation. In some embodiments, the viral particle comprises a ligand for OX40, or functional fragment thereof, coupled to its native transmembrane domain or a heterologous transmembrane domain.

[0359] CD134, also known as OX40, is a member of the TNFR-superfamily of receptors which is expressed on activated T cells. OX40 may promote cell division and survival. 0X40 is a secondary costimulatory molecule, expressed after 24 to 72 hours following activation; its ligand, OX40L, is also not expressed on resting antigen presenting cells, but is following their activation. In some embodiments, the viral particle comprises a ligand for OX40, or functional fragment thereof, coupled to its native transmembrane domain or a heterologous transmembrane domain.

[0360] CD137, also known as 4-1BB, is a member of the tumor necrosis factor (TNF) receptor family. CD137 is expressed on activated T cells. In addition, CD137 expression is found on dendritic cells, follicular dendritic cells, natural killer cells, granulocytes and cells of blood vessel walls at sites of inflammation. The best characterized activity of CD137 is its costimulatory activity for activated T cells. Crosslinking of CD137 enhances T cell proliferation, IL-2 secretion survival and cytolytic activity. In some embodiments, the viral particle comprises a ligand for 4-1BB, or functional fragment thereof, coupled to its native transmembrane domain or a heterologous transmembrane domain. 4-1BBL is a cytokine that belongs to the tumor necrosis factor (TNF)

ligand family. This transmembrane cytokine is a bidirectional signal transducer that acts as a ligand for 4-1BB, which is a costimulatory receptor molecule in T lymphocytes. 4-1BBL has been shown to reactivate anergic T lymphocytes in addition to promoting T lymphocyte proliferation.

[0361] Viral particles comprising one or more activation or co-stimulation molecule(s) may be made by engineering the packaging cell line by methods provided by WO 2016/139463; or by expression of the T-cell activation or co-stimulation molecule(s) from a polycistronic helper vector as described in Int'l Pat. Pub. No. WO 2020/106992 A1, both of which are incorporated herein by reference in their entireties.

3. Adhesion Molecules

[0362] In some embodiments, the viral particle comprises an adhesion molecule. As used herein, the term " adhesion molecule" refers to a subset of cell surface molecules involved in the binding of cells with other cells. Adhesion cells may help to form more stable interactions, such as an immunological synapse, between immune cells. The immunological synapse is a stable adhesive junction between a polarized immune effector cell and an antigen-bearing cell. In some embodiments, the adhesion molecule may provide a costimulatory signal to the target cell. In some embodiments, adhesion molecules include, but are not limited to, CD58, HHLA2, ICAM-1, OX40L, 4-1BBL, CD40, CD155, CD70, HVEM, GITRL, ICOSL, CD30L, SLAM, Ly-9, CD84, Ly108, MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, and B7-H6. In some embodiments, the adhesion molecule includes, but is not limited to, binding agents, such as scFvs, antibodies, single-domain antibodies, antibody fragments, and nanobodies that bind to any of the adhesion or costimulatory molecules described herein. In some embodiments, these binding agents may include anti-CD28, anti-CD2, anti-CD28H, anti-LFA-1, anti-OX40, anti-4-1BB, anti-CD40L, anti-DNAM-1, anti-CD27, anti-ICOS, anti-LIGHT, anti-GITR, anti-CD30, anti-SLAM, anti-Ly-9, anti-CD84, anti-Ly108, anti-NKG2D, anti-NKp46, anti-NKp44, anti-NKp30, anti-CD244, anti-NKp80, anti-TCRα chain, anti-TCRβ chain, anti-TCRγ chain, and anti-TCRδ chain agents.

[0363] In some embodiments, the adhesion molecule binds to CD2. CD2 is also known as T11, LFA-2, and the erythrocyte rosette receptor and is a surface protein expressed on T lymphocytes and NK cells. CD2 is a natural ligand for CD58. In addition to performing adhesion functions, engagement of CD2 provides a costimulatory signal that may enhance activation and effector

functions. In some embodiments, the lentiviral particle comprises a molecule that binds to CD2. In some embodiments, the lentiviral particle comprises an antibody, single domain antibody, antibody fragment, and/or nanobody specific for CD2. In some embodiments, the lentiviral particle comprises CD58, or a functional portion thereof that binds to CD2.

[0364] In some embodiments, the adhesion molecule is CD58. In some embodiments, the co-stimulation molecule is a CD58 polypeptide comprising the amino acid sequence of SEQ ID NO: 80. In some embodiments, the co-stimulation molecule is a CD58 polypeptide comprising an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 80.

[0365] In some embodiments, the CD58 polypeptide is encoded by the nucleotide sequence of SEQ ID NO: 81. In some embodiments, the CD58 polypeptide is encoded by a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 81.

4. Additional Non-Viral Proteins

[0366] In some embodiments, the viral particle comprises at least one non-viral protein. In some embodiments, the viral particle comprises at least one non-viral protein in addition to those described supra.

[0367] In some embodiments, the viral particle comprises a targeting ligand. In some embodiments, the viral particle comprises CD19, or a functional fragment thereof, coupled to its native transmembrane domain or a heterologous transmembrane domain. In some embodiments, CD19 acts as a ligand for blinatumomab, thus providing an adapter for coupling the particle to T-cells via the anti-CD3 moiety of blinatumomab. In some embodiments, another type of particle surface ligand can serve to couple an appropriately surface engineered lentiviral particle to a T-cell using a multispecific antibody comprising a binding moiety for the particle surface ligand. In some embodiments, the multispecific antibody is a bispecific antibody, for example, a Bispecific T-cell engager (BiTE).

[0368] In some embodiments, the non-viral protein is a cytokine. In some embodiments, the cytokine may be selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, and any combination thereof. Where the non-viral protein used is a soluble protein (such as an scFv or a

cytokine) it may be tethered to the surface of the viral particle by fusion to a transmembrane domain, such as the transmembrane domain of CD8. Alternatively, it may be indirectly tethered to the lentiviral particle by use of a transmembrane protein engineered to bind the soluble protein. Further inclusion of one or more cytoplasmic residues may increase the stability of the fusion protein.

[0369] The mitogenic transduction enhancer and/or cytokine- based transduction enhancer may comprise a "spacer sequence" to connect the antigen-binding domain with the transmembrane domain. A flexible spacer allows the antigen-binding domain to orient in different directions to facilitate binding. As used herein, the term "coupled to" refers to a chemical linkage, a direct C-terminal to N-terminal fusion of two protein; chemical linkage to a non-peptide space; chemical linkage to a polypeptide space; and C-terminal to N-terminal fusion of two protein via peptide bonds to a polypeptide spacer, e.g., a spacer sequence.

[0370] The spacer sequence may, for example, comprise an lgG1 Fc region, an lgG1 hinge or a human CD8 stalk or the mouse CD8 stalk. The spacer may alternatively comprise an alternative linker sequence which has similar length and/or domain spacing properties as an lgG1 Fc region, an lgG1 hinge or a CD8 stalk. A human lgG1 spacer may be altered to remove Fc binding motifs. In some embodiments, the spacer sequence may be derived from a human protein.

[0371] In some embodiments, the spacer sequence comprises a CD8 derived hinge.

[0372] In some embodiments, the spacer sequence comprises a 'short' hinge. The short hinge is described as hinge region comprising fewer nucleotides relative to CAR hinge regions known in the art.

[0373] The transmembrane domain is the sequence of the mitogenic transduction enhancer and/or cytokine-based transduction enhancer that spans the membrane. The transmembrane domain may comprise a hydrophobic alpha helix. The transmembrane domain may be derived from CD28. In some embodiments, the transmembrane domain is derived from a human protein.

[0374] The viral particle of the present invention may comprise a cytokine-based transduction enhancer in the viral envelope. In some embodiments, the cytokine-based transduction enhancer is derived from the host cell during viral particle production. In some embodiments, the cytokine-based transduction enhancer is made by the host cell and expressed at the cell surface. When the

nascent viral particle buds from the host cell membrane, the cytokine-based transduction enhancer may be incorporated in the viral envelope as part of the packaging cell-derived lipid bilayer.

[0375] The cytokine-based transduction enhancer may comprise a cytokine domain and a transmembrane domain. It may have the structure C-S-TM, where C is the cytokine domain, S is an optional spacer domain (e.g., a spacer sequence) and TM is the transmembrane domain. The spacer domain and transmembrane domains are as defined above.

[0376] The cytokine domain may comprise a T-cell activating cytokine, such as from IL2, IL7 and IL15, or a functional fragment thereof. As used herein, a "functional fragment" of a cytokine is a fragment of a polypeptide that retains the capacity to bind its particular receptor and activate T-cells.

[0377] IL2 is one of the factors secreted by T cells to regulate the growth and differentiation of T cells and certain B cells. IL2 is a lymphokine that induces the proliferation of responsive T cells. It is secreted as a single glycosylated polypeptide, and cleavage of a signal sequence is required for its activity. Solution NMR suggests that the structure of IL2 comprises a bundle of 4 helices (termed A-D), flanked by 2 shorter helices and several poorly defined loops. Residues in helix A, and in the loop region between helices A and B, are important for receptor binding.

IV. METHODS AND USES THEREOF

[0378] In some embodiments, provided herein are methods of using the polycistronic constructs or nucleotide vectors disclosed herein. In some embodiments, the provided methods deliver the polycistronic constructs to cells for expression of the provided systems including a cytosolic FRB, synthetic cytokine receptor and CAR. In some embodiments, the polycistronic constructs are contained within a viral vector and the viral vector is used to transduce target cells. In some embodiments, the provided methods are carried out ex vivo or in vitro to engineer target cells with the polycistronic construct. In some embodiments, the engineered cells are administered to a subject. In some embodiments, the provided methods are carried out in vivo and a viral vector containing the polycistronic construct is introduced to a subject for in vivo targeted delivery of the polycistronic vector to a target cell, such as a T cell.

A. Methods of Transducing a Cell

[0379] In some embodiments, provided herein is a method of transducing a cell comprising contacting a target cell with a particle, such as a viral vector, containing any of the polycistronic constructs provided herein. In some embodiments, the target cell comprises a stem cell. In some embodiments, the stem cell comprises an induced pluripotent stem cell (iPSC). In some embodiments, the target cell comprises a progenitor cell. In some embodiments, the progenitor cell comprises a peripheral blood mononuclear cell (PBMC). In some embodiments, the target cell comprises a T cell. In some embodiments, the T cell comprises a CD4+ or CD8+ T cell. In some embodiments, the method further comprises contacting the target cell with a (i) a guide RNA (gRNA) targeting a target site in an endogenous gene, and (ii) an RNA-guided endonuclease, thereby inserting the nucleotide sequence into the endogenous gene.

[0380] In some aspects, the polynucleotides described herein may be delivered to cells *in vivo*. In some embodiments, a polynucleotide encoding the elements of the polycistronic construct disclosed herein is administered to a subject directly via administration of a particle comprising the polynucleotide. In some embodiments, the particle is a viral particle. In some embodiments, the viral particle comprises an anti-CD3 scFv and a cocal glycoprotein and is capable of delivering the polynucleotide to cells *in vivo*. In some embodiments, the polynucleotide encodes a cytosolic FRB, a synthetic cytokine receptor, and a chimeric antigen receptor (CAR). The polynucleotides disclosed herein may be administered to the subject which allows the production of the various construct components (e.g. FRB, synthetic cytokine receptor, and CAR) *in vivo*. In some embodiments, the administration of such polynucleotide generates similar effect *in vivo* as direct administration of *ex vivo* engineered cells expressing the FRB, synthetic cytokine receptor, and CAR. In some embodiments, the administration of such polynucleotide improves the *in vivo* transduction efficiency of a particle. In some embodiments, the polynucleotide is an mRNA.

[0381] The polynucleotides described herein may also be delivered to cells *ex vivo*. Viral particles described herein may be used *ex vivo*, either in convention cell manufacturing processes or in an extracorporeal or bedside process as described, e.g., in Int'l Pat. Pub. No. WO 2022/072885 A1. In one embodiment, the disclosure provides an *ex vivo* method of transducing target cells, comprising contacting the target cells with the particle according to the present disclosure. In some embodiments, the particles described herein may be used to transduce cells that

have not been previously activated. For example, the particles described herein may be useful for transducing cells that have not been previously contacted with cell activation beads or activation reagents (e.g. Dynabeads or other reagents comprising anti-CD3 and/or anti-CD28 antibodies or binding fragments thereof).

[0382] In some embodiments, the disclosure provides a method of delivering a nucleic acid to a cell in an ex vivo CAR T manufacturing process. Such methods typically involve the isolation of PBMCs from a patient via leukapheresis. These cells are washed and optionally further purified via one or more selection steps to isolate particular T cell populations of interest. In some aspects, these might include CD4+ and/or CD8+ T cells. The washed and/or purified cells may be optionally activated and then transduced using a lentiviral vector. The activation step may comprise contacting the cells with an exogenous activation agent such as anti-CD3 and anti-CD28 antibodies bound to a substrate or using unbound antibodies. Illustrative activation agents include anti-CD3 and anti-CD28-presenting beads and/or soluble polymers. After transduction, the cells may be optionally further washed and cultured until harvest. Methods of manufacturing engineered cell therapies, including CAR T cells, are known in the art (*see e.g.,* Abou-el-Enein, M. *et al.* Blood Cancer Discov (2021), Vol 2(5): 408-422; Arcangeli, S. *et al.* Front. Immunol (19 Jun 2020), Vol. 11 (1217) 1-13; Ghassemi, S. *et al.* Nat Biomed Eng (Feb 2022), Vol 6(2): 118-128; Vormittag, P. *et al.* Curr Opin Biotechnol (Oct 2018), Vol. 54: 164-181; each of which is herein incorporated by reference). Illustrative methods of autologous CAR T manufacturing are disclosed in US Patent Publication Nos. 2019/0269727, 2016/0122782, 2021/0163893, and US 2017/0037369, each of which is incorporated herein in its entirety.

[0383] In some embodiments, the disclosure provides a method of delivering a nucleic acid to a cell in an ex-vivo closed-loop manufacturing process. In some embodiments, an ex-vivo manufacturing process is an extracorporeal process. In exemplary embodiments, the lentiviral vectors disclosed herein permit delivery of a nucleic acid to a target cell during a closed-loop process. Exemplary methods of closed-loop and/or extracorporeal processes are disclosed in US Patent Publication No. 2021/0244871 and WO2022072885, both of which are incorporated herein in their entirety. In some embodiments, the lentiviral vectors as disclosed herein may be used to transduce cells ex vivo. For example, in exemplary closed-loop manufacturing processes, cells are obtained from a subject, washed, incubated and/or contacted with lentiviral particles, optionally

washed again, and infused into the subject in a closed-loop system. In such embodiments, the lentiviral particles as disclosed herein are useful even without prior activation of the cells and are capable of binding to the cells in a short incubation and/or contacting step. In some embodiments, the incubation and/or contacting step is approximately or less than one hour. In some embodiments, the incubation and/or contacting step is approximately or less than two hours, approximately or less than three hours, approximately or less than four hours, or approximately or less than five hours. In some embodiments, the incubation and/or contacting step is less than 12 hours or less than 24 hours. In some embodiments, a nucleic acid is delivered to a cell by transduction with a lentiviral vector such that the nucleic acid enters the cell ex-vivo. In some embodiments, a nucleic acid is delivered to a cell by contacting the lentiviral vector to the surface of the cell. In such embodiments, the nucleic acid may enter the cell ex-vivo or in vivo after the cells (complexed with the lentiviral vector) are infused back into the subject.

[0384] In some embodiments, the lentiviral vectors as disclosed herein eliminate the need for an ex-vivo activation step. In such embodiments, the isolated cells could be transduced directly after leukapheresis, washing, or selection. It is contemplated that the surface engineering described herein enables the lentiviral particles disclosed herein to activate and transduce cells in a single step. In such embodiments, the lentiviral particles disclosed herein may enable a short or truncated manufacturing process, reducing the time spent in ex-vivo manufacturing by eliminating one or more unit operations (e.g. activation prior to transduction) and/or reducing the amount of time necessary in post-transduction cell culture.

B. Methods of Expressing a Receptor

[0385] In some embodiments, provided herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell. In some embodiments, the methods include contacting a target cell with a particle, such as a viral vector, containing any of the polycistronic constructs provided herein. In some embodiments, the contacting is carried out ex vivo or in vitro. In some embodiments, the contacting is carried out in vivo in a subject by administering the polynucleotide constructs or particles, such as viral vectors, containing the same to a subject.

[0386] In some embodiments, the target cell comprises a stem cell.

[0387] In some embodiments, the stem cell comprises an induced pluripotent stem cell (iPSC).

[0388] In some embodiments, the target cell comprises a progenitor cell.

[0389] In some embodiments, the progenitor cell comprises a peripheral blood mononuclear cell (PBMC).

[0390] In some embodiments, the target cell comprises a T cell.

[0391] In some embodiments, the T cell comprises a CD4+ or CD8+ T cell.

[0392] In some embodiments, the method is performed ex vivo or in vitro.

[0393] In some embodiments, the method is performed in vivo.

[0394] In some embodiments, provided herein is a cell produced by any of the methods provided herein.

C. Methods of Administering

[0395] Also provided herein are methods of administering any of the provided cells engineered with a provided polycistronic construct to a subject. Also provided herein are methods of administering to a subject any of the provided particles, such as viral vectors, for example a lentiviral vector. In some embodiments, the subject has a disease or condition and the methods of administering are for treating a disease or condition. In any of subject methods, the cells or particles are administered as pharmaceutical compositions. In some embodiments, the compositions are for use in treating a disease or condition. Also provided are uses of provided compositions for treating a disease or condition in a subject. Such methods and uses include therapeutic methods and uses, for example, involving administration of the engineered cells or particles (e.g. viral vectors), or compositions containing the same, to a subject having a disease of condition. In some cases, the disease or condition is a tumor or cancer. In some embodiments, the cells or pharmaceutical composition thereof is administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of the cells or pharmaceutical compositions thereof in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject.

[0396] In some embodiments, engineered cells that contain any of the provided polycistronic constructs that encode a cytosolic FRB, a synthetic cytokine receptor, and a chimeric antigen

receptor (CAR) may be administered to a subject to treat a disease or condition. In some embodiments, particles, such as viral vectors (e.g. lentiviral vectors) may be directly administered to a subject for in vivo targeted delivery of the polycistronic construct to target cells for the production of the various construct components (e.g. FRB, synthetic cytokine receptor, and CAR) *in vivo*. The disclosed cells or particles (e.g. viral vectors) may be administered in a number of ways depending upon whether local or systemic treatment is desired.

[0397] In the case of adoptive cell therapy, methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions.

[0398] In general, administration may be topical, parenteral, or enteral. The compositions of the disclosure are typically suitable for parenteral administration. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue- penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrastemal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, intratumoral, intrasynovial injection or infusions; and kidney dialytic infusion techniques. In an embodiment, parenteral administration of the compositions of the present disclosure comprises intravenous administration. In some embodiments, the viral particle is administered by intraperitoneal injection of the viral particle. In some embodiments, the viral particle is administered by intra-nodal injection - that is, the viral particle may be administered via injection into a lymph node, such as an inguinal lymph node. In some embodiments, the viral particle is administered by injection of the viral particle into tumor sites (i.e. intratumoral). In some embodiments, the viral particle is administered subcutaneously. In some embodiments, the viral particle is administered systemically. In some embodiments, the viral particle is administered intravenously. In some embodiments, the viral particle is administered intraarterially. In some embodiments, the viral particle is a lentiviral particle.

[0399] Formulations of a pharmaceutical composition suitable for parenteral administration typically generally comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and the like. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. Parenteral formulations also include aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. Illustrative parenteral administration forms include solutions or suspensions in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, or in a liposomal preparation. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In some embodiments, the solution intended for subcutaneous administration includes hyaluronidase.

[0400] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for

example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0401] The present polycistronic constructs may be administered in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0402] In certain embodiments, in the context of infusing differentiated cells or transgenic differentiated cells according to the disclosure, a subject is administered the range of about one million to about 100 billion cells, such as, e.g., 1 million to about 50 billion cells (e.g., about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (e.g., about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (e.g., about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges, and/or such a number of

cells per kilogram of body weight of the subject. For example, in some embodiments the administration of the cells or population of cells can comprise administration of about 10^3 to about 10^9 cells per kg body weight including all integer values of cell numbers within those ranges.

[0403] In some embodiments, provided herein is a method of administering to a subject any of the cells provided herein.

[0404] In some embodiments, provided herein is a method of administering to a subject any of the viral vectors provided herein.

[0405] In some embodiments, the CAR encoded by the provided polycistronic construct is targeted to an antigen associated with a disease or condition and the methods include administering the cells (e.g. by adoptive cell therapy) or viral vector, such as lentiviral vectors, to a subject that has or is suspected of having the disease or condition. In some embodiments, among provided methods are methods for treating a subject suffering from cancer, including the step of administering a provided cell engineered with any of the provided polycistronic constructs of the disclosure to the subject, wherein the cancer is treated in the subject. In some embodiments, among provided methods are methods for treating a subject suffering from cancer, including the step of administering a provide viral vector, such as lentiviral particle, incorporating any of the provided polycistronic constructs of the disclosure to the subject, wherein the cancer is treated in the subject.

[0406] In some embodiments, the cancer is a solid tumor, such as a melanoma, non-small cell lung cancer, or breast cancer. The methods of the present disclosure may include treating any cancer, including, without limitation, acute granulocytic leukemia, acute lymphocytic leukemia, acute myelogenous leukemia, adenocarcinoma, adenosarcoma, adrenal cancer, adrenocortical carcinoma, anal cancer, anaplastic astrocytoma, angiosarcoma, appendix cancer, astrocytoma, basal cell carcinoma, B-cell lymphoma, bile duct cancer, bladder cancer, bone cancer, bone marrow cancer, bowel cancer, brain cancer, brain stem glioma, brain tumor, breast cancer, carcinoid tumors, cervical cancer, cholangiocarcinoma, chondrosarcoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, colon cancer, colorectal cancer, craniopharyngioma, cutaneous lymphoma, cutaneous melanoma, diffuse astrocytoma, ductal carcinoma in situ, endometrial cancer, ependymoma, epithelioid sarcoma, esophageal cancer, Ewing sarcoma, extrahepatic bile duct cancer, eye cancer, fallopian tube cancer, fibrosarcoma, gallbladder cancer, gastric cancer, gastrointestinal cancer, gastrointestinal carcinoid cancer, gastrointestinal stromal tumors, general,

germ cell tumor, gestational trophoblastic disease, glioblastoma multiforme, glioma, hairy cell leukemia, head and neck cancer, hemangioendothelioma, Hodgkin lymphoma, Hodgkin lymphoma, Hodgkin's disease, hypopharyngeal cancer, infiltrating ductal carcinoma, infiltrating lobular carcinoma, inflammatory breast cancer, intestinal cancer, intrahepatic bile duct cancer, invasive/infiltrating breast cancer, islet cell cancer, jaw cancer, kaposi sarcoma, kidney cancer, laryngeal cancer, leiomyosarcoma, leptomeningeal metastases, leukemia, lip cancer, liposarcoma, liver cancer, lobular carcinoma in situ, low-grade astrocytoma, lung cancer, lymph node cancer, lymphoma, male breast cancer, medullary carcinoma, medulloblastoma, melanoma, meningioma, Merkel cell carcinoma, mesenchymal chondrosarcoma, mesenchymal mesothelioma, metastatic breast cancer, metastatic melanoma, metastatic squamous neck cancer, mixed gliomas, mouth cancer, mucinous carcinoma, mucosal melanoma, multiple myeloma, mycosis fungoides, myelodysplastic syndrome, nasal cavity cancer, nasopharyngeal cancer, neck cancer, neuroblastoma, neuroendocrine tumors, non-Hodgkin lymphoma, non-small cell lung cancer, oat cell cancer, ocular cancer, ocular melanoma, oligodendroglioma, oral cancer, oral cavity cancer, oropharyngeal cancer, osteogenic sarcoma, osteosarcoma, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian primary peritoneal carcinoma, ovarian sex cord stromal tumor, Paget's disease, pancreatic cancer, papillary carcinoma, paranasal sinus cancer, parathyroid cancer, pelvic cancer, penile cancer, peripheral nerve cancer, peritoneal cancer, pharyngeal cancer, pheochromocytoma, pilocytic astrocytoma, pineal region tumor, pineoblastoma, pituitary tumors, primary central nervous system, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma, bone sarcoma, soft-tissue sarcoma, uterine, sinus cancer, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cancer, spinal column cancer, spinal cord cancer, spinal tumor, squamous cell carcinoma, stomach cancer, synovial sarcoma, T-cell lymphoma, testicular cancer, throat cancer, thymoma/thymic carcinoma, thyroid cancer, tongue cancer, tonsil cancer, transitional cell cancer, triple-negative breast cancer, tubal cancer, tubular carcinoma, undiagnosed cancer, ureteral cancer, uterine adenocarcinoma, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer.

[0407] In some embodiments, the CAR encoded by the provided polycistronic construct is a CAR that targets a ligand that is able to bind to an antigen on the surface of a cell associated with a disease or condition. In some embodiments, the CAR of any of the provided embodiments is an

anti-FITC CAR directed against FITC and the ligand is a bifunctional ligand composed of FITC and a binding molecule that is able to bind to a surface molecule or receptor on the target cell. In some embodiments, the method further includes administering the bifunctional ligand to tag a cancer cell in the subject, wherein the bifunctional ligand specifically binds a molecule expressed on a tumor. In some embodiments, the bifunctional ligand is FITC-folate. In some embodiments, the cancer is an osteosarcoma.

[0408] In some embodiments, the method further comprises administering a bifunctional ligand to tag a cancer cell in the subject, wherein the bifunctional ligand specifically binds a molecule expressed on a tumor. In some embodiments, the bifunctional ligand comprises FITC-folate.

[0409] In some of any embodiments, the method further including administering a non-physiological ligand to the subject. In some embodiments, the non-physiological ligand is able to bind to the synthetic cytokine receptor and induce gamma cytokine signaling in the cell. In some embodiments, the nonphysiological ligand includes rapamycin or a rapamycin analog.

1. Non-physiological ligand

[0410] In various embodiments of the compositions and methods of the disclosure, the system comprises a non-physiological ligand. Illustrative small molecules useful as ligands include, without limitation: rapamycin, fluorescein, fluorescein isothiocyanate (FITC), 4-[(6-methylpyrazin-2-yl) oxy] benzoic acid (aMPOB), folate, rhodamine, acetazolamide, and a CA9 ligand.

[0411] In some embodiments, the synthetic cytokine receptor is activated by a ligand. In some embodiments, the ligand is a non-physiological ligand.

[0412] In some embodiments, the non-physiological ligand is a rapalog.

[0413] In some embodiments, the non-physiological ligand is rapamycin.

[0414] In some embodiments, the non-physiological ligand is AP21967.

[0415] In some embodiments, the non-physiological ligand is FK506.

[0416] In some embodiments, the non-physiological ligand is FK1012. In some embodiments, the non-physiological ligand is AP1510. In some embodiments, the non-physiological ligand is AP1903. In some embodiments, the non-physiological ligand is AP20187. In some embodiments, the non-physiological ligand is cyclosporin-A (CsA). In some embodiments, the non-physiological ligand is coumermycin.

[0417] In some embodiments, the synthetic cytokine receptor complex activated by folate, fluorescein, aMPOB, acetazolamide, a CA9 ligand, tacrolimus, rapamycin, a rapalog (a rapamycin analog), CD28 ligand, poly(his) tag, Strep-tag, FLAG-tag, VS-tag, Myc-tag, HA-tag, NE-tag, biotin, digoxigenin, dinitrophenol, or a derivative thereof.

[0418] In some embodiments, the non-physiological ligand may be an inorganic or organic compound that is less than 1000 Daltons.

[0419] In some embodiments, the ligand may be rapamycin or a rapamycin analog (rapalog). In some embodiments, the rapalog comprises variants of rapamycin having one or more of the following modifications relative to rapamycin: demethylation, elimination or replacement of the methoxy at C7, C42 and/or C29; elimination, derivatization or replacement of the hydroxy at C13, C43 and/or C28; reduction, elimination or derivatization of the ketone at C14, C24 and/or C30; replacement of the 6-membered pipecolate ring with a 5-membered prolyl ring; and alternative substitution on the cyclohexyl ring or replacement of the cyclohexyl ring with a substituted cyclopentyl ring.

[0420] Thus, in some embodiments, the rapalog is everolimus, novolimus, pimecrolimus, ridaforolimus, tacrolimus, temsirolimus, umirolimus, zotarolimus, Temsirolimus (CCI-779), C20-methallylrapamycin, C16-(S)-3-methylindolerapamycin, C16-(S)-3-methylindolerapamycin (C16-iRap), AP21967 (A/C Heterodimerizer, Takara Bio®), sodium mycophenolic acid, benidipine hydrochloride, rapamine, AP23573 (Ridaforolimus), AP1903 (Rimiducid), or metabolites, derivatives, and/or combinations thereof.

[0421] In some embodiments, the ligand comprises FK1012 (a semisynthetic dimer of FK506), tacrolimus (FK506), FKCsA (a composite of FK506 and cyclosporine), rapamycin, coumermycin, gibberellin, HaXS dimerizer (chemical dimerizers of HaloTag and SNAP-tag), TMP-HTag (trimethoprim haloenzyme protein dimerizer), or ABT-737 or functional derivatives thereof.

[0422] In some embodiments, the non-physiological ligand is present or provided in an amount from 0 nM to 1000 nM such as, *e.g.*, 0.05 nM, 0.1 nM, 0.5. nM, 1.0 nM, 5.0 nM, 10.0 nM, 15.0 nM, 20.0 nM, 25.0 nM, 30.0 nM, 35.0 nM, 40.0 nM, 45.0 nM, 50.0 nM, 55.0 nM, 60.0 nM, 65.0 nM, 70.0 nM, 75.0 nM, 80.0 nM, 90.0 nM, 95.0 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, or 1000 nM, or an amount that is within a range defined by any two of the aforementioned amounts.

[0423] In some embodiments, the non-physiological ligand is AP21967 and is present or provided at 10 nM. In some embodiments, the non-physiological ligand is AP21967 and is present or provided at 20 nM. In some embodiments, the non-physiological ligand is AP21967 and is present or provided at 50 nM. In some embodiments, the non-physiological ligand is AP21967 and is present or provided at 100 nM.

[0424] In some embodiments, the non-physiological ligand is rapamycin and is present or provided at 1 nM. In some embodiments, the non-physiological ligand is rapamycin and is present or provided at 10 nM. In some embodiments, the non-physiological ligand is rapamycin and is present or provided at 20 nM. In some embodiments, the non-physiological ligand is rapamycin and is present or provided at 50 nM.

[0425] In some embodiments, the non-physiological ligand is a rapalog and is present or provided at 1 nM. In some embodiments, the non-physiological ligand is a rapalog and is present or provided at 10 nM. In some embodiments, the non-physiological ligand is a rapalog and is present or provided at 20 nM. In some embodiments, the non-physiological ligand is a rapalog and is present or provided at 50 nM. In some embodiments, the non-physiological ligand is a rapalog and is present or provided at 100 nM.

[0426] In some embodiments, the non-physiological ligand is present or provided at 1 nM.

[0427] In some embodiments, the non-physiological ligand is present or provided at 10 nM.

[0428] In some embodiments, the non-physiological ligand is present or provided at 100 nM.

[0429] In some embodiments, the non-physiological ligand is present or provided at 1000 nM.

V. DEFINITIONS

[0430] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0431] As used herein, the singular forms “a”, “an”, and “the” are include the plural forms as well, unless the context indicates otherwise. The conjugation “and/or” denotes all possible combinations of one or more of listed items.

[0432] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0433] It is understood that aspects and embodiments of the invention described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

[0434] As used herein, "optional" or "optionally" means that the subsequently described event or circumstance does or does not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, an optionally substituted group means that the group is unsubstituted or is substituted.

[0435] The term “composition” refers to any mixture of two or more products, substances, or compounds, including cells or antibodies. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof. The preparation is generally in such form as to permit the biological activity of the active ingredient (e.g. antibody) to be effective.

[0436] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0437] As used herein, combination refers to any association between or among two or more items. The combination can be two or more separate items, such as two compositions or two collections, can be a mixture thereof, such as a single mixture of the two or more items, or any variation thereof. The elements of a combination are generally functionally associated or related.

[0438] As used herein, a kit is a packaged combination that optionally includes other elements, such as additional agents and instructions for use of the combination or elements thereof, for a purpose including, but not limited to, therapeutic uses.

[0439] “Subject” as used herein refers to the recipient of polycistronic construct or other agent. The term includes mammal, such as primate, mouse, rat, dog, cat, cow, horse, goat, camel, sheep or a pig, preferably a human.

[0440] "Treat," "treating" or "treatment" as used herein refers to any type of action or administration that imparts a benefit to a subject that has a disease or disorder, including improvement in the condition of the patient (*i.e.*, improvement, reduction, or amelioration of one or more symptoms, and partial or complete response to treatment).

[0441] The term "effective amount" refers to an amount effective to generate a desired biochemical, cellular, or physiological response. The term "therapeutically effective amount" refer to the amount, dosage, or dosage regime of a therapy effective to cause a desire treatment effect. As used herein, an "individual" or a "subject" is a mammal. A "mammal" for purposes of treatment includes humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, *etc.* In some embodiments, the individual or subject is human.

[0442] "Polynucleotide" as used herein refers to a biopolymer composed of two or more nucleotide monomers covalently bonded through ester linkages between the phosphoryl group of one nucleotide and the hydroxyl group of the sugar component of the next nucleotide in a chain. DNA and RNA are non-limiting examples of polynucleotides.

[0443] "Polypeptide" as used herein refers to a polymer consisting of amino acid residues chained together by peptide bonds, forming part of (or the whole of) a protein.

[0444] It will be understood by a skilled person that numerous different polynucleotides and nucleic acids can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described herein to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed.

[0445] Nucleic acids may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3′ and/or 5′ ends of the molecule. For the purposes of the use as described herein, it is to be understood that the polynucleotides may be modified by any method available in

the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides of interest.

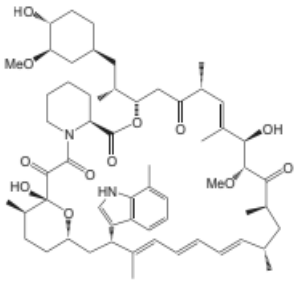
[0446] The term "variant" means a polynucleotide or polypeptide having at least one substitution, insertion, or deletion in its sequence compared to a reference polynucleotide or polypeptide. A "functional variant" is a variant that retains one or functions of the reference polynucleotide or polypeptide.

[0447] As used herein the term "sequence identity", or "identity" in relation to polynucleotides or polypeptide sequences, refers to the extent to which two optimally aligned polynucleotides or polypeptide sequences match at each position in the alignment across the full length of the reference sequence. The "percent identity" is the number of matched positions in the optimal alignment, divided by length of the reference sequence plus the sum of the lengths of any gaps in the reference sequence in the alignment. The optimal alignment is the alignment that results in the maximum percent identity. Alignment of sequences to determine percent identity can be accomplished by a number of well-known methods, including for example by using mathematical algorithms, such as, for example, those in the BLAST suite or Clustal Omega sequence analysis programs. Unless noted otherwise, the term "sequence identity" in the claims refers to sequence identity as calculated by BLAST version 2.12.0 using default parameters. And, unless noted otherwise, the alignment is an alignment of all or a portion of the polynucleotide or polypeptide sequences of interest across the full length of the reference sequence.

[0448] As used herein, "small molecule" refers to a low molecular weight (<1000 Daltons), organic compound. Small molecules may bind specific biological macromolecules and can have a variety of biological functions or applications including, but not limited to, serving as cell signaling molecules, drugs, secondary metabolites, or various other modes of action.

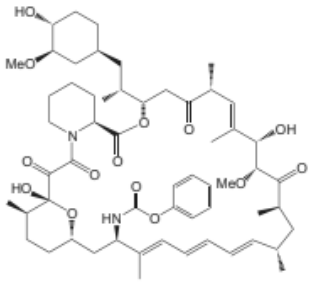
[0449] The term "analog" in relation to a small molecule refers to a compound having a structure and/or function similar to that of another compound but differing from it in respect to a certain component. The analog may differ in one or more atoms, functional groups, or substructures, which are replaced with other atoms, groups, or substructures. Despite a high structural and/or functional similarity, analogs can have different physical, chemical, physiochemical, biochemical, or pharmacological properties.

[0450] The term "rapalog" is an art-recognized group of analogs of rapamycin analog that share structural and functional similarity to rapamycin. Certain rapalogs are known to share some but not all functional attributes of rapamycin. For example, some rapalogs are suitable for uses as a non-physiological ligand because they promote dimerization but have substantially no immunosuppressive activity (*e.g.*, AP21967, AP23102, or iRAP).



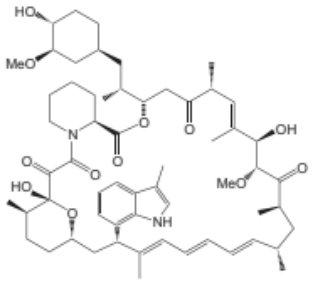
AP21967

[0451] An illustrative rapalog of the disclosure is AP21967 .



AP23102

[0452] An illustrative rapalog of the disclosure is AP23102 .



iRap

[0453] An illustrative rapalog of the disclosure is iRAP .

[0454] The term "cell population" refers to mixture of cells suspended in solution, attached to a substrate, or stored in a container. The characteristics of a cell population as a whole can be studied with bulk measurements of sample volumes having a plurality of cells. Flow cytometry methods

may be employed to reduce problems with background fluorescence which are encountered in bulk cell population measurements.

[0455] As used herein, the term "engineered" refers to a cell that has been stably transduced with a heterologous polynucleotide or subjected to gene editing to introduce, delete, or modify polynucleotides in the cell, or cells transiently transduced with a polynucleotide in a manner that causes a stable phenotypic change in the cell.

VI. EXEMPLARY EMBODIMENTS

[0456] Among the provided embodiments are:

1. A polycistronic construct comprising in 5' to 3' order (a) a first expression cassette comprising a nucleotide sequence encoding FRB, (b) a second expression cassette comprising a nucleotide sequence encoding a synthetic cytokine gamma chain polypeptide, (c) a third expression cassette comprising a nucleotide sequence encoding a synthetic cytokine beta chain polypeptide, and (d) a fourth expression cassette comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein each of the expression cassettes are separated by a nucleotide sequence encoding a cleavage site sequence.
2. The polycistronic construct of embodiment 1, wherein the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50.
3. The polycistronic construct of embodiment 1 or embodiment 2, wherein the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NOs:3, 13, or 50.
4. The polycistronic construct of any one of embodiments 1 to 3, wherein the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51.
5. The polycistronic construct of any one of embodiments 1 to 4, wherein the FRB comprises the amino acid sequence of SEQ ID NOs:4, 14, or 51.
6. The polycistronic construct of any one of embodiments 1 to 5, wherein the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:15.
7. The polycistronic construct of any one of embodiments 1 to 6, wherein the nucleotide encoding the synthetic cytokine gamma chain polypeptide comprises the nucleotide sequence of SEQ ID NO:15.
8. The polycistronic construct of any one of embodiments 1 to 7, wherein the synthetic cytokine gamma chain polypeptide comprises interleukin 2 receptor subunit γ (IL2RG).
9. The polycistronic construct of embodiment 8, wherein the IL2RG comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:16.
10. The polycistronic construct of embodiment 8 or embodiment 9, wherein the IL2RG comprises the amino acid sequence of SEQ ID NO:16.

11. The polycistronic construct of any one of embodiments 1 to 10, wherein the second expression cassette further comprises a nucleotide sequence encoding FRB.
12. The polycistronic construct of embodiment 11, wherein the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:13.
13. The polycistronic construct of embodiment 11 or embodiment 12, wherein the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NO:13.
14. The polycistronic construct of any one of embodiments 11 to 13, wherein the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14.
15. The polycistronic construct of any one of embodiments 1 to 14, wherein the FRB comprises the amino acid sequence of SEQ ID NO: 14.
16. The polycistronic construct of any one of embodiments 1 to 15, wherein the second expression cassette is codon optimized.
17. The polycistronic construct of any one of embodiments 1 to 16, wherein the second expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:11.
18. The polycistronic construct of any one of embodiments 1 to 17, wherein the second expression cassette comprises the nucleotide sequence of SEQ ID NO:11.
19. The polycistronic construct of any one of embodiments 1 to 18, wherein the second expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:12.
20. The polycistronic construct of any one of embodiments 1 to 19, wherein the second expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NO:12.
21. The polycistronic construct of any one of embodiments 1 to 15, wherein the second expression cassette further comprises a nucleotide sequence encoding FKBP12.
22. The polycistronic construct of embodiment 21, wherein the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:21 or 55.
23. The polycistronic construct of embodiment 21 or embodiment 22, wherein the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NOs:21 or 55.
24. The polycistronic construct of any one of embodiments 21 to 23, wherein the FKBP12 comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:22.
25. The polycistronic construct of any one of embodiments 21 to 24, wherein the FKBP12 comprises the amino acid sequence of SEQ ID NO: 22.
26. The polycistronic construct of any one of embodiments 1 to 25, wherein the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:23 or 61.
27. The polycistronic construct of any one of embodiments 1 to 26, wherein the nucleotide encoding the synthetic cytokine beta chain polypeptide comprises the nucleotide sequence of SEQ ID NOs:23 or 61.
28. The polycistronic construct of any one of embodiments 1 to 27, wherein the synthetic cytokine beta chain polypeptide comprises interleukin 2 receptor subunit β (IL2RB).

29. The polycistronic construct of embodiment 28, wherein the IL2RB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:24 or 62.
30. The polycistronic construct of embodiment 28 or embodiment 29, wherein the IL2RB comprises the amino acid sequence of SEQ ID NOs:24 or 62.
31. The polycistronic construct of any one of embodiments 1 to 30, wherein the third expression cassette further comprises a nucleotide sequence encoding FKBP12.
32. The polycistronic construct of embodiment 31, wherein the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 21.
33. The polycistronic construct of embodiment 31 or embodiment 32, wherein the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NO: 21.
34. The polycistronic construct of any one of embodiments 31 to 33, wherein the FKBP12 comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:20.
35. The polycistronic construct of any one of embodiments 31 to 34, wherein the FKBP12 comprises the amino acid sequence of SEQ ID NO: 20.
36. The polycistronic construct of any one of embodiments 1 to 35, wherein the third expression cassette is codon optimized.
37. The polycistronic construct of any one of embodiments 1 to 36, wherein the third expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:19.
38. The polycistronic construct of any one of embodiments 1 to 37, wherein the third expression cassette comprises the nucleotide sequence of SEQ ID NO:19.
39. The polycistronic construct of any one of embodiments 1 to 38, wherein the third expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:20.
40. The polycistronic construct of any one of embodiments 1 to 39, wherein the third expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NO:20.
41. The polycistronic construct of any one of embodiments 1 to 40, wherein the third expression cassette further comprises a nucleotide sequence encoding FRB.
42. The polycistronic construct of embodiment 41, wherein the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:13.
43. The polycistronic construct of embodiment 41 or embodiment 42, wherein the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NO:13.
44. The polycistronic construct of any one of embodiments 41 to 43, wherein the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14.
45. The polycistronic construct of any one of embodiments 1 to 44, wherein the FRB comprises the amino acid sequence of SEQ ID NO: 14.
46. The polycistronic construct of any one of embodiments 1 to 45, wherein the CAR comprises an scFv domain.
47. The polycistronic construct of embodiment 46, wherein the scFv domain comprises anti-fluorescein isothiocyanate (FITC) E2.

48. The polycistronic construct of embodiment 46 or embodiment 47, wherein the scFv domain comprises a light chain variable domain (VL), a linker, and a heavy chain variable domain (VH).
49. The polycistronic construct of embodiment 48, wherein the scFv VL comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:30 or 65.
50. The polycistronic construct of embodiment 48 or embodiment 49, wherein the scFv VL comprises the nucleotide sequence of SEQ ID NOs:30 or 65.
51. The polycistronic construct of any one of embodiments 48 to 50, wherein the scFv VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:31.
52. The polycistronic construct of any one of embodiments 48 to 51, wherein the scFv VL comprises the amino acid sequence of SEQ ID NO:31.
53. The polycistronic construct of any one of embodiments 48 to 52, wherein the scFv VH comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:34 or 67.
54. The polycistronic construct of any one of embodiments 48 to 53, wherein the scFv VH comprises the nucleotide sequence of SEQ ID NOs:34 or 67.
55. The polycistronic construct of any one of embodiments 48 to 54, wherein the scFv VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:35.
56. The polycistronic construct of any one of embodiments 48 to 55, wherein the scFv VH comprises the amino acid sequence of SEQ ID NO:35.
57. The polycistronic construct of any one of embodiments 48 to 56, wherein the scFv linker comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:32 or 66.
58. The polycistronic construct of any one of embodiments 48 to 57, wherein the scFv linker comprises the nucleotide sequence of SEQ ID NOs:32 or 66.
59. The polycistronic construct of any one of embodiments 48 to 58, wherein the scFv linker comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:33.
60. The polycistronic construct of any one of embodiments 48 to 59, wherein the scFv linker comprises the amino acid sequence of SEQ ID NO:33.
61. The polycistronic construct of any one of embodiments 46 to 60, wherein the scFv comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:28 or 64.
62. The polycistronic construct of any one of embodiments 46 to 61, wherein the scFv comprises the nucleotide sequence of SEQ ID NOs:28 or 64.
63. The polycistronic construct of any one of embodiments 46 to 62, wherein the scFv comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:29.
64. The polycistronic construct of any one of embodiments 46 to 63, wherein the scFv comprises the amino acid sequence of SEQ ID NO:29.
65. The polycistronic construct of any one of embodiments 1 to 64, wherein the CAR comprises a hinge domain.

66. The polycistronic construct of embodiment 65, wherein the hinge domain comprises a short hinge or a medium hinge domain.
67. The polycistronic construct of embodiment 65 or embodiment 66, wherein the hinge domain comprises a CD8 or an IgG.
68. The polycistronic construct of embodiment 67, wherein the CD8 hinge comprises CD8α hinge.
69. The polycistronic construct of embodiment 68, wherein the CD8α hinge comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID Nos:38 or 114.
70. The polycistronic construct of embodiment 68 or embodiment 69, wherein the CD8α hinge comprises the nucleotide sequence of SEQ ID Nos: 38 or 114.
71. The polycistronic construct of any of embodiments 68 to 70, wherein the CD8α hinge comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:39 or 115.
72. The polycistronic construct of any of embodiments 68 to 71, wherein the CD8α hinge comprises the amino acid sequence of SEQ ID NOs: 39 or 115.
73. The polycistronic construct of any one of embodiments 1 to 72, wherein the CAR comprises a transmembrane domain.
74. The polycistronic construct of embodiment 73, wherein the transmembrane domain comprises a CD8 or a CD28.
75. The polycistronic construct of embodiment 74, wherein the CD8 transmembrane domain comprises CD8α transmembrane domain.
76. The polycistronic construct of any one of embodiments 73 to 75, wherein the transmembrane domain comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:40.
77. The polycistronic construct of any one of embodiments 73 to 76, wherein the transmembrane domain comprises the nucleotide sequence of SEQ ID NO:40.
78. The polycistronic construct of any one of embodiments 73 to 77, wherein the transmembrane domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:41.
79. The polycistronic construct of any one of embodiments 73 to 78 wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO:41.
80. The polycistronic construct of any one of embodiments 1 to 79, wherein the CAR comprises an endodomain.
81. The polycistronic construct of embodiment 80, wherein the endodomain comprises a costimulatory molecule.
82. The polycistronic construct of embodiment 80 or embodiment 81, wherein the endodomain comprises 4-1BB, CD3ζ, and/or CD28.
83. The polycistronic construct of embodiment 82, wherein the 4-1BB endodomain comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:42 or 69.
84. The polycistronic construct of embodiment 82 or embodiment 83, wherein the 4-1BB endodomain comprises the nucleotide sequence of SEQ ID NOs: 42 or 69.

85. The polycistronic construct of any one of embodiments 82 to 84, wherein the 4-1BB endodomain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:43.
86. The polycistronic construct of any one of embodiments 82 to 85, wherein the 4-1BB endodomain comprises the amino acid sequence of SEQ ID NO:43.
87. The polycistronic construct of any one of embodiments 82 to 86, wherein the CD3ζ endodomain comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118.
88. The polycistronic construct of any one of embodiments 82 to 87, wherein the CD3ζ endodomain comprises the nucleotide sequence of SEQ ID NOs: 46, 70, 100 or 118.
89. The polycistronic construct of any one of embodiments 82 to 88, wherein the CD3ζ endodomain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:47.
90. The polycistronic construct of any one of embodiments 82 to 89, wherein the CD3ζ endodomain comprises the amino acid sequence of SEQ ID NO:47.
91. The polycistronic construct of any one of embodiments 1 to 90, wherein the fourth expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82.
92. The polycistronic construct of any one of embodiments 1 to 91, wherein the fourth expression cassette comprises the nucleotide sequence of SEQ ID NOs: 26, 63, 71, or 82.
93. The polycistronic construct of any one of embodiments 1 to 92, wherein the fourth expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 27, 72 or 127.
94. The polycistronic construct of any one of embodiments 1 to 93, wherein the fourth expression cassette encodes the amino acid sequence of SEQ ID NOs: 27, 72 or 127.
95. The polycistronic construct of embodiment 1, wherein each of the cleavage site sequences comprises a 2A cleavable linker sequence.
96. The polycistronic construct of embodiment 95, wherein each nucleotide encoding a 2A cleavable linker sequences is different.
97. The polycistronic construct of embodiment 95 or embodiment 96, wherein the 2A cleavable linker is independently a T2A, P2A, E2A or F2A cleavage site.
98. The polycistronic construct of embodiment 95 or embodiment 97, wherein the 2A cleavable linker is independently a P2A or a T2A.
99. The polycistronic construct of any one of embodiments 95 to 98, wherein at least one 2A cleavable linker is a P2A and the nucleotide sequence encoding the P2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 17, 25, 52, or 58.
100. The polycistronic construct of embodiment 99, wherein the nucleotide sequence encoding the P2A cleavable linker is set forth in SEQ ID NOs: 17, 25, 52, or 58.
101. The polycistronic construct of any one of embodiments 97 to 100 wherein the P2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:18.
102. The polycistronic construct of embodiment 101, wherein the P2A cleavable linker comprises the sequence set forth in SEQ ID NO:18.

103. The polycistronic construct of any one of embodiments 95 to 102, wherein at least one 2A cleavable linker is a T2A and the nucleotide sequence encoding the T2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:9.
104. The polycistronic construct of any one of embodiments 95 to 103, wherein the nucleotide sequence encoding the T2A cleavable linker is set forth in SEQ ID NO:9.
105. The polycistronic construct of any one of embodiments 97, 98, 103 and 104, wherein the T2A cleavable linker comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:10.
106. The polycistronic construct of any one of embodiments 95 to 105, wherein the T2A cleavable linker comprising the sequence set forth in SEQ ID NO:10.
107. The polycistronic construct of any one of embodiments 1 to 106, wherein at least one of the cleavage site sequences comprises a furin cleavage site sequence.
108. The polycistronic construct of embodiment 107, wherein the furin cleavage site sequence is located between the first expression cassette and the second expression cassette.
109. The polycistronic construct of embodiment 107 or embodiment 108, wherein the nucleotide sequence encoding the furin cleavage site sequence comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:7.
110. The polycistronic construct of any one of embodiments 107 to 109, wherein the nucleotide sequence encoding the furin cleavage site sequence comprises the sequence set forth in SEQ ID NO: 7.
111. The polycistronic construct of any one of embodiments 107 to 110, wherein the furin cleavage site sequence comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 8.
112. The polycistronic construct of any one of embodiments 106 to 111, wherein the furin cleavage site sequence comprises the amino acid sequence of SEQ ID NO: 8.
113. The polycistronic construct of any one of embodiments 1 to 112, wherein the cleavage site sequence comprises a furin cleavage site sequence and a T2A cleavage sequence (furinT2A).
114. The polycistronic construct of any one of embodiments 1 to 113, wherein the nucleotide sequence encoding the cleavage site sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 5.
115. The polycistronic construct of any one of embodiments 1 to 114, wherein the nucleotide sequence encoding the cleavage site sequence comprises the nucleotide sequence of SEQ ID NO: 5.
116. The polycistronic construct of any one of embodiments 1 to 115, wherein the cleavage site sequence comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 6.
117. The polycistronic construct of any one of embodiments 1 to 116, wherein the cleavage site sequence comprises the amino acid sequence of SEQ ID NO:6.
118. The polycistronic construct of any one of embodiments 1 to 109, wherein the first expression cassette and second expression cassette are separated by a furinT2A, the second expression cassette and third expression cassette are separated by a P2A, and the third expression cassette and fourth expression cassette are separated by a P2A.
119. The polycistronic construct of any one of embodiments 1 to 118, wherein the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:1.

120. The polycistronic construct of any one of embodiments 1 to 119, wherein the construct comprises the nucleotide sequence of SEQ ID NO:1.
121. The polycistronic construct of any one of embodiments 1 to 120, wherein the construct encodes a polypeptide comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:2.
122. The polycistronic construct of any one of embodiments 1 to 121, wherein the construct encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2.
123. The polycistronic construct of any of embodiments 1 to 118, wherein the construct comprises a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 48.
124. The polycistronic construct of any one of embodiments 1 to 118 or 123, wherein the polycistronic construct comprises the nucleotide sequence of SEQ ID NO: 48.
125. The polycistronic construct of any one of embodiments 1 to 118, 123, or 124, wherein the polycistronic construct encodes a polypeptide comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 49.
126. The polycistronic construct of any one of embodiments 1 to 118 or 123 to 125, wherein the construct encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 49.
127. A viral vector comprising the polycistronic construct of any one of embodiments 1 to 126.
128. The viral vector of embodiment 127, wherein the viral vector is a lentiviral vector.
129. The viral vector of embodiment 127 or embodiment 128, wherein the viral vector further comprises one or more surface T cell activating agents.
130. The viral vector of embodiment 129, wherein the one or more surface T cell activating agents comprise CD58, anti-CD3, or CD80.
131. A cell comprising the viral vector of any one of embodiments 127 to 130.
132. The cell of embodiment 131, wherein the cell comprises a stem cell or a progenitor cell.
133. The cell of embodiment 132, wherein the stem cell comprises an induced pluripotent stem cell (iPSC).
134. The cell of embodiment 131, wherein the progenitor cell comprises a peripheral blood mononuclear cell (PBMC).
135. The cell of embodiment 131, wherein the cell comprises a T cell.
136. The cell of embodiment 131, wherein the cell comprises a cytotoxic innate lymphocyte (CIL) cell.
137. The cell of embodiment 131, wherein the cell comprises a natural killer (NK) cell.
138. A method of transducing a cell comprising contacting a target cell with any of the polycistronic constructs of any of embodiments 1 to 126.
139. A method of transducing a cell comprising contacting a target cell with the viral vector of any one of embodiments 127-130.
140. The method of embodiment 138 or embodiment 139, wherein the target cell comprises a stem cell.
141. The method of embodiment 140, wherein the stem cell comprises an induced pluripotent stem cell (iPSC).
142. The method of embodiment 138 or embodiment 139, wherein the target cell comprises a progenitor cell.

143. The method of embodiment 142, wherein the progenitor cell comprises a peripheral blood mononuclear cell (PBMC).
144. The method of embodiment 138 or embodiment 139, wherein the target cell comprises a T cell.
145. The method of embodiment 144, wherein the T cell comprises a CD4+ or CD8+ T cell.
146. The method of any one of embodiments 138 to 145, further comprising contacting the target cell with a (i) a guide RNA (gRNA) targeting a target site in an endogenous gene, and (ii) an RNA-guided endonuclease, thereby inserting the nucleotide sequence into the endogenous gene.
147. A method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell.
148. A method of expressing a a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell, the method comprising contacting the target cell with the viral vector of any one of embodiments 127-130.
149. The method of embodiment 147 or embodiment 148, wherein the target cell comprises a stem cell.
150. The method of embodiment 149, wherein the stem cell comprises an induced pluripotent stem cell (iPSC).
151. The method of embodiment 147 or embodiment 148, wherein the target cell comprises a progenitor cell.
152. The method of embodiment 151, wherein the progenitor cell comprises a peripheral blood mononuclear cell (PBMC).
153. The method of embodiment 147 or embodiment 148, wherein the target cell comprises a T cell.
154. The method of embodiment 153, wherein the T cell comprises a CD4+ or CD8+ T cell.
155. The method of any one of embodiments 138 to 154, wherein the method is performed ex vivo or in vitro.
156. The method of any one of embodiments 138 to 154, wherein the method is performed in vivo.
157. A method of transducing a T cell, the method comprising contacting the T cell with a viral vector comprising one or more T cell activating agents and a polycistronic construct of any one of embodiments 1-126, wherein the one or more T cell activating agents bind a receptor on the T cell.
158. A method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a T cell, the method comprising contacting the T cell with a viral vector comprising one or more T cell activating agents and a polycistronic construct of any one of embodiments 1-126, wherein the one or more T cell activating agents bind a receptor on the T cell.
159. A method of delivering a payload to a T cell, the method comprising contacting the T cell with a viral vector comprising one or more T cell activating agents and a polycistronic construct of any one of embodiments 1-126, wherein the one or more T cell activating agents bind a receptor on the T cell.
160. The method of any one of embodiments 157 to 159, wherein the T cell comprises a CD4+ or CD8+ T cell.
161. The method of any one of embodiments 157 to 160, wherein the method is performed ex vivo or in vitro.
162. The method of any one of embodiments 157 to 160, wherein the method is performed in vivo.

163. The method of any one of embodiments 157 to 162, wherein the one or more T cell activating agents comprise CD58, anti-CD3, or CD80.
164. The method of any one of embodiments 157 to 163, wherein the viral vector comprises a lentiviral vector.
165. A cell produced by the method of any one of embodiments 138 to 164.
166. A method of administering to a subject the cell of embodiment 165.
167. A method of administering to a subject the viral vector of any one of embodiments 127 to 130.
168. The method of embodiment 166 or embodiment 167, wherein the method treats a disease or condition in the subject.
169. The method of embodiment 168, wherein the disease or condition is treatable by the chimeric antigen receptor (CAR) encoded by the polycistronic construct,.
170. The method of embodiment 169, wherein the CAR is an anti-FITC CAR and the CAR is targeted to a cell of the disease or condition by administering a bifunctional ligand comprising FITC and a ligand that specifically binds a molecule expressed on the cell of the disease or condition.
171. The method of any of embodiments 168 to 170, wherein the disease or condition is a cancer.
172. The method of embodiment 171, wherein the cancer is a solid tumor.
173. The method of any of embodiments 170 to 172, wherein the cell is a cancer cell.
174. The method of embodiment 166 or embodiment 167, further comprising administering to the subject a bifunctional ligand to tag a cancer cell in the subject, wherein the bifunctional ligand specifically binds a molecule expressed on a tumor.
175. The method of embodiment 174, wherein the bifunctional ligand comprises a fluorescein isothiocyanate (FITC) moiety and the chimeric antigen receptor (CAR) encoded by the polycistronic construct is an anti-FITC CAR.
176. The method of any of embodiments 167 to 174, wherein the bifunctional ligand comprises FITC-folate.
177. The method of any one of embodiments 167 to 176, further comprising administering to the subject a non-physiological ligand, optionally wherein the non-physiological ligand binds to a synthetic cytokine receptor composed of the synthetic gamma chain polypeptide and the synthetic cytokine beta chain polypeptide encoded by the polycistronic construct.
178. The method of embodiment 177, wherein the non-physiological ligand comprises rapamycin or a rapamycin analog.
179. The method of embodiment 177 or embodiment 178, wherein binding of the non-physiological ligand to the synthetic cytokine receptor stimulates an intracellular cytokine signal in cells transduced to express the synthetic cytokine receptor.
180. The method of embodiment 177 or embodiment 178, wherein binding of the non-physiological ligand to the synthetic cytokine receptor promotes proliferation of cells transduced to express the synthetic cytokine receptor.

VII. EXAMPLES

[0457] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1 Generation and Assessment of RACR-TagCAR Polynucleotide Polycistronic Constructs

[0458] This example describes the generation of polycistronic constructs and their evaluation for RACR and CAR expression levels in order to elucidate the impact of the vector orientation on TagCAR activity.

[0459] A series of 8 polycistronic constructs were generated as shown in **Table E.1** and **FIGS. 2A-2B**. In all constructs, the polynucleotide of the TagCAR encoded a CAR with the following components in N-terminal to C-terminal order: an scFv (e.g. anti-FITC E2), a hinge (spacer), a transmembrane domain, and an endodomain with a costimulatory signaling domain and a CD3zeta signaling domain (Z). The constructs differed in the hinge (spacer) domain, either IgG4 hinge (IgG4H) or CD8alpha hinge (CD8H); transmembrane domain, either CD28 TM or CD8 TM; and costimulatory domain, either 41BB costimulatory domain or CD28 costimulatory domain. The constructs also differed in the placement of the TagCAR, being present either as the front or end of the construct transgene sequence. The individual polynucleotide components of the construct were separated by a 2A cleavage site sequence.

[0460] Table E1: Polycistronic Construct Design

Construct Name	**Construct design**
Construct A.1	(E2_IgG4H_CD28TM_41BBZ)-FRB-RACR
Construct A.2	FRB-RACR-(E2_IgG4H_CD28TM_41BBZ)
Construct B.1	(E2_IgG4H_CD28TM_28Z)-FRB-RACR
Construct B.2	FRB-RACR-(E2_IgG4H_CD28TM_28Z)
Construct C.1	(E2_CD8HTM_41BBz)-FRB-RACR
Construct C.2	FRB-RACR-(E2_CD8HTM_41BBz)
Construct D.1	(E2_CD8HTM_28Z)-FRB-RACR
Construct D.2	FRB-RACR-(E2_CD8HTM_28Z)

[0461] For lentiviral vector production, 293T producer cells were transfected with plasmids expressing viral vector proteins (gag/pol, rev) and a transfer plasmid encoding each of the above payload constructs. The lentiviral vectors also were engineered to encode CD58, anti-CD3, and CD80 surface proteins to mediate T cell activation. Following viral vector production, the cell culture was centrifuged to pellet the cells and the supernatant containing crude virus was collected.

[0462] On day 0, PBMCs from 3 donors were seeded in a 24-well plate at $2x10^6$ cells/mL in media containing 50 U/mL of IL-2 and transduced with lentivirus at an MOI of 2 or 10. On day 3, virus was washed out and cells were stained by flow cytometry for CD25 as a marker of T cell activation and also were split into separate plates and exposed to either 50 U/mL IL-2 + either 10 nM rapamycin or no rapamycin. On day 7, cells were stained to assess CAR expression, lysed for western blot, or were used for co-culture killing and expansion assays.

[0463] T cell activation (**FIG. 3A**) and T cell transduction (**FIG. 3B**) increased with particle dose, with higher activation and transduction occurring with MOI 10 (light bars) than MOI 2 (dark bars). Results in **FIG. 3A** also showed that T cell activation (as determined by % of CD25+ of CD8+ or CD4+ T cells) was similar across all lentiviral vector preparations. Likewise, **FIG. 3B** showed that T cell transduction (as determined by % CAR expression of CD8+ and CD4+ T cells) also was similar across all lentiviral vector preparations. Together, the results indicate surprisingly that the orientation of the components in the polynucleotide constructs do not impact the percentage of activated CD8+ and CD4+ T cells or the percentage of transduced CD8+ and CD4+ TagCAR T cells.

[0464] In contrast, Western Blot analysis of lysates showed that placing FRB in the first position in the transgene resulted in higher FRB protein expression (**FIG. 3C**), whether the cells were grown in IL2 only without rapamycin or in IL-2 + rapamycin. These results establish that constructs in which FRB is in the front position relative to the RACR CAR sequences are superior for ensuring high level expression of the FRB.

[0465] Based on the superiority of FRB as the first transgene, lentiviral vectors with Construct C.2 and Construct D.2 oriented transgenes were further analyzed following transduction of T cells. On day 0, PBMCs from 3 donors were seeded in a 24-well plate at $2x10^6$ cells/mL in media containing 50 U/mL of IL-2 and transduced with lentivirus at an MOI of 10. On day 3, virus was washed out and cells were split into separate plates and exposed to either 50 U/mL IL-2, 10 nM

rapamycin or 50 U/mL IL-2 +10 nM rapamycin. On day 7, 11 and 14 cells were stained to assess CAR expression. As shown in **FIG. 4**, CAR T cells transduced with either transgene were enriched and expanded with rapamycin.

[0466] Transduced T cells from Day 10 after transduction were assayed in a killing assay. On the day prior (Day 10), $6x10^3$ FRα+ MDA-MB-231 targets cells that were labeled with mCherry were plated in a 96-well plate in folic acid-free media. On day 11, transduced CAR-T cells were added to target cells at a 1:1 or 1:4 effector to target (E:T) ratio. Then Folate-Fluorescein (FITC-folate) was added at 0, 0.1 or 10 nM to each well. At Day 12, supernatant was collected and used for analysis of cytokines. As shown in **FIG. 5A** (IgG4 hinge) and **FIG. 5B** (CD8 hinge), CAR T cells with a CD8alpha hinge exhibited superior activity at eliminating FRα+ target cells at low doses of the Folate-Fluorescein across all donors, regardless of prior expansion in rapamycin. More specifically, tumor object area at 96hrs decreased when co-cultured with cells transduced with the following constructs (top to bottom in **FIG. 5A**): Construct A.2 + FITC-Folate expanded in IL-2 only; Construct A.1; Construct A.2; Construct A.1 + FITC-Folate expanded in IL-2 + 10 nM rapamycin; Construct A.2 + FITC-Folate expanded in IL-2 + 10 nM rapamycin; Construct A.1 + FITC-Folate expanded in IL-2 only. Tumor object area at 96hrs decreased when co-cultured with transduced with the following constructs (top to bottom in **FIG. 5B**): Construct B.2; Construct B.1; Construct B.2 + FITC-Folate expanded in IL-2 + 10 nM rapamycin; Construct B.2 + FITC-Folate expanded in IL-2 only; Construct B.1 + FITC-Folate expanded in IL-2 + 10 nM rapamycin; Construct B.1 + FITC-Folate expanded in IL-2 only. Tumor object area at 96hrs decreased when co-cultured with transduced with the following constructs (top to bottom in **FIG. 6A**): Construct C.1; Construct C.2; Construct C.1 + FITC-Folate expanded in IL-2 only; Construct C.2 + FITC-Folate expanded in IL-2 only; Construct C.1 + FITC-Folate expanded in IL-2 and 10 nM rapamycin; Construct C.2 + FITC-Folate expanded in IL-2 and 10 nM rapamycin. Tumor object area at 96hrs decreased when co-cultured with transduced with the following constructs (top to bottom in **FIG. 6B**): Construct D.2; Construct D.1; Construct D.1 + FITC-Folate expanded in IL-2 and 10 nM rapamycin; Construct D.2 + FITC-Folate expanded in IL-2 and 10 nM rapamycin; Construct D.1 + FITC-Folate expanded in IL-2 only; Construct D.2 + FITC-Folate expanded in IL-2 only.

[0467] Pro-inflammatory cytokine production and T cell proliferation also was increased in co-culture with CD8alpha hinge CAR candidates in the presence of FITC-folate (data not shown).

[0468] Results also showed that CAR T cells transduced with lentiviral vectors containing a transgene encoding a CAR with a 4-1BB costimulatory domain also demonstrated enhanced persistence following repeat stimulation with FRα+ tumor cells in the presence of FITC-folate (data now shown). Further, mice treated by in vivo administration of a lentiviral vector encoding 41BB endodomain CAR candidates in the presence of FITC-folate demonstrated improved regression/control of FRα+solid tumors, increased circulating CAR-T cell enrichment/expansion, and survival compared to mice treated with lentiviral vectors encoding CD28 endodomain CAR candidates.

[0469] These results additionally indicate that constructs encoding a CAR with a CD8hinge and 4-1BB costimulatory domain also are superior.

[0470] Based on the above analysis, Construct C.2 was selected for further analysis.

Example 2 Generation and Assessment of RACR-TagCAR Polynucleotide Polycistronic Constructs with different FRB Orientations

[0471] To further assess the impact of a construct with FRB-RACR in the front position of the transgene, the selected Construct C.2 was compared to a construct in which FRB-RACR was oriented at the end of the transgene. The polynucleotide orientations explored in the present example is shown in **Table E2**.

[0472] Table E2

<table>
<tr><td>Polynucleotide construct</td><td colspan="4">N-terminus → C-terminus</td></tr>
<tr><td rowspan="2">Construct V (FRB-RACR at End)</td><td rowspan="2">CD19-CAR</td><td rowspan="2">FRB</td><td colspan="2">RACR</td></tr>
<tr><td>FKBP12-IL2Rγ</td><td>FRB-IL2Rβ</td></tr>
<tr><td rowspan="2">Construct C.2</td><td rowspan="2">FRB</td><td colspan="2">RACR</td><td rowspan="2">Tag-CAR</td></tr>
<tr><td>FKBP12-IL2Rγ</td><td>FRB-IL2Rβ</td></tr>
</table>

[0473] On day 0, PBMCs from 3 donors were seeded in a 24-well plate at $1x10^6$ cells/mL in medium containing 250 U/mL of IL2 and transduced with lentivirus encoding the above constructs. On day 3, cells were counted and stained for CD25 to confirm T cell activation by the lentiviral vector or cells were transferred to separate plates for stimulation with IL2 and rapamycin. On day

7, cells were either counted again and stained to assess CAR expression, lysed for western blot, or stimulated with IL2 (250 U/mL) and rapamycin (10nM). On days 11, 14, and 18, cells were counted, stained to assess CAR expression or stimulated with IL2 (250 U/mL) and rapamycin (10nM).

[0474] PBMCs transduced with lentiviral vector containing Construct V or Construct C.2 both expressed CAR (**FIG. 6A**). Consistent with the results in Example 1, enhanced FRB and RACR expression was observed by Western Blot in PBMCs transduced with Construct C.2 compared to Construct V (data not shown). The enhanced expression of FRB correlated with enhanced proliferation of PBMCs transduced with Construct C.2 compared to Construct V in the presence of rapamycin in transduced cells from each donor. (**FIG. 6B**). These data demonstrate that cells containing Construct C.2 responded better to rapamycin stimulation compared to cells containing Construct V.

Example 3 Generation and Characterization of Additional Polycistronic Construct

[0475] Although FRB expression seems to be rescued in cells transduced with lentiviral vectors containing Construct C.2, attempts were made to modify the constructs to further improve expression in transduced cells of FRB as well the synthetic cytokine receptor (RACR) composed of FRB-IL2Rgamma and FKBP12-IL2Rbeta. An increase in FRB and the synthetic cytokine receptor could enhance rapamycin mediated CAR T cell enrichment and expansion.

[0476] A polynucleotide construct was generated by rearranging the orientation of the polynucleotide compared to Construct C.2. Specifically, the FRB and FKBP12 were swapped to encode a synthetic gamma chain polypeptide composed of FRB fused toIL2Rγ and a synthetic beta chain polypeptide composed of FKBP12 sequence fused to IL2Rβ. Further, a furin T2A linker was included in place of P2A between the first and second transgenes of the construct, namely between FRB and FRB:IL2Rγ. Finally, the polynucleotide encoding the synthetic cytokine receptor (RACR) components was codon optimized. The resultant optimized polynucleotide construct is shown as Construct C.2U in **FIG. 7A** and SEQ ID NO:1.

[0477] Expression and Activity of T cells transduced with lentiviral vectors containing Construct C.2 or Construct C.2U was assessed. Lentiviral vector was produced generally as described in Example 1.

[0478] On day 0, PBMCs from five different donors were seeded in a 24-well plate at $1x10^6$ cells/mL and transduced with lentivirus containing construct C.2 polynucleotide or Construct C.2U polynucleotide. On day 3, cells were stained for CD25. On day 7, cells were either stained to assess expression of the RACR-TagCAR (FITC-CAR). On day 8, cells were collected and lysed for western blot.

[0479] The percentage of CD25 + cells was comparable between T cells transduced with lentiviral vectors with either Construct C.2 or Construct C.2U transgenes (**FIG. 7B**). On day 7, Construct C.2U transduced T cells expressed higher levels of the CAR compared to Construct C.2 transduced T cells, both as the percentage of CAR expressing T cells (**FIG. 7C**)and as a mean fluorescence intensity (MFI) (**FIG. 7D**).

[0480] T cells transduced with lentivirus containing polynucleotide Construct C.2U exhibited higher detectable expression by Western Blot of FRB (**FIG. 7E**) and RACR (FRB-IL2Rγ and FKBP12-IL2Rβ) (**FIG. 7F**).

[0481] In a separate experiment, activation and transduction of gated T cell populations was assessed by flow cytometry on day 3 and day 7 post-transduction (**FIG. 8A**). Following transduction, the percentage of CD8+ and CD4+ T cells as a percentage of CD25+ cells on day 3 of activation increased (**FIG. 8A**, left graph). Also, the percentage RACR-TagCAR+ CD4+ or CD8+ T cells increased following transduction at an MOI 10 (**FIG. 8A**, right graph). Representative flow plots on day 7 post-transduction show an increased percentage of CD8+ T cells express RACR-TagCAR, particularly at MOI 10 (**FIG. 8B**).

[0482] To assess impact on functional activity of transduced cells, PBMCs at 1 x 10^6 cells/mL in 24-well plates were transduced with lentivirus containing polynucleotide Construct C.2 or C.2U on day 0. On day 3, cells were stimulated with IL2 (250 U/mL) or rapamycin (10nM) and stained for CD25. On day 7 cells were stimulated with IL2 only, rapamycin only, AP21967 (50nM) only, or rapamycin and IL2.

[0483] T cells transduced with Construct C.2U transgene lentiviral vector demonstrated enhanced CAR T cell expansion in the presence of IL2 or rapamycin beginning on day 3 compared to T cells transduced with Construct C.2 transgene lentiviral vector. Results from one representative donor is shown in **FIG. 9A**. Construct C.2U transgene transduced T cells also

demonstrated enhanced CAR T cell expansion in the presence of rapamycin or rapamycin analog AP21967 as shown in **FIG. 9B**.

Example 4 Characterization of RACR-TagCAR T cells

[0484] This example demonstrates characterization (*e.g.*, activation, transduction, cytotoxicity, etc.) of primary human T cells transduced with Construct C.2U transgene lentiviral vector described in Example 3.

[0485] PBMCs from 3 healthy donors were transduced *in vitro* with lentivirus vectors containing Construct C.2U transgene at a multiplicity of infection (MOI) 2 or 10 in the absence of T cell activating reagents.

[0486] Next, rapamycin-mediated RACR activation of RACR-TagCAR T cells was assessed. PBMCs from 2 healthy donors were cultured with Construct C.2U transgene lentiviral vector at MOI 10. PBMCs were split into two co-cultures. One co-culture contained 50U/mL of IL-2 and the other co-culture contained 50U/mL of IL-2 and 10 nM rapamycin, which were added on day 3 of co-culture, and enrichment and expansion of T cells was measured on days 7, 11 and 14 by flow cytometry (**FIG. 10A**). Rapamycin-mediated RACR activation of T cells resulted in increased enrichment of RACR-TagCAR expressing T cells across days 11 and 14 (**FIG. 10B**, left graph). Rapamycin-mediated RACR activation of T cells also resulted in increased expansion of RACR-TagCAR expressing T cells across days 11 and 14 (**FIG. 10B**, right graph).

Example 5 RACR-TagCAR T cells demonstrate *in vitro* tumor killing

[0487] T cell anti-tumor activity and persistence was assessed using a co-culture approach with folate receptor (FR)-expressing MDA-MB-231 breast carcinoma cells and titrated doses of FITC-Folate. T cells transduced with Construct C.2U transgene lentiviral vector. On Day 11 post-transduction, rapamycin-expanded T cells were co-cultured with mCherry+FRα+MDA-MB-231 tumor cells in the presence of 0.1 nM FITC-Folate or 0.1 nM FITC-Folate and10nM rapamycin. Breast carcinoma cells were quantified over time using the Incucyte® Live-Cell Analysis System. At time points indicated with the arrows in **FIG. 11A** and **FIG. 11B**, breast carcinoma cells were reintroduced (tumor re-challenge). RACR-TagCAR T cells repeatedly killed FRα+ MDA-MB-231 breast carcinoma cells in the presence of FITC-Folate with improved activity upon rapamycin

treatment (**FIG. 11A**). Further, RACR-TagCAR T cell proliferation persisted across the course of the experiment and with each tumor re-challenge (**FIG. 11B**).

Example 6 Rapamycin Activated Cytokine Receptor (RACR) activation of TagCAR T cells (RACR-TagCAR T cells) demonstrate *in vivo* tumor killing

[0488] RACR-TagCAR T cells were tested for their ability to control tumors *in vivo* in a mouse model receiving *ex vivo* transduced RACR-TagCAR T cells (**FIG. 12A**). To assess *in vivo* anti-tumor activity, NSG MHCI/II^{DKO} mice were injected with FRα+ MDA-MB-231 breast carcinoma cells. Once tumors reached a size of 100 mm^3, mice received an i.v. injection of 5e6 or 10e6 RACR-TagCAR T cells that were transduced *ex vivo* with CD58/CD3/CD80 surface-engineered lentiviral particles containing Construct C.2 transgene encoding FRB-RACR-TagCAR. Mice were then dosed twice per week with FITC-Folate. Efficacy was measured by tumor regression using digital calipers and plotted as shown in **FIG. 12B**. Administration of both 5e6 and 10e6 RACR-TagCAR T cell numbers rapidly cleared FRα+ solid tumors in mice (**FIG. 12B**).

[0489] RACR-TagCAR lentivirus particles were tested for their ability to control tumors *in vivo* in a mouse model. Mice were administered CD58/CD3/CD80 surface-engineered lentiviral particles containing Construct C.2U transgene encoding FRB-RACR-TagCAR vectors (**FIG. 13A**). Female NSG MHCI/IIDKO mice were engrafted with FRα+MDA-MB-231 tumors in the flank and tumors were allowed to grow until 100-200 mm^3 in size. Mice were humanized with healthy human donor PBMCs (25e6 or 100e6) followed by administration of CD58/CD3/CD80 surface-engineered lentiviral particles containing Construct C.2U transgene encoding the FRB-RACR-TagCAR vectors. In this experiment, the lentivirus particles were administered at a dose of 100e6 transfecting units (TU) of TagCAR vector or 25e6 TU TagCAR vector. Mice were treated with FITC-Folate twice per week and efficacy was determined by assessing tumor regression and circulating RACR-TagCAR T cells in blood by flow cytometry from weekly blood draws. RACR-TagCAR T cells were detected in blood as early as day 7 (**FIG. 13B**). Administration of the lentiviral vector particles also controlled FRα+ solid tumors in mice as evidenced by a reduction in tumor volume (**FIG. 13C**).

[0490] In a separate experiment, RACR-TagCAR lentivirus particles were tested for their ability to control tumors *in vivo* in the mouse model described in **FIG. 13A**. In this study, mice

were administered CD58/CD3/CD80 surface-engineered lentiviral particles containing Construct C.2U at doses of 0.2e6 TU TagCAR vector, 1.0e6 TU TagCAR vector or 5.0e6 TU TagCAR vector. Efficacy was determined by monitoring tumor regression and circulating RACR-TagCAR T cells as described above. There was dose-dependent control of solid tumors, and mice that received 5.0e6 TU of lentiviral particles had the greatest tumor reduction by Day 25 (**FIG. 14A**). Additionally, there was a dose-dependent effect on the amount of RACR-TagCAR T cells detected in the blood of the mice. RACR-TagCAR T cells were detected around Day 6 and by Day 25 mice that received 5.0e6 TU of lentiviral particles had the greatest number of circulating RACR-TagCAR T cells (**FIG. 14B**). The results are consistent with an observation that the dose dependent control of well-established solid tumor correlated with generation of large numbers of TagCAR T cells *in vivo.*

[0491] These results establish that the TagCAR T cells can be generated in vivo with administration of the lentiviral particles and mediate tumor control and regression with FITC-folate administration. The studies herein and above establish the utility of a TagCAR T cell system for ex vivo or in vivo delivery to T cells and administration of a tumor tag, which can provide utility in the context of additional antigen-targeting tumor tags.

[0492] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

VIII. SEQUENCES

#	SEQUENCE	Description
1.	ACATGTCTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCAC TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGT CAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGA GCTCGTTTAGTGAACCGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTG GCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTA GTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACC AGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGG GGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGAT GGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCG GTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGG AGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATA TAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGG AAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCACCGCACAGCAAGCG GCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTAT ATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGA AGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTT GGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGAC AATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC TGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAAC TCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAG ATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTT AATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTAT TGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGG TATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAAGAATAGTTTTTGC TGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCC ACCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG AGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCTCGACGGTATCGGTTAACTTT TAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAG CAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTATC GATTGCCTGACGCGTAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGGATCAAGGT CAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCT GCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCT GTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTC CCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGA AATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGC GCTTCTGCTCCCCGAGCTCTATATAAGAGCCCACAACCCCTCACTCGGCGCGTGAACAC AATTCTGCAGTCGAAGGCGTACCGTCACTTACGAGTCGGTAGCCTGCAGGGCCGCCACC ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTTGGCGAGAGGAA CGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAATGATGGAGAGGGGACCTC AGACACTGAAGGAGACCTCCTTCAACCAGGCCTATGGCAGAGACCTGATGGAGGCCCAG GAGTGGTGCAGGAAGTACATGAAGTCTGGCAATGTGAAGGACCTGCTGCAGGCCTGGGA	Construct C.2U Full Vector nucleotide

	TCTGTACTATCACGTGTTTCGGAGAATCAGCAAGGCTAGCAGAAGAAAGAGAGGCAGCG GCGAGGGCCGAGGCAGCCTGCTGACCTGCGGTGATGTGGAAGAAAACCCGGGCCCCATG CCCTTGCCCGTGACCGCGTTGCTCCTGCCCTTGGCTCTACTGCTGCACGCCGCTAGACC CATCCTGTGGCACGAGATGTGGCACGAGGGCCTGGAGGAGGCTAGCAGACTGTACTTCG GCGAGAGAAACGTGAAGGGCATGTTCGAGGTGCTGGAGCCCCTGCACGCCATGATGGAG AGAGGCCCTCAGACCCTGAAGGAGACAAGCTTCAACCAAGCCTACGGCAGAGACCTGAT GGAGGCCCAAGAGTGGTGCAGAAAGTACATGAAGAGCGGCAACGTGAAGGACCTGCTGC AAGCCTGGGACCTGTACTACCACGTGTTCAGAAGAATCAGCAAGGGCAGCAATACAAGC AAGGAAAACCCCTTCCTGTTCGCCCTGGAGGCCGTGGTGATCAGCGTGGGCAGCATGGG CCTGATCATCAGCCTGCTGTGCGTGTACTTCTGGCTGGAGAGAACCATGCCTAGAATCC CCACCCTGAAGAACCTGGAGGACCTGGTGACCGAGTACCACGGCAACTTCAGCGCCTGG AGCGGCGTGAGCAAGGGCCTGGCCGAGAGCCTGCAGCCCGACTACAGCGAGCGACTGTG CCTGGTGAGCGAGATTCCCCCTAAGGGCGGGGCCTTGGGTGAGGGACCCGGGGCAAGCC CGTGCAATCAGCACAGCCCCTACTGGGCCCCCCCCTGTTACACCCTGAAGCCCGAGACC GGCAGCGGAGCCACCAATTTCAGCCTCCTGAAACAAGCCGGTGACGTTGAAGAGAACCC CGGCCCCATGCCCCTGGGGTTGCTGTGGTTGGGACTCGCCCTCCTCGGCGCCCTGCACG CTCAAGCCGGCGTTCAAGTGGAGACTATCTCCCCCGGCGACGGCAGAACCTTCCCTAAG AGAGGGCAGACCTGCGTGGTGCACTACACCGGCATGCTGGAGGACGGCAAGAAGTTCGA CAGCAGCAGAGACAGAAACAAGCCCTTCAAGTTCATGCTGGGCAAGCAAGAGGTGATCA GAGGCTGGGAGGAGGGTGTGGCCCAAATGAGCGTGGGGCAGAGAGCCAAATTGACCATC TCACCCGACTACGCTTACGGGGCCACCGGCCATCCCGGCATCATCCCCCCCCACGCCAC CCTGGTGTTCGACGTGGAGCTGCTGAAGCTGGGCGAGGGCAAGGACACCATCCCCTGGC TGGGCCACCTGCTGGTGGGCCTGAGCGGCGCCTTCGGCTTCATCATCCTGGTGTACCTG CTGATCAACTGCAGAAACACCGGCCCCTGGCTGAAGAAGGTGCTGAAGTGCAACACCCC CGACCCTAGCAAGTTCTTCTCTCAGCTGAGCAGCGAGCACGGCGGCGACGTGCAGAAGT GGCTGAGCAGCCCATTTCCTAGCAGCAGCTTTAGCCCCGGCGGCCTAGCTCCCGAGATC AGCCCCCTGGAAGTACTGGAGAGAGATAAGGTGACACAGCTGCTGCTGCAGCAAGACAA GGTGCCCGAGCCCGCTAGCCTGAGCAGCAACCACAGCCTGACAAGCTGCTTCACCAACC AAGGCTACTTCTTCTTCCACCTGCCCGACGCCCTGGAGATCGAGGCCTGCCAAGTGTAC TTCACCTACGATCCGTACAGCGAGGAAGACCCCGACGAGGGAGTGGCCGGCGCCCCTAC CGGCAGCTCGCCACAACCGCTGCAACCTCTGAGCGGCGAGGACGACGCCTACTGCACCT TCCCTAGCAGAGATGATCTGCTGCTCTTTTCCCCTAGCTTGCTGGGGGGCCCTTCGCCT CCAAGTACTGCCCCCGGAGGTAGCGGGGCCGGCGAAGAAAGAATGCCCCCAAGCCTGCA AGAAAGAGTGCCTAGAGACTGGGACCCTCAACCACTGGGCCCACCCACTCCGGGCGTGC CCGACCTGGTAGATTTTCAGCCCCCTCCCGAGCTGGTGCTACGCGAGGCCGGCGAAGAG GTGCCCGACGCTGGGCCAAGAGAGGGTGTGTCATTCCCCTGGAGCCGACCCCCCGGTCA AGGCGAGTTCAGAGCCCTGAACGCTAGACTGCCCCTGAACACCGACGCCTACCTGAGCC TGCAAGAGCTGCAAGGCCAAGACCCCACCCACCTGGTGGGATCCGGAGCAACAAACTTT TCTCTGCTGAAGCAGGCCGGCGATGTGGAAGAAAACCCTGGACCTCTGCTGCTGGTGAC AAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGATCCCCTCCGTGCTGA CCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAATCAGCTGTTCCGGC TCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAGCACCCTGGCAAGGC CCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGTGCCTGATCGGTTCT CCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCAGTCCGAGGAC GAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTCTGTTCGGCAC CGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCAGGATCTGGAG AGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAGGAAACCTGGTGCAGCCA GGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACCTTCGGCTCTTTTAGCAT GTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGTGGCCGGCCTGAGCGCCC GGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGAT AACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGT GTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTT ATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCCACCACCACACCTGCT CCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCTGAGACCTGA	

	GGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCG ACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTGAGCCTGGTG ATCACACTGTACTGCAAGCGCGGCCGGAAGAAGCTGCTCTACATCTTTAAGCAGCCATT CATGCGCCCCGTGCAGACCACACAGGAGGAGGACGGCTGCTCCTGTCGGTTTCCAGAGG AGGAGGAGGGAGGATGTGAGCTGAGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCC TATCAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTA CGACGTGCTGGATAAGAGGAGGGGAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAA AGAACCCACAGGAGGGCCTGTATAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTAC TCCGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTATCA GGGCCTGAGCACCGCCACAAAGGACACATACGATGCCCTGCACATGCAGGCCCTGCCTC CAAGGTGACGCCGGCGCTAGTGTCGACGTAGTGGTACCTTTAAGACCAATGACTTACAA GGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTC ACTCCCAACGAAGACAAGATCTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAG ATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAG CTTGCCTTGAGTGCTTCAATGTGTGTGTTGGTTTTTTGTGTGTCGTCGTCTCACTTGGA TCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAA TTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAG TGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGGGAAATGTGTGCAGCTCTGGCCCGTGT CTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAA CTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAAC GTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTC GCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCG CCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGAT GGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCC GTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAG GTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCT GCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTC GTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGAT GACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCC ATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTG ACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATAC CAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACG GCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGA TGCTCGATGAGTTTTTCTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGAT TTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCAT GACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGA TCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAA AAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCC GAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGT AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC CTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAG ACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGC CCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAA AGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGG AACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTG TCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCC TTTTGCTC	
2.	MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQ EWCRKYMKSGNVKDLLQAWDLYYHVFRRISKASRRKRGSGEGRGSLLTCGDVEENPGPM PLPVTALLLPLALLLHAARPILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMME RGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSNTS KENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKNLEDLVTEYHGNFSAW	Construct C.2U polypeptide

	SGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET GSGATNFSLLKQAGDVEENPGPMPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPK RGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLGEGKDTIPWLGHLLVGLSGAFGFIILVYL LINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEI SPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVY FTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSP PSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEE VPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLVGSGATNF SLLKQAGDVEENPGPLLLVTSLLLCELPHPAFLLIPSVLTQPSSVSAAPGQKVTISCSG STSNIGNNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSED EADYYCAAWDDSLSEFLFGTGTKLTVLGSTSGSGKPGSGEGSTKGQVQLVESGGNLVQP GGSLRLSCAASGFTFGSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRD NAKNSVYLQMNSLRVEDTAVYYCARRSYDSSGYWGHFYSYMDVWGQGTLVTVSSTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLV ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
3.	ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTTGGCGAGAGGAA CGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAATGATGGAGAGGGGACCTC AGACACTGAAGGAGACCTCCTTCAACCAGGCCTATGGCAGAGACCTGATGGAGGCCCAG GAGTGGTGCAGGAAGTACATGAAGTCTGGCAATGTGAAGGACCTGCTGCAGGCCTGGGA TCTGTACTATCACGTGTTTCGGAGAATCAGCAAG	FRB nucleotide
4.	MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQ EWCRKYMKSGNVKDLLQAWDLYYHVFRRISK	FRB polypeptide
5.	AGAAGAAAGAGAGGCAGCGGCGAGGGCCGAGGCAGCCTGCTGACCTGCGGTGATGTGGA AGAAAACCCGGGCCCC	Furin T2A nucleotide
6.	RRKRGSGEGRGSLLTCGDVEENPGP	Furin T2A polypeptide
7.	AGAAGAAAGAGA	Furin site nucleotide
8.	RRKR	Furin site polypeptide
9.	GAGGGCCGAGGCAGCCTGCTGACCTGCGGTGATGTGGAAGAAAACCCGGGCCCC	T2A nucleotide
10.	EGRGSLLTCGDVEENPGP	T2A polypeptide
11.	ATCCTGTGGCACGAGATGTGGCACGAGGGCCTGGAGGAGGCTAGCAGACTGTACTTCGG CGAGAGAAACGTGAAGGGCATGTTCGAGGTGCTGGAGCCCCTGCACGCCATGATGGAGA GAGGCCCTCAGACCCTGAAGGAGACAAGCTTCAACCAAGCCTACGGCAGAGACCTGATG GAGGCCCAAGAGTGGTGCAGAAAGTACATGAAGAGCGGCAACGTGAAGGACCTGCTGCA AGCCTGGGACCTGTACTACCACGTGTTCAGAAGAATCAGCAAGGGCAGCAATACAAGCA AGGAAAACCCCTTCCTGTTCGCCCTGGAGGCCGTGGTGATCAGCGTGGGCAGCATGGGC CTGATCATCAGCCTGCTGTGCGTGTACTTCTGGCTGGAGAGAACCATGCCTAGAATCCC CACCCTGAAGAACCTGGAGGACCTGGTGACCGAGTACCACGGCAACTTCAGCGCCTGGA GCGGCGTGAGCAAGGGCCTGGCCGAGAGCCTGCAGCCCGACTACAGCGAGCGACTGTGC CTGGTGAGCGAGATTCCCCCTAAGGGCGGGGCCTTGGGTGAGGGACCCGGGGCAAGCCC GTGCAATCAGCACAGCCCCTACTGGGCCCCCCCCTGTTACACCCTGAAGCCCGAGACC	FRB:IL2RG nucleotide
12.	ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM EAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSNTSKENPFLFALEAVVISVGSMG LIISLLCVYFWLERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLC LVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET	FRB:IL2RG polypeptide

13.	ATCCTGTGGCACGAGATGTGGCACGAGGGCCTGGAGGAGGCTAGCAGACTGTACTTCGG CGAGAGAAACGTGAAGGGCATGTTCGAGGTGCTGGAGCCCCTGCACGCCATGATGGAGA GAGGCCCTCAGACCCTGAAGGAGACAAGCTTCAACCAAGCCTACGGCAGAGACCTGATG GAGGCCCAAGAGTGGTGCAGAAAGTACATGAAGAGCGGCAACGTGAAGGACCTGCTGCA AGCCTGGGACCTGTACTACCACGTGTTCAGAAGAATCAGCAAG	FRB nucleotide
14.	ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM EAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISK	FRB polypeptide
15.	GGCAGCAATACAAGCAAGGAAAACCCCTTCCTGTTCGCCCTGGAGGCCGTGGTGATCAG CGTGGGCAGCATGGGCCTGATCATCAGCCTGCTGTGCGTGTACTTCTGGCTGGAGAGAA CCATGCCTAGAATCCCCACCCTGAAGAACCTGGAGGACCTGGTGACCGAGTACCACGGC AACTTCAGCGCCTGGAGCGGCGTGAGCAAGGGCCTGGCCGAGAGCCTGCAGCCCGACTA CAGCGAGCGACTGTGCCTGGTGAGCGAGATTCCCCCTAAGGGCGGGGCCTTGGGTGAGG GACCCGGGGCAAGCCCGTGCAATCAGCACAGCCCCTACTGGGCCCCCCCCTGTTACACC CTGAAGCCCGAGACC	IL2RG nucleotide
16.	GSNTSKENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKNLEDLVTEYHG NFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYT LKPET	IL2RG polypeptide
17.	GCCACCAATTTCAGCCTCCTGAAACAAGCCGGTGACGTTGAAGAGAACCCCGGCCCC	P2A nucleotide
18.	ATNFSLLKQAGDVEENPGP	P2A polypeptide
19.	GGCGTTCAAGTGGAGACTATCTCCCCCGGCGACGGCAGAACCTTCCCTAAGAGAGGGCA GACCTGCGTGGTGCACTACACCGGCATGCTGGAGGACGGCAAGAAGTTCGACAGCAGCA GAGACAGAAACAAGCCCTTCAAGTTCATGCTGGGCAAGCAAGAGGTGATCAGAGGCTGG GAGGAGGGTGTGGCCCAAATGAGCGTGGGGCAGAGAGCCAAATTGACCATCTCACCCGA CTACGCTTACGGGGCCACCGGCCATCCCGGCATCATCCCCCCCCACGCCACCCTGGTGT TCGACGTGGAGCTGCTGAAGCTGGGCGAGGGCAAGGACACCATCCCCTGGCTGGGCCAC CTGCTGGTGGGCCTGAGCGGCGCCTTCGGCTTCATCATCCTGGTGTACCTGCTGATCAA CTGCAGAAACACCGGCCCCTGGCTGAAGAAGGTGCTGAAGTGCAACACCCCCGACCCTA GCAAGTTCTTCTCTCAGCTGAGCAGCGAGCACGGCGGCGACGTGCAGAAGTGGCTGAGC AGCCCATTTCCTAGCAGCAGCTTTAGCCCCGGCGGCCTAGCTCCCGAGATCAGCCCCCT GGAAGTACTGGAGAGAGATAAGGTGACACAGCTGCTGCTGCAGCAAGACAAGGTGCCCG AGCCCGCTAGCCTGAGCAGCAACCACAGCCTGACAAGCTGCTTCACCAACCAAGGCTAC TTCTTCTTCCACCTGCCCGACGCCCTGGAGATCGAGGCCTGCCAAGTGTACTTCACCTA CGATCCGTACAGCGAGGAAGACCCCGACGAGGGAGTGGCCGGCGCCCCTACCGGCAGCT CGCCACAACCGCTGCAACCTCTGAGCGGCGAGGACGACGCCTACTGCACCTTCCCTAGC AGAGATGATCTGCTGCTCTTTTCCCCTAGCTTGCTGGGGGGCCCTTCGCCTCCAAGTAC TGCCCCCGGAGGTAGCGGGGCCGGCGAAGAAAGAATGCCCCCAAGCCTGCAAGAAAGAG TGCCTAGAGACTGGGACCCTCAACCACTGGGCCCACCCACTCCGGGCGTGCCCGACCTG GTAGATTTTCAGCCCCCTCCCGAGCTGGTGCTACGCGAGGCCGGCGAAGAGGTGCCCGA CGCTGGGCCAAGAGAGGGTGTGTCATTCCCCTGGAGCCGACCCCCCGGTCAAGGCGAGT TCAGAGCCCTGAACGCTAGACTGCCCCTGAACACCGACGCCTACCTGAGCCTGCAAGAG CTGCAAGGCCAAGACCCCACCCACCTGGTG	FKBP12:IL2RB nucleotide
20.	GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGW EEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLGEGKDTIPWLGH LLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLS SPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGY FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPS RDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDL VDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQE LQGQDPTHLV	FKBP12:IL2RB polypeptide

21.	GGCGTTCAAGTGGAGACTATCTCCCCCGGCGACGGCAGAACCTTCCCTAAGAGAGGGCA GACCTGCGTGGTGCACTACACCGGCATGCTGGAGGACGGCAAGAAGTTCGACAGCAGCA GAGACAGAAACAAGCCCTTCAAGTTCATGCTGGGCAAGCAAGAGGTGATCAGAGGCTGG GAGGAGGGTGTGGCCCAAATGAGCGTGGGGCAGAGAGCCAAATTGACCATCTCACCCGA CTACGCTTACGGGGCCACCGGCCATCCCGGCATCATCCCCCCCCACGCCACCCTGGTGT TCGACGTGGAGCTGCTGAAGCTG	FKBP12 nucleotide
22.	GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGW EEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKL	FKBP12 polypeptide
23.	GGCAAGGACACCATCCCCTGGCTGGGCCACCTGCTGGTGGGCCTGAGCGGCGCCTTCGG CTTCATCATCCTGGTGTACCTGCTGATCAACTGCAGAAACACCGGCCCCTGGCTGAAGA AGGTGCTGAAGTGCAACACCCCCGACCCTAGCAAGTTCTTCTCTCAGCTGAGCAGCGAG CACGGCGGCGACGTGCAGAAGTGGCTGAGCAGCCCATTTCCTAGCAGCAGCTTTAGCCC CGGCGGCCTAGCTCCCGAGATCAGCCCCCTGGAAGTACTGGAGAGAGATAAGGTGACAC AGCTGCTGCTGCAGCAAGACAAGGTGCCCGAGCCCGCTAGCCTGAGCAGCAACCACAGC CTGACAAGCTGCTTCACCAACCAAGGCTACTTCTTCTTCCACCTGCCCGACGCCCTGGA GATCGAGGCCTGCCAAGTGTACTTCACCTACGATCCGTACAGCGAGGAAGACCCCGACG AGGGAGTGGCCGGCGCCCCTACCGGCAGCTCGCCACAACCGCTGCAACCTCTGAGCGGC GAGGACGACGCCTACTGCACCTTCCCTAGCAGAGATGATCTGCTGCTCTTTTCCCCTAG CTTGCTGGGGGGCCCTTCGCCTCCAAGTACTGCCCCCGGAGGTAGCGGGGCCGGCGAAG AAAGAATGCCCCCAAGCCTGCAAGAAAGAGTGCCTAGAGACTGGGACCCTCAACCACTG GGCCCACCCACTCCGGGCGTGCCCGACCTGGTAGATTTTCAGCCCCCTCCCGAGCTGGT GCTACGCGAGGCCGGCGAAGAGGTGCCCGACGCTGGGCCAAGAGAGGGTGTGTCATTCC CCTGGAGCCGACCCCCCGGTCAAGGCGAGTTCAGAGCCCTGAACGCTAGACTGCCCCTG AACACCGACGCCTACCTGAGCCTGCAAGAGCTGCAAGGCCAAGACCCCACCCACCTGGT G	IL2RB nucleotide
24.	GKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSE HGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHS LTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSG EDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPL GPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPL NTDAYLSLQELQGQDPTHLV	IL2RB polypeptide
25.	GCAACAAACTTTTCTCTGCTGAAGCAGGCCGGCGATGTGGAAGAAAACCCTGGACCT	P2A nucleotide
26.	CTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGAT CCCCTCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAA TCAGCTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAG CACCCTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGT GCCTGATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGAC TGCAGTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAG TTTCTGTTCGGCACCGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAA GCCAGGATCTGGAGAGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAGGAA ACCTGGTGCAGCCAGGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACCTTC GGCTCTTTTAGCATGTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGTGGC CGGCCTGAGCGCCCGGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCTTCA CCATCTCCCGGGATAACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGGGTG GAGGACACAGCCGTGTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTGGGG CCACTTTTACTCTTATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCCA CCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTG TCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACT GGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGC TCCTGAGCCTGGTGATCACACTGTACTGCAAGCGCGGCCGGAAGAAGCTGCTCTACATC TTTAAGCAGCCATTCATGCGCCCCGTGCAGACCACACAGGAGGAGGACGGCTGCTCCTG	TagCAR nucleotide

	TCGGTTTCCAGAGGAGGAGGAGGGAGGATGTGAGCTGAGAGTGAAGTTCTCTAGGAGCG CCGATGCCCCTGCCTATCAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTGGGC CGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGGAAGAGACCCAGAGATGGGAGG CAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTATAATGAGCTGCAGAAGGACAAGA TGGCCGAGGCCTACTCCGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACAC GATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGGACACATACGATGCCCTGCACAT GCAGGCCCTGCCTCCAAGGTGA	
27.	LLLVTSLLLCELPHPAFLLIPSVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWYQQ HPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSEDEADYYCAAWDDSLSE FLFGTGTKLTVLGSTSGSGKPGSGEGSTKGQVQLVESGGNLVQPGGSLRLSCAASGFTF GSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRDNAKNSVYLQMNSLRV EDTAVYYCARRSYDSSGYWGHFYSYMDVWGQGTLVTVSSTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	TagCAR polypeptide
28.	TCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAATCAG CTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAGCACC CTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGTGCCT GATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCA GTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTC TGTTCGGCACCGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCA GGATCTGGAGAGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAGGAAACCT GGTGCAGCCAGGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACCTTCGGCT CTTTTAGCATGTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGTGGCCGGC CTGAGCGCCCGGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCTTCACCAT CTCCCGGGATAACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGGGTGGAGG ACACAGCCGTGTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTGGGGCCAC TTTTACTCTTATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCC	E2 scFv nucleotide
29.	SVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWYQQHPGKAPKLMIYDVSKRPSGVP DRFSGSKSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLFGTGTKLTVLGSTSGSGKP GSGEGSTKGQVQLVESGGNLVQPGGSLRLSCAASGFTFGSFSMSWVRQAPGGGLEWVAG LSARSSLTHYADSVKGRFTISRDNAKNSVYLQMNSLRVEDTAVYYCARRSYDSSGYWGH FYSYMDVWGQGTLVTVSS	E2 scFv polypeptide
30.	TCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAATCAG CTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAGCACC CTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGTGCCT GATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCA GTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTC TGTTCGGCACCGGCACAAAGCTGACCGTGCTG	E2 scFv VL nucleotide
31.	SVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWYQQHPGKAPKLMIYDVSKRPSGVP DRFSGSKSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLFGTGTKLTVL	E2 scFv VL polypeptide
32.	GGCTCTACAAGCGGATCCGGCAAGCCAGGATCTGGAGAGGGCAGCACAAAGGGA	E2 scFv linker nucleotide
33.	GSTSGSGKPGSGEGSTKG	E2 scFv linker polypeptide
34.	CAGGTGCAGCTGGTGGAGAGCGGAGGAAACCTGGTGCAGCCAGGAGGCTCCCTGCGCCT GTCTTGTGCCGCCAGCGGCTTTACCTTCGGCTCTTTTAGCATGTCCTGGGTGCGCCAGG CACCTGGAGGAGGACTGGAGTGGGTGGCCGGCCTGAGCGCCCGGTCTAGCCTGACACAC TATGCCGACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGATAACGCCAAGAATAGCGT	E2 scFv VH nucleotide

	GTACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGTGTACTATTGCGCCAGGC GCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTTATATGGACGTGTGGGGA CAGGGCACCCTGGTGACAGTGAGCTCC	
35.	QVQLVESGGNLVQPGGSLRLSCAASGFTFGSFSMSWVRQAPGGGLEWVAGLSARSSLTH YADSVKGRFTISRDNAKNSVYLQMNSLRVEDTAVYYCARRSYDSSGYWGHFYSYMDVWG QGTLVTVSS	E2 scFv VH polypeptide
36.	ACCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCT GTCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGAC TGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTG CTCCTGAGCCTGGTGATCACACTGTACTGC	CD8 HTM nucleotide
37.	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVL LLSLVITLYC	CD8 HTM polypeptide
38.	ACCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCT GTCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGAC TGGATTTCGCCTGCGACATCTAC	CD8 Hinge nucleotide
39.	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY	CD8 Hinge polypeptide
40.	ATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTGAGCCTGGTGATCACACT GTACTGC	CD8 transmembrane nucleotide
41.	IWAPLAGTCGVLLLSLVITLYC	CD8 transmembrane polypeptide
42.	AAGCGCGGCCGGAAGAAGCTGCTCTACATCTTTAAGCAGCCATTCATGCGCCCCGTGCA GACCACACAGGAGGAGGACGGCTGCTCCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGAT GTGAGCTG	41BB nucleotide
43.	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	41BB polypeptide
44.	ATGTTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGCTATAGCCTGCTGGTGAC CGTGGCCTTTATCATCTTCTGGGTG	CD28 nucleotide
45.	MFWVLVVVGGVLACYSLLVTVAFIIFWV	CD28 polypeptide
46.	AGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCCTATCAGCAGGGACAGAACCAGCT GTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGG GAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTAT AATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACTCCGAGATCGGCATGAAGGGAGA GAGGCGCCGGGGCAAGGGACACGATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGG ACACATACGATGCCCTGCACATGCAGGCCCTGCCTCCAAGG	CD3ζ nucleotide
47.	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	CD3ζ polypeptide
48.	ACATGTCTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCAC TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGT CAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGA GCTCGTTTAGTGAACCGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTG	Construct C.2 Full Vector nucleotide

GCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTA
GTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC
AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACC
AGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGG
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GCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTAT
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	CCATGTTACACACTGAAGCCCGAGACCGGCTCTGGCGCCACAAACTTCAGCCTGCTGAA GCAAGCAGGCGACGTGGAAGAAAATCCAGGACCTATGGCACTGCCTGTGACCGCCCTGC TGCTGCCACTGGCCCTGCTGCTGCACGCAGCAAGGCCTATCCTGTGGCACGAAATGTGG CATGAAGGCCTGGAGGAGGCAAGCAGGCTGTATTTTGGCGAGCGGAATGTGAAAGGAAT GTTTGAAGTCCTGGAACCTCTGCATGCCATGATGGAAAGGGGACCACAGACACTGAAGG AGACCAGCTTTAACCAGGCCTATGGAAGGGACCTGATGGAGGCACAGGAGTGGTGCCGG AAGTACATGAAGTCCGGAAATGTGAAAGACCTGCTGCAGGCCTGGGATCTGTATTATCA CGTGTTCAGGCGCATCTCTAAGGGCAAGGATACCATCCCCTGGCTGGGACACCTGCTGG TGGGACTGAGCGGAGCCTTTGGCTTCATCATCCTGGTGTACCTGCTGATCAACTGCAGA AATACCGGCCCTTGGCTGAAGAAGGTGCTGAAGTGTAACACACCAGACCCCTCCAAGTT CTTTTCTCAGCTGTCTAGCGAGCACGGCGGCGATGTGCAGAAGTGGCTGTCCTCTCCTT TTCCAAGCTCCTCTTTCAGCCCAGGAGGACTGGCACCAGAGATCTCCCCCCTGGAGGTG CTGGAGAGGGACAAGGTGACCCAGCTGCTGCTGCAGCAGGATAAGGTGCCCGAGCCTGC CAGCCTGAGCTCCAACCACAGCCTGACATCCTGCTTTACCAATCAGGGCTATTTCTTTT TCCACCTGCCAGACGCCCTGGAGATCGAGGCCTGTCAGGTGTACTTCACCTATGATCCA TACTCCGAAGAGGACCCAGATGAGGGAGTGGCCGGCGCCCCCACAGGCTCTAGCCCACA GCCACTGCAGCCTCTGTCTGGAGAGGACGATGCCTACTGTACCTTTCCCAGCCGCGACG ATCTGCTGCTGTTCAGCCCTTCCCTGCTGGGAGGACCATCTCCACCTAGCACAGCACCA GGAGGAAGCGGGGCAGGGGAGGAGCGGATGCCACCATCTCTGCAGGAGAGGGTGCCTAG GGACTGGGATCCTCAGCCACTGGGACCTCCAACCCCTGGAGTGCCAGACCTGGTGGATT TTCAGCCCCCTCCAGAGCTGGTGCTGCGGGAGGCAGGAGAGGAGGTGCCTGACGCAGGA CCACGCGAGGGCGTGAGCTTTCCATGGTCCAGGCCACCTGGACAGGGAGAGTTCAGAGC CCTGAACGCCAGGCTGCCTCTGAATACAGACGCCTATCTGTCTCTGCAGGAGCTGCAGG GCCAGGATCCAACCCACCTGGTGGGATCCGGAGCAACAAACTTTTCTCTGCTGAAGCAG GCCGGCGATGTGGAAGAAAACCCTGGACCTCTGCTGCTGGTGACAAGCCTGCTGCTGTG CGAGCTGCCTCACCCAGCCTTTCTGCTGATCCCCTCCGTGCTGACCCAGCCTAGCTCCG TGTCTGCCGCACCAGGACAGAAGGTGACAATCAGCTGTTCCGGCTCTACCAGCAACATC GGCAACAATTACGTGAGCTGGTACCAGCAGCACCCTGGCAAGGCCCCAAAGCTGATGAT CTACGACGTGTCCAAGAGGCCATCTGGAGTGCCTGATCGGTTCTCCGGCTCTAAGAGCG GCAATTCCGCCTCTCTGGACATCAGCGGACTGCAGTCCGAGGACGAGGCAGATTACTAT TGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTCTGTTCGGCACCGGCACAAAGCTGAC CGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCAGGATCTGGAGAGGGCAGCACAAAGG GACAGGTGCAGCTGGTGGAGAGCGGAGGAAACCTGGTGCAGCCAGGAGGCTCCCTGCGC CTGTCTTGTGCCGCCAGCGGCTTTACCTTCGGCTCTTTTAGCATGTCCTGGGTGCGCCA GGCACCTGGAGGAGGACTGGAGTGGGTGGCCGGCCTGAGCGCCCGGTCTAGCCTGACAC ACTATGCCGACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGATAACGCCAAGAATAGC GTGTACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGTGTACTATTGCGCCAG GCGCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTTATATGGACGTGTGGG GACAGGGCACCCTGGTGACAGTGAGCTCCACCACCACACCTGCTCCTAGACCACCTACA CCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCTGAGACCTGAGGCCTGTAGACCTGC CGCTGGAGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGG CTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTGAGCCTGGTGATCACACTGTACTGC AAGCGCGGCCGGAAGAAGCTGCTCTACATCTTTAAGCAGCCATTCATGCGCCCCGTGCA GACCACACAGGAGGAGGACGGCTGCTCCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGAT GTGAGCTGAGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCCTATCAGCAGGGACAG AACCAGCTGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAA GAGGAGGGGAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGG GCCTGTATAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACTCCGAGATCGGCATG AAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTATCAGGGCCTGAGCACCGC CACAAAGGACACATACGATGCCCTGCACATGCAGGCCCTGCCTCCAAGGTGACGCCGGC GCTAGTGTCGACGTAGTGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCT TAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGAC AAGATCTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAG CTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCT	

	TCAATGTGTGTGTTGGTTTTTTGTGTGTCGTCGTCTCACTTGGATCCCATCCCGCCCCT AACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATG CAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTT GGAGGCCTAGGCTTTTGGGAAATGTGTGCAGCTCTGGCCCGTGTCTCAAAATCTCTGAT GTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATA AACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATT AAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGC AATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTG AAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTG GCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATG CATGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATAT CCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTC GATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGC AATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGC TGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTC AGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAA TAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATC CTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATA TGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTT TCTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTT TAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCA GCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTT CAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACT TCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCT GCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGA TAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAA CGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCC GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAC GAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACC TCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAAC GCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC	
49.	MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQ EWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSGATNFSLLKQAGDVEENPGPMPLGLL WLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKP FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELL KLGEGSNTSKENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKNLEDLVT EYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAP PCYTLKPETGSGATNFSLLKQAGDVEENPGPMALPVTALLLPLALLLHAARPILWHEMW HEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCR KYMKSGNVKDLLQAWDLYYHVFRRISKGKDTIPWLGHLLVGLSGAFGFIILVYLLINCR NTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEV LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDP YSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAP GGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAG PREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLVGSGATNFSLLKQ AGDVEENPGPLLLVTSLLLCELPHPAFLLIPSVLTQPSSVSAAPGQKVTISCSGSTSNI GNNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSEDEADYY CAAWDDSLSEFLFGTGTKLTVLGSTSGSGKPGSGEGSTKGQVQLVESGGNLVQPGGSLR LSCAASGFTFGSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRDNAKNS VYLQMNSLRVEDTAVYYCARRSYDSSGYWGHFYSYMDVWGQGTLVTVSSTTTPAPRPPT PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQ	Construct C.2 polypeptide

	NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
50.	ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTTGGCGAGAGGAA CGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAATGATGGAGAGGGGACCTC AGACACTGAAGGAGACCTCCTTCAACCAGGCCTATGGCAGAGACCTGATGGAGGCCCAG GAGTGGTGCAGGAAGTACATGAAGTCTGGCAATGTGAAGGACCTGCTGCAGGCCTGGGA TCTGTACTATCACGTGTTTCGGAGAATCAGCAAGGGCTCCGGC	FRB nucleotide
51.	MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQ EWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSG	FRB polypeptide
52.	GCCACCAACTTCAGCCTGCTGAAGCAGGCAGGCGATGTGGAGGAGAATCCAGGACCT	P2A nucleotide
53.	ATGCCACTGGGACTGCTGTGGCTGGGACTGGCCCTGCTGGGCGCCCTGCACGCCCAGGC AGGAGTGCAGGTGGAGACCATCTCCCCAGGCGACGGACGCACATTTCCAAAGAGGGGAC AGACCTGCGTGGTGCACTACACAGGCATGCTGGAGGATGGCAAGAAGTTCGACAGCTCC CGCGATCGGAATAAGCCCTTTAAGTTCATGCTGGGCAAGCAGGAAGTGATCAGAGGATG GGAGGAGGGAGTGGCACAGATGTCCGTGGGACAGAGGGCCAAGCTGACCATCTCTCCTG ACTACGCCTATGGAGCAACAGGACACCCAGGAATCATCCCACCTCACGCCACCCTGGTG TTTGATGTGGAGCTGCTGAAGCTGGGCGAGGGCTCTAACACAAGCAAGGAGAATCCTTT TCTGTTCGCACTGGAGGCAGTGGTCATCTCCGTGGGCTCTATGGGCCTGATCATCAGCC TGCTGTGCGTGTACTTTTGGCTGGAGAGAACCATGCCAAGGATCCCCACACTGAAGAAC CTGGAGGACCTGGTGACCGAGTATCACGGCAATTTCAGCGCCTGGTCCGGCGTGTCTAA GGGACTGGCAGAGTCCCTGCAGCCAGATTACTCTGAGCGGCTGTGCCTGGTGTCCGAGA TCCCACCCAAGGGAGGCGCCCTGGGAGAGGGACCAGGAGCCAGCCCTTGCAACCAGCAC TCCCCATATTGGGCCCCTCCATGTTACACACTGAAGCCCGAGACCGGCTCTGGC	FKBP12:IL2RG nucleotide
54.	MPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSS RDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLV FDVELLKLGEGSNTSKENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKN LEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQH SPYWAPPCYTLKPETGSG	FKBP12:IL2RG polypeptide
55.	GGAGTGCAGGTGGAGACCATCTCCCCAGGCGACGGACGCACATTTCCAAAGAGGGGACA GACCTGCGTGGTGCACTACACAGGCATGCTGGAGGATGGCAAGAAGTTCGACAGCTCCC GCGATCGGAATAAGCCCTTTAAGTTCATGCTGGGCAAGCAGGAAGTGATCAGAGGATGG GAGGAGGGAGTGGCACAGATGTCCGTGGGACAGAGGGCCAAGCTGACCATCTCTCCTGA CTACGCCTATGGAGCAACAGGACACCCAGGAATCATCCCACCTCACGCCACCCTGGTGT TTGATGTGGAGCTGCTGAAGCTG	FKBP12 nucleotide
56.	ATGCCACTGGGACTGCTGTGGCTGGGACTGGCCCTGCTGGGCGCCCTGCACGCCCAGGC AGGAGTGCAGGTGGAGACCATCTCCCCAGGCGACGGACGCACATTTCCAAAGAGGGGAC AGACCTGCGTGGTGCACTACACAGGCATGCTGGAGGATGGCAAGAAGTTCGACAGCTCC CGCGATCGGAATAAGCCCTTTAAGTTCATGCTGGGCAAGCAGGAAGTGATCAGAGGATG GGAGGAGGGAGTGGCACAGATGTCCGTGGGACAGAGGGCCAAGCTGACCATCTCTCCTG ACTACGCCTATGGAGCAACAGGACACCCAGGAATCATCCCACCTCACGCCACCCTGGTG TTTGATGTGGAGCTGCTGAAGCTGGGCGAGGGCTCTAACACAAGCAAGGAGAATCCTTT TCTGTTCGCACTGGAGGCAGTGGTCATCTCCGTGGGCTCTATGGGCCTGATCATCAGCC TGCTGTGCGTGTACTTTTGGCTGGAGAGAACCATGCCAAGGATCCCCACACTGAAGAAC CTGGAGGACCTGGTGACCGAGTATCACGGCAATTTCAGCGCCTGGTCCGGCGTGTCTAA GGGACTGGCAGAGTCCCTGCAGCCAGATTACTCTGAGCGGCTGTGCCTGGTGTCCGAGA TCCCACCCAAGGGAGGCGCCCTGGGAGAGGGACCAGGAGCCAGCCCTTGCAACCAGCAC TCCCCATATTGGGCCCCTCCATGTTACACACTGAAGCCCGAGACC	FKBP12:IL2RG nucleotide
57.	MPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSS RDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLV FDVELLKLGEGSNTSKENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKN LEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQH SPYWAPPCYTLKPET	FKBP12:IL2RG polypeptide

58.	GCCACAAACTTCAGCCTGCTGAAGCAAGCAGGCGACGTGGAAGAAAATCCAGGACCT	P2A nucleotide
59.	ATGGCACTGCCTGTGACCGCCCTGCTGCTGCCACTGGCCCTGCTGCTGCACGCAGCAAG GCCTATCCTGTGGCACGAAATGTGGCATGAAGGCCTGGAGGAGGCAAGCAGGCTGTATT TTGGCGAGCGGAATGTGAAAGGAATGTTTGAAGTCCTGGAACCTCTGCATGCCATGATG GAAAGGGGACCACAGACACTGAAGGAGACCAGCTTTAACCAGGCCTATGGAAGGGACCT GATGGAGGCACAGGAGTGGTGCCGGAAGTACATGAAGTCCGGAAATGTGAAAGACCTGC TGCAGGCCTGGGATCTGTATTATCACGTGTTCAGGCGCATCTCTAAGGGCAAGGATACC ATCCCCTGGCTGGGACACCTGCTGGTGGGACTGAGCGGAGCCTTTGGCTTCATCATCCT GGTGTACCTGCTGATCAACTGCAGAAATACCGGCCCTTGGCTGAAGAAGGTGCTGAAGT GTAACACACCAGACCCCTCCAAGTTCTTTTCTCAGCTGTCTAGCGAGCACGGCGGCGAT GTGCAGAAGTGGCTGTCCTCTCCTTTTCCAAGCTCCTCTTTCAGCCCAGGAGGACTGGC ACCAGAGATCTCCCCCCTGGAGGTGCTGGAGAGGGACAAGGTGACCCAGCTGCTGCTGC AGCAGGATAAGGTGCCCGAGCCTGCCAGCCTGAGCTCCAACCACAGCCTGACATCCTGC TTTACCAATCAGGGCTATTTCTTTTTCCACCTGCCAGACGCCCTGGAGATCGAGGCCTG TCAGGTGTACTTCACCTATGATCCATACTCCGAAGAGGACCCAGATGAGGGAGTGGCCG GCGCCCCCACAGGCTCTAGCCCACAGCCACTGCAGCCTCTGTCTGGAGAGGACGATGCC TACTGTACCTTTCCCAGCCGCGACGATCTGCTGCTGTTCAGCCCTTCCCTGCTGGGAGG ACCATCTCCACCTAGCACAGCACCAGGAGGAAGCGGGGCAGGGGAGGAGCGGATGCCAC CATCTCTGCAGGAGAGGGTGCCTAGGGACTGGGATCCTCAGCCACTGGGACCTCCAACC CCTGGAGTGCCAGACCTGGTGGATTTTCAGCCCCCTCCAGAGCTGGTGCTGCGGGAGGC AGGAGAGGAGGTGCCTGACGCAGGACCACGCGAGGGCGTGAGCTTTCCATGGTCCAGGC CACCTGGACAGGGAGAGTTCAGAGCCCTGAACGCCAGGCTGCCTCTGAATACAGACGCC TATCTGTCTCTGCAGGAGCTGCAGGGCCAGGATCCAACCCACCTGGTG	FRB:IL2RB nucleotide
60.	MALPVTALLLPLALLLHAARPILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMM ERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGKDT IPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGD VQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSC FTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDA YCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPT PGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA YLSLQELQGQDPTHLV	FRB:IL2RB polypeptide
61.	GGGCAAGGATACCATCCCCTGGCTGGGACACCTGCTGGTGGGACTGAGCGGAGCCTTTG GCTTCATCATCCTGGTGTACCTGCTGATCAACTGCAGAAATACCGGCCCTTGGCTGAAG AAGGTGCTGAAGTGTAACACACCAGACCCCTCCAAGTTCTTTTCTCAGCTGTCTAGCGA GCACGGCGGCGATGTGCAGAAGTGGCTGTCCTCTCCTTTTCCAAGCTCCTCTTTCAGCC CAGGAGGACTGGCACCAGAGATCTCCCCCCTGGAGGTGCTGGAGAGGGACAAGGTGACC CAGCTGCTGCTGCAGCAGGATAAGGTGCCCGAGCCTGCCAGCCTGAGCTCCAACCACAG CCTGACATCCTGCTTTACCAATCAGGGCTATTTCTTTTTCCACCTGCCAGACGCCCTGG AGATCGAGGCCTGTCAGGTGTACTTCACCTATGATCCATACTCCGAAGAGGACCCAGAT GAGGGAGTGGCCGGCGCCCCCACAGGCTCTAGCCCACAGCCACTGCAGCCTCTGTCTGG AGAGGACGATGCCTACTGTACCTTTCCCAGCCGCGACGATCTGCTGCTGTTCAGCCCTT CCCTGCTGGGAGGACCATCTCCACCTAGCACAGCACCAGGAGGAAGCGGGGCAGGGGAG GAGCGGATGCCACCATCTCTGCAGGAGAGGGTGCCTAGGGACTGGGATCCTCAGCCACT GGGACCTCCAACCCCTGGAGTGCCAGACCTGGTGGATTTTCAGCCCCCTCCAGAGCTGG TGCTGCGGGAGGCAGGAGAGGAGGTGCCTGACGCAGGACCACGCGAGGGCGTGAGCTTT CCATGGTCCAGGCCACCTGGACAGGGAGAGTTCAGAGCCCTGAACGCCAGGCTGCCTCT GAATACAGACGCCTATCTGTCTCTGCAGGAGCTGCAGGGCCAGGATCCAACCCACCTGG TG	IL2RB nucleotide
62.	GKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSE HGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHS LTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSG EDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPL	IL2RB polypeptide

	GPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPL NTDAYLSLQELQGQDPTHLV	
63.	CTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGAT CCCCTCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAA TCAGCTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAG CACCCTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGT GCCTGATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGAC TGCAGTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAG TTTCTGTTCGGCACCGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAA GCCAGGATCTGGAGAGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAGGAA ACCTGGTGCAGCCAGGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACCTTC GGCTCTTTTAGCATGTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGTGGC CGGCCTGAGCGCCCGGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCTTCA CCATCTCCCGGGATAACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGGGTG GAGGACACAGCCGTGTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTGGGG CCACTTTTACTCTTATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCCA CCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTG TCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACT GGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGC TCCTGAGCCTGGTGATCACACTGTACTGCAAGCGCGGCCGGAAGAAGCTGCTCTACATC TTTAAGCAGCCATTCATGCGCCCCGTGCAGACCACACAGGAGGAGGACGGCTGCTCCTG TCGGTTTCCAGAGGAGGAGGAGGGAGGATGTGAGCTGAGAGTGAAGTTCTCTAGGAGCG CCGATGCCCCTGCCTATCAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTGGGC CGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGGAAGAGACCCAGAGATGGGAGG CAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTATAATGAGCTGCAGAAGGACAAGA TGGCCGAGGCCTACTCCGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACAC GATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGGACACATACGATGCCCTGCACAT GCAGGCCCTGCCTCCAAGGTGA	TagCAR nucleotide
64.	TCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAATCAG CTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAGCACC CTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGTGCCT GATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCA GTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTC TGTTCGGCACCGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCA GGATCTGGAGAGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAGGAAACCT GGTGCAGCCAGGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACCTTCGGCT CTTTTAGCATGTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGTGGCCGGC CTGAGCGCCCGGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCTTCACCAT CTCCCGGGATAACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGGGTGGAGG ACACAGCCGTGTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTGGGGCCAC TTTTACTCTTATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCC	E2 scFv nucleotide
65.	TCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAATCAG CTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAGCACC CTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGTGCCT GATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCA GTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTC TGTTCGGCACCGGCACAAAGCTGACCGTGCTG	E2 scFv VL nucleotide
66.	GGCTCTACAAGCGGATCCGGCAAGCCAGGATCTGGAGAGGGCAGCACAAAGGGA	E2 scFv linker nucleotide
67.	CAGGTGCAGCTGGTGGAGAGCGGAGGAAACCTGGTGCAGCCAGGAGGCTCCCTGCGCCT GTCTTGTGCCGCCAGCGGCTTTACCTTCGGCTCTTTTAGCATGTCCTGGGTGCGCCAGG CACCTGGAGGAGGACTGGAGTGGGTGGCCGGCCTGAGCGCCCGGTCTAGCCTGACACAC TATGCCGACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGATAACGCCAAGAATAGCGT	E2 scFv VH nucleotide

	GTACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGTGTACTATTGCGCCAGGC GCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTTATATGGACGTGTGGGGA CAGGGCACCCTGGTGACAGTGAGCTCC	
68.	ACCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCT GTCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGAC TGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTG CTCCTGAGCCTGGTGATCACACTGTACTGC	CD8 HTM nucleotide
69.	AAGCGCGGCCGGAAGAAGCTGCTCTACATCTTTAAGCAGCCATTCATGCGCCCCGTGCA GACCACACAGGAGGAGGACGGCTGCTCCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGAT GTGAGCTG	41BB nucleotide
70.	AGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCCTATCAGCAGGGACAGAACCAGCT GTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGG GAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTAT AATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACTCCGAGATCGGCATGAAGGGAGA GAGGCGCCGGGGCAAGGGACACGATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGG ACACATACGATGCCCTGCACATGCAGGCCCTGCCTCCAAGG	CD3ζ nucleotide
71.	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAG GCCGGATGTCGTGATGACCCAGACCCCCCTCAGCCTCCCAGTGTCCCTCGGTGACCAGG CTTCTATTAGTTGCAGATCCAGCCAGTCCCTCGTGCACTCTAACGGTAATACCTACCTG AGATGGTATCTCCAGAAGCCCGGACAGAGCCCTAAGGTGCTGATCTACAAAGTCTCCAA CCGGGTGTCTGGAGTCCCTGACCGCTTCTCAGGGAGCGGTTCCGGCACCGACTTCACCC TGAAGATCAACCGGGTGGAGGCCGAAGACCTCGGCGTCTATTTCTGCTCTCAGAGTACA CATGTGCCCTGGACCTTCGGCGGAGGGACCAAGCTGGAGATCAAAAGCTCCGCAGACGA TGCCAAGAAAGATGCCGCTAAGAAAGACGATGCTAAGAAAGACGATGCAAAGAAAGACG GTGGCGTGAAGCTGGATGAAACCGGAGGAGGTCTCGTCCAGCCAGGAGGAGCCATGAAG CTGAGTTGCGTGACCAGCGGATTCACCTTTGGGCACTACTGGATGAACTGGGTGCGACA GTCCCCAGAGAAGGGGCTCGAATGGGTCGCTCAGTTCAGGAACAAACCCTACAATTATG AGACATACTATTCAGACAGCGTGAAGGGCAGGTTTACTATCAGTAGAGACGATTCCAAA TCTAGCGTGTACCTGCAGATGAACAATCTCAGGGTCGAAGATACAGGCATCTACTATTG CACAGGGGCATCCTATGGTATGGAGTATCTCGGTCAGGGGACAAGCGTCACAGTCAGTT TCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGCGACCACCAACA CCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGC GGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGG CGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGC AACCACAGGAACCGTTTCTCTGTTGTTAAACGGGGCAGAAAGAAACTCCTGTATATATT CAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCC GATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCA GACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACG AAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAA AGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATG GCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGA TGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGC AGGCCCTGCCCCCTCGCTAA	TagCAR nucleotide
72.	MALPVTALLLPLALLLHAARPDVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYL RWYLQKPGQSPKVLIYKVSNRVSGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFCSQST HVPWTFGGGTKLEIKSSADDAKKDAAKKDDAKKDDAKKDGGVKLDETGGGLVQPGGAMK LSCVTSGFTFGHYWMNWVRQSPEKGLEWVAQFRNKPYNYETYYSDSVKGRFTISRDDSK SSVYLQMNNLRVEDTGIYYCTGASYGMEYLGQGTSVTVSFVPVFLPAKPTTTPAPRPPT PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC NHRNRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	TagCAR polypeptide
73.	GCCACCATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGC CGCCAGGCCGGATGTCGTGATGACCCAGACCCCCCTCAGCCTCCCAGTGTCCCTCGGTG	Nucleotide insert

	ACCAGGCTTCTATTAGTTGCAGATCCAGCCAGTCCCTCGTGCACTCTAACGGTAATACC TACCTGAGATGGTATCTCCAGAAGCCCGGACAGAGCCCTAAGGTGCTGATCTACAAAGT CTCCAACCGGGTGTCTGGAGTCCCTGACCGCTTCTCAGGGAGCGGTTCCGGCACCGACT TCACCCTGAAGATCAACCGGGTGGAGGCCGAAGACCTCGGCGTCTATTTCTGCTCTCAG AGTACACATGTGCCCTGGACCTTCGGCGGAGGGACCAAGCTGGAGATCAAAAGCTCCGC AGACGATGCCAAGAAAGATGCCGCTAAGAAAGACGATGCTAAGAAAGACGATGCAAAGA AAGACGGTGGCGTGAAGCTGGATGAAACCGGAGGAGGTCTCGTCCAGCCAGGAGGAGCC ATGAAGCTGAGTTGCGTGACCAGCGGATTCACCTTTGGGCACTACTGGATGAACTGGGT GCGACAGTCCCCAGAGAAGGGGCTCGAATGGGTCGCTCAGTTCAGGAACAAACCCTACA ATTATGAGACATACTATTCAGACAGCGTGAAGGGCAGGTTTACTATCAGTAGAGACGAT TCCAAATCTAGCGTGTACCTGCAGATGAACAATCTCAGGGTCGAAGATACAGGCATCTA CTATTGCACAGGGGCATCCTATGGTATGGAGTATCTCGGTCAGGGGACAAGCGTCACAG TCAGTTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGCGACCA CCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCG GCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTACA TCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTT TACTGCAACCACAGGAACCGTTTCTCTGTTGTTAAACGGGGCAGAAAGAAACTCCTGTA TATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTA GCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGG AGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCT AGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGG GGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGAT AAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGG GCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTC ACATGCAGGCCCTGCCCCCTCGCTAA	
74.	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLRWYLQKPGQSPKVLIYKVSNR VSGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFCSQSTHVPWTFGGGTKLEIKSSADDA KKDAAKKDDAKKDDAKKDGGVKLDETGGGLVQPGGAMKLSCVTSGFTFGHYWMNWVRQS PEKGLEWVAQFRNKPYNYETYYSDSVKGRFTISRDDSKSSVYLQMNNLRVEDTGIYYCT GASYGMEYLGQGTSVTVSFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRFSVVKRGRKKLLYIFK QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR	TagCAR amino acid sequence without signal peptide
75.	MALPVTALLLPLALLLHAARP	CAR amino acid signal peptide
76.	MDPQCTMGLSNILFVMAFLLSGAAPLKIQAYFNETADLPCQFANSQNQSLSELVVFWQD QENLVLNEVYLGKEKFDSVHSKYMGRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPT GMIRIHQMNSELSVLANFSQPEIVPISNITENVYINLTCSSIHGYPEPKKMSVLLRTKN STIEYDGVMQKSQDNVTELYDVSISLSVSFPDVTSNMTIFCILETDKTRLLSSPFSIEL EDPQPPPDHIPWITAVLPTVIICVMVFCLILWKWKKKKRPRNSYKCGTNTMEREESEQT KKREKIHIPERSDEAQRVFKSSKTSSCDKSDTCF	CD86 amino acid
77.	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSGVIHVTKEVKEVATLSCGHNVSVEEL AQTRIYWQKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVV LKYEKDAFKREHLAEVTLSVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEPHLSWLE NGEELNAINTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTTKQ EHFPDNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV	CD80 amino acid
78.	ATGGATCCCCAGTGCACTATGGGACTGAGTAACATTCTCTTTGTGATGGCCTTCCTGCT CTCTGGTGCTGCTCCTCTGAAGATTCAAGCTTATTTCAATGAGACTGCAGACCTGCCAT GCCAATTTGCAAACTCTCAAAACCAAAGCCTGAGTGAGCTAGTAGTATTTTGGCAGGAC CAGGAAAACTTGGTTCTGAATGAGGTATACTTAGGCAAAGAGAAATTTGACAGTGTTCA TTCCAAGTATATGGGCCGCACAAGTTTTGATTCGGACAGTTGGACCCTGAGACTTCACA	CD86 nucleotide

	ATCTTCAGATCAAGGACAAGGGCTTGTATCAATGTATCATCCATCACAAAAAGCCCACA GGAATGATTCGCATCCACCAGATGAACTCTGAACTGTCAGTGCTTGCTAACTTCAGTCA ACCTGAAATAGTACCAATTTCTAATATAACAGAAAATGTGTACATAAATTTGACCTGCT CATCTATACACGGTTACCCAGAACCTAAGAAGATGAGTGTTTTGCTAAGAACCAAGAAC TCAACTATCGAGTATGATGGTGTTATGCAGAAATCTCAAGATAATGTCACAGAACTGTA CGACGTTTCCATCAGCTTGTCTGTTTCATTCCCTGATGTTACGAGCAATATGACCATCT TCTGTATTCTGGAAACTGACAAGACGCGGCTTTTATCTTCACCTTTCTCTATAGAGCTT GAGGACCCTCAGCCTCCCCCAGACCACATTCCTTGGATTACAGCTGTACTTCCAACAGT TATTATATGTGTGATGGTTTTCTGTCTAATTCTATGGAAATGGAAGAAGAAGAAGCGGC CTCGCAACTCTTATAAATGTGGAACCAACACAATGGAGAGGGAAGAGAGTGAACAGACC AAGAAAAGAGAAAAAATCCATATACCTGAAAGGTCTGATGAAGCCCAGCGTGTTTTTAA AAGTTCGAAGACATCTTCATGCGACAAAAGTGATACATGTTTT	
79.	ATGGGCCACACACGGAGGCAGGGAACATCACCATCCAAGTGTCCATACCTCAATTTCTT TCAGCTCTTGGTGCTGGCTGGTCTTTCTCACTTCTGTTCAGGTGTTATCCACGTGACCA AGGAAGTGAAAGAAGTGGCAACGCTGTCCTGTGGTCACAATGTTTCTGTTGAAGAGCTG GCACAAACTCGCATCTACTGGCAAAAGGAGAAGAAAATGGTGCTGACTATGATGTCTGG GGACATGAATATATGGCCCGAGTACAAGAACCGGACCATCTTTGATATCACTAATAACC TCTCCATTGTGATCCTGGCTCTGCGCCCATCTGACGAGGGCACATACGAGTGTGTTGTT CTGAAGTATGAAAAAGACGCTTTCAAGCGGGAACACCTGGCTGAAGTGACGTTATCAGT CAAAGCTGACTTCCCTACACCTAGTATATCTGACTTTGAAATTCCAACTTCTAATATTA GAAGGATAATTTGCTCAACCTCTGGAGGTTTTCCAGAGCCTCACCTCTCCTGGTTGGAA AATGGAGAAGAATTAAATGCCATCAACACAACAGTTTCCCAAGATCCTGAAACTGAGCT CTATGCTGTTAGCAGCAAACTGGATTTCAATATGACAACCAACCACAGCTTCATGTGTC TCATCAAGTATGGACATTTAAGAGTGAATCAGACCTTCAACTGGAATACAACCAAGCAA GAGCATTTTCCTGATAACCTGCTCCCATCCTGGGCCATTACCTTAATCTCAGTAAATGG AATTTTTGTGATATGCTGCCTGACCTACTGCTTTGCCCCAAGATGCAGAGAGAGAAGGA GGAATGAGAGATTGAGAAGGGAAAGTGTACGCCCTGTA	CD80 nucleotide
80.	MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQ KDKVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKFFL YVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHRGLIMYSWDCPMEQCKRNSTSIYFKM ENDLPQKIQCTLSNPLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLYMNG ILKCDRKPDRTNSN	CD58 amino acid
81.	ATGGTTGCTGGGAGCGACGCGGGGCGGGCCCTGGGGGTCCTCAGCGTGGTCTGCCTGCT GCACTGCTTTGGTTTCATCAGCTGTTTTTCCCAACAAATATATGGTGTTGTGTATGGGA ATGTAACTTTCCATGTACCAAGCAATGTGCCTTTAAAAGAGGTCCTATGGAAAAAACAA AAGGATAAAGTTGCAGAACTGGAAAATTCTGAGTTCAGAGCTTTCTCATCTTTTAAAAA TAGGGTTTATTTAGACACTGTGTCAGGTAGCCTCACTATCTACAACTTAACATCATCAG ATGAAGATGAGTATGAAATGGAATCGCCAAATATTACTGATACCATGAAGTTCTTTCTT TATGTGCTTGAGTCTCTTCCATCTCCCACACTAACTTGTGCATTGACTAATGGAAGCAT TGAAGTCCAATGCATGATACCAGAGCATTACAACAGCCATCGAGGACTTATAATGTACT CATGGGATTGTCCTATGGAGCAATGTAAACGTAACTCAACCAGTATATATTTTAAGATG GAAAATGATCTTCCACAAAAAATACAGTGTACTCTTAGCAATCCATTATTTAATACAAC ATCATCAATCATTTTGACAACCTGTATCCCAAGCAGCGGTCATTCAAGACACAGATATG CACTTATACCCATACCATTAGCAGTAATTACAACATGTATTGTGCTGTATATGAATGGT ATTCTGAAATGTGACAGAAAACCAGACAGAACCAACTCCAAT	CD58 nucleotide
82.	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCT GATCCCCTCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGA CAATCAGCTGTTCCGGCTCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAG CAGCACCCTGGCAAGGCCCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGG AGTGCCTGATCGGTTCTCCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCG GACTGCAGTCCGAGGACGAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCC GAGTTTCTGTTCGGCACCGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGG CAAGCCAGGATCTGGAGAGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAG GAAACCTGGTGCAGCCAGGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACC	TagCAR nucleotide

	TTCGGCTCTTTTAGCATGTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGT GGCCGGCCTGAGCGCCCGGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCT TCACCATCTCCCGGGATAACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGG GTGGAGGACACAGCCGTGTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTG GGGCCACTTTTACTCTTATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCT CCACCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCT CTGTCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGG ACTGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGC TGCTCCTGAGCCTGGTGATCACACTGTACTGCAAGCGCGGCCGGAAGAAGCTGCTCTAC ATCTTTAAGCAGCCATTCATGCGCCCCGTGCAGACCACACAGGAGGAGGACGGCTGCTC CTGTCGGTTTCCAGAGGAGGAGGAGGGAGGATGTGAGCTGAGAGTGAAGTTCTCTAGGA GCGCCGATGCCCCTGCCTATCAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTG GGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGGAAGAGACCCAGAGATGGG AGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTATAATGAGCTGCAGAAGGACA AGATGGCCGAGGCCTACTCCGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGA CACGATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGGACACATACGATGCCCTGCA CATGCAGGCCCTGCCTCCAAGG	
83.	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCT GATCCCCGATATCGTGCTGACCCAGTCCCCCGCCATCCTGTCCGCCTCTCCTGGAGAGA AGGTGACCATGACATGTCGGGCCAGCTCCTCTGTGAACTACATGGACTGGTATCAGAAG AAGCCTGGCAGCTCCCCCAAGCCTTGGATCTACGCCACCTCCAATCTGGCCTCTGGAGT GCCAGCAAGATTCAGCGGATCCGGATCTGGCACAAGCTATTCCCTGACCATCTCCAGGG TGGAGGCAGAGGATGCAGCAACATACTATTGCCAGCAGTGGTCTTTCAACCCCCCTACA TTTGGCGGCGGCACCAAGCTGGAGATCAAGGGCTCTACCAGCGGAGGAGGAAGCGGAGG AGGATCCGGAGGCGGCGGCTCTAGCGAGGTGCAGCTGCAGCAGTCCGGAGCAGAGCTGG TGAAGCCTGGAGCCTCTGTGAAGATGAGCTGTAAGGCCTCCGGCTACACCTTCACATCT TATAATATGCACTGGGTGAAGCAGACACCAGGACAGGGACTGGAGTGGATCGGAGCAAT CTACCCTGGCAACGGCGACACCAGCTATAATCAGAAGTTTAAGGGCAAGGCCACCCTGA CAGCCGATAAGTCCTCTAGCACAGCCTACATGCAGCTGTCCTCTCTGACCAGCGAGGAC TCCGCCGATTACTATTGCGCCCGGTCCAACTACTATGGCAGCTCCTATTGGTTCTTTGA CGTGTGGGGAGCAGGAACAACCGTGACCGTGTCTAGCGCTGCAGGAGGCGGAGGATCTG GAGGCGGCGGCGGAGACTACAAAGACGATGACGACAAGTTCGAAGCAAAGCCAACCACC ACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCT GAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACTGGATT TCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTG AGCCTGGTGATCACACTGTACTGCAAGAGAGGCAGGAAGAAGCTGCTGTATATCTTTAA GCAGCCCTTCATGCGCCCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGTCGGT TTCCAGAGGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCAGCCGGTCCGCCGAT GCCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAG AGAGGAGTACGACGTGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGGGAGGCAAGC CTCGGAGAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCC GAGGCCTATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGG CCTGTACCAGGGCCTGAGCACCGCCACAAAGGACACCTATGATGCCCTGCACATGCAGG CCCTGCCCCCTCGG	CD20 CAR nucleotide (with flag)
84.	MLLLVTSLLLCELPHPAFLLIPDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQK KPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPT FGGGTKLEIKGSTSGGGSGGGSGGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTS YNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSED SADYYCARSNYYGSSYWFFDVWGAGTTVTVSSAAGGGSGGGGGDYKDDDDKFEAKPTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	CD20 CAR polypeptide (with flag sequence)

85.	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCT GATCCCCGATATCGTGCTGACCCAGTCCCCCGCCATCCTGTCCGCCTCTCCTGGAGAGA AGGTGACCATGACATGTCGGGCCAGCTCCTCTGTGAACTACATGGACTGGTATCAGAAG AAGCCTGGCAGCTCCCCCAAGCCTTGGATCTACGCCACCTCCAATCTGGCCTCTGGAGT GCCAGCAAGATTCAGCGGATCCGGATCTGGCACAAGCTATTCCCTGACCATCTCCAGGG TGGAGGCAGAGGATGCAGCAACATACTATTGCCAGCAGTGGTCTTTCAACCCCCCTACA TTTGGCGGCGGCACCAAGCTGGAGATCAAGGGCTCTACCAGCGGAGGAGGAAGCGGAGG AGGATCCGGAGGCGGCGGCTCTAGCGAGGTGCAGCTGCAGCAGTCCGGAGCAGAGCTGG TGAAGCCTGGAGCCTCTGTGAAGATGAGCTGTAAGGCCTCCGGCTACACCTTCACATCT TATAATATGCACTGGGTGAAGCAGACACCAGGACAGGGACTGGAGTGGATCGGAGCAAT CTACCCTGGCAACGGCGACACCAGCTATAATCAGAAGTTTAAGGGCAAGGCCACCCTGA CAGCCGATAAGTCCTCTAGCACAGCCTACATGCAGCTGTCCTCTCTGACCAGCGAGGAC TCCGCCGATTACTATTGCGCCCGGTCCAACTACTATGGCAGCTCCTATTGGTTCTTTGA CGTGTGGGGAGCAGGAACAACCGTGACCGTGTCTAGCGCAAAGCCAACCACCACACCTG CTCCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCTGAGACCT GAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTG CGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTGAGCCTGG TGATCACACTGTACTGCAAGAGAGGCAGGAAGAAGCTGCTGTATATCTTTAAGCAGCCC TTCATGCGCCCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGTCGGTTTCCAGA GGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCAGCCGGTCCGCCGATGCCCCTG CCTACCAGCAGGGCCAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGGAG TACGACGTGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGGGAGGCAAGCCTCGGAG AAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCT ATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTAC CAGGGCCTGAGCACCGCCACAAAGGACACCTATGATGCCCTGCACATGCAGGCCCTGCC CCCTCGG	CD20 CAR nucleotide (without flag)
86.	MLLLVTSLLLCELPHPAFLLIPDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQK KPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPT FGGGTKLEIKGSTSGGGSGGGSGGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTS YNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSED SADYYCARSNYYGSSYWFFDVWGAGTTVTVSSAKPTTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR	CD20 CAR polypeptide (without flag sequence)
87.	GATATCGTGCTGACCCAGTCCCCCGCCATCCTGTCCGCCTCTCCTGGAGAGAAGGTGAC CATGACATGTCGGGCCAGCTCCTCTGTGAACTACATGGACTGGTATCAGAAGAAGCCTG GCAGCTCCCCCAAGCCTTGGATCTACGCCACCTCCAATCTGGCCTCTGGAGTGCCAGCA AGATTCAGCGGATCCGGATCTGGCACAAGCTATTCCCTGACCATCTCCAGGGTGGAGGC AGAGGATGCAGCAACATACTATTGCCAGCAGTGGTCTTTCAACCCCCCTACATTTGGCG GCGGCACCAAGCTGGAGATCAAGGGCTCTACCAGCGGAGGAGGAAGCGGAGGAGGATCC GGAGGCGGCGGCTCTAGCGAGGTGCAGCTGCAGCAGTCCGGAGCAGAGCTGGTGAAGCC TGGAGCCTCTGTGAAGATGAGCTGTAAGGCCTCCGGCTACACCTTCACATCTTATAATA TGCACTGGGTGAAGCAGACACCAGGACAGGGACTGGAGTGGATCGGAGCAATCTACCCT GGCAACGGCGACACCAGCTATAATCAGAAGTTTAAGGGCAAGGCCACCCTGACAGCCGA TAAGTCCTCTAGCACAGCCTACATGCAGCTGTCCTCTCTGACCAGCGAGGACTCCGCCG ATTACTATTGCGCCCGGTCCAACTACTATGGCAGCTCCTATTGGTTCTTTGACGTGTGG GGAGCAGGAACAACCGTGACCGTGTCTAGC	CD20 scFv nucleotide
88.	DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPA RFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGSTSGGGSGGGS GGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYP GNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVW GAGTTVTVSS	CD20 scFv polypeptide

89.	GATATCGTGCTGACCCAGTCCCCCGCCATCCTGTCCGCCTCTCCTGGAGAGAAGGTGAC CATGACATGTCGGGCCAGCTCCTCTGTGAACTACATGGACTGGTATCAGAAGAAGCCTG GCAGCTCCCCCAAGCCTTGGATCTACGCCACCTCCAATCTGGCCTCTGGAGTGCCAGCA AGATTCAGCGGATCCGGATCTGGCACAAGCTATTCCCTGACCATCTCCAGGGTGGAGGC AGAGGATGCAGCAACATACTATTGCCAGCAGTGGTCTTTCAACCCCCCTACATTTGGCG GCGGCACCAAGCTGGAGATCAAG	CD20 scFv VL nucleotide
90.	DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGVPA RFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIK	CD20 scFv VL polypeptide
91.	GGCTCTACCAGCGGAGGAGGAAGCGGAGGAGGATCCGGAGGCGGCGGCTCTAGC	218 linker nucleotide
92.	GSTSGGGSGGGSGGGGSS	218 linker polypeptide
93.	GAGGTGCAGCTGCAGCAGTCCGGAGCAGAGCTGGTGAAGCCTGGAGCCTCTGTGAAGAT GAGCTGTAAGGCCTCCGGCTACACCTTCACATCTTATAATATGCACTGGGTGAAGCAGA CACCAGGACAGGGACTGGAGTGGATCGGAGCAATCTACCCTGGCAACGGCGACACCAGC TATAATCAGAAGTTTAAGGGCAAGGCCACCCTGACAGCCGATAAGTCCTCTAGCACAGC CTACATGCAGCTGTCCTCTCTGACCAGCGAGGACTCCGCCGATTACTATTGCGCCCGGT CCAACTACTATGGCAGCTCCTATTGGTTCTTTGACGTGTGGGGAGCAGGAACAACCGTG ACCGTGTCTAGC	CD20 scFv VH nucleotide
94.	EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTS YNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTV TVSS	CD20 scFv VH polypeptide
95.	GACTACAAAGACGATGACGACAAG	Flag nucleotide
96.	DYKDDDDK	Flag polypeptide
97.	GCAAAGCCAACCACCACACCTGCTCCTAGACCACCTACACCCGCTCCTACCATCGCCAG CCAGCCTCTGTCTCTGAGACCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACA CCAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGC GGCGTGCTGCTCCTGAGCCTGGTGATCACACTGTACTGC	CD8 HTM nucleotide
98.	AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYC	CD8 HTM polypeptide
99.	AAGAGAGGCAGGAAGAAGCTGCTGTATATCTTTAAGCAGCCCTTCATGCGCCCTGTGCA GACCACACAGGAGGAGGACGGCTGCAGCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGAT GCGAGCTG	41BB nucleotide
100	CGCGTGAAGTTCAGCCGGTCCGCCGATGCCCCTGCCTACCAGCAGGGCCAGAACCAGCT GTATAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGG GAAGGGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTAC AATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTATTCTGAGATCGGCATGAAGGGAGA GAGGCGCCGGGGCAAGGGACACGATGGCCTGTACCAGGGCCTGAGCACCGCCACAAAGG ACACCTATGATGCCCTGCACATGCAGGCCCTGCCCCCTCGG	CD3ζ nucleotide
101	ACATGTCTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCAC TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC AGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGT CAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGA GCTCGTTTAGTGAACCGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTG GCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTA GTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC	Construct C.3U Full Vector nucleotide

AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACC
AGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGG
GGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGAT
GGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCG
GTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGG
AGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA
ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATA
TAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGG
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GCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTAT
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AGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTT
GGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGAC
AATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA
CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC
TGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAAC
TCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAG
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TGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGG
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AGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCTCGACGGTATCGGTTAACTTT
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GATTGCCTGACGCGTAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGGATCAAGGT
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AATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGC
GCTTCTGCTCCCCGAGCTCTATATAAGAGCCCACAACCCCTCACTCGGCGCGTGAACAC
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CCACCCTGAAGAACCTGGAGGACCTGGTGACCGAGTACCACGGCAACTTCAGCGCCTGG
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CCTGGTGAGCGAGATTCCCCCTAAGGGCGGGGCCTTGGGTGAGGGACCCGGGGCAAGCC
CGTGCAATCAGCACAGCCCCTACTGGGCCCCCCCCTGTTACACCCTGAAGCCCGAGACC
GGCAGCGGAGCCACCAATTTCAGCCTCCTGAAACAAGCCGGTGACGTTGAAGAGAACCC
CGGCCCCATGCCCCTGGGGTTGCTGTGGTTGGGACTCGCCCTCCTCGGCGCCCTGCACG

CTCAAGCCGGCGTTCAAGTGGAGACTATCTCCCCCGGCGACGGCAGAACCTTCCCTAAG
AGAGGGCAGACCTGCGTGGTGCACTACACCGGCATGCTGGAGGACGGCAAGAAGTTCGA
CAGCAGCAGAGACAGAAACAAGCCCTTCAAGTTCATGCTGGGCAAGCAAGAGGTGATCA
GAGGCTGGGAGGAGGGTGTGGCCCAAATGAGCGTGGGGCAGAGAGCCAAATTGACCATC
TCACCCGACTACGCTTACGGGGCCACCGGCCATCCCGGCATCATCCCCCCCCACGCCAC
CCTGGTGTTCGACGTGGAGCTGCTGAAGCTGGGCGAGGGCAAGGACACCATCCCCTGGC
TGGGCCACCTGCTGGTGGGCCTGAGCGGCGCCTTCGGCTTCATCATCCTGGTGTACCTG
CTGATCAACTGCAGAAACACCGGCCCCTGGCTGAAGAAGGTGCTGAAGTGCAACACCCC
CGACCCTAGCAAGTTCTTCTCTCAGCTGAGCAGCGAGCACGGCGGCGACGTGCAGAAGT
GGCTGAGCAGCCCATTTCCTAGCAGCAGCTTTAGCCCCGGCGGCCTAGCTCCCGAGATC
AGCCCCCTGGAAGTACTGGAGAGAGATAAGGTGACACAGCTGCTGCTGCAGCAAGACAA
GGTGCCCGAGCCCGCTAGCCTGAGCAGCAACCACAGCCTGACAAGCTGCTTCACCAACC
AAGGCTACTTCTTCTTCCACCTGCCCGACGCCCTGGAGATCGAGGCCTGCCAAGTGTAC
TTCACCTACGATCCGTACAGCGAGGAAGACCCCGACGAGGGAGTGGCCGGCGCCCCTAC
CGGCAGCTCGCCACAACCGCTGCAACCTCTGAGCGGCGAGGACGACGCCTACTGCACCT
TCCCTAGCAGAGATGATCTGCTGCTCTTTTCCCCTAGCTTGCTGGGGGGCCCTTCGCCT
CCAAGTACTGCCCCCGGAGGTAGCGGGGCCGGCGAAGAAAGAATGCCCCCAAGCCTGCA
AGAAAGAGTGCCTAGAGACTGGGACCCTCAACCACTGGGCCCACCCACTCCGGGCGTGC
CCGACCTGGTAGATTTTCAGCCCCCTCCCGAGCTGGTGCTACGCGAGGCCGGCGAAGAG
GTGCCCGACGCTGGGCCAAGAGAGGGTGTGTCATTCCCCTGGAGCCGACCCCCCGGTCA
AGGCGAGTTCAGAGCCCTGAACGCTAGACTGCCCCTGAACACCGACGCCTACCTGAGCC
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TCTCTGCTGAAGCAGGCCGGCGATGTGGAAGAAAACCCTGGACCTCTGCTGCTGGTGAC
CTCCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGATCCCCGACATCCAGA
TGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGCGACAGAGTGACCATCTCCTGT
AGGGCCTCTCAGGATATCAGCAAGTACCTGAACTGGTATCAGCAGAAGCCAGATGGCAC
AGTGAAGCTGCTGATCTACCACACCTCCAGGCTGCACTCTGGAGTGCCAAGCCGGTTCT
CCGGATCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACCTGGAGCAGGAGGAT
ATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATATACATTCGGCGGAGGAAC
CAAGCTGGAGATCACCGGATCCACATCTGGAAGCGGCAAGCCAGGAAGCGGAGAGGGAT
CCACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGGTGGCACCATCCCAG
TCTCTGAGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACTACGGCGTGTCCTG
GATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGTGATCTGGGGCTCTGAGA
CCACATACTATAATTCTGCCCTGAAGAGCCGCCTGACCATCATCAAGGACAACTCCAAG
TCTCAGGTGTTTCTGAAGATGAATAGCCTGCAGACCGACGATACAGCCATCTACTATTG
CGCCAAGCACTACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGCCAGGGCACCT
CCGTGACAGTGTCTAGCGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCGACTTC
TGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGCTGGTGACCGTGGC
CTTTATCATCTTCTGGGTGAAGAGAGGCAGGAAGAAGCTGCTGTATATCTTTAAGCAGC
CCTTCATGCGCCCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGTCGGTTTCCA
GAGGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCAGCCGGTCCGCCGATGCCCC
TGCCTACCAGCAGGGCCAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGG
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AGAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGC
CTATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGT
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CCACCTAGGTGACGCCGGCGCTAGTGTCGACGTAGTGGTACCTTTAAGACCAATGACTT
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AAAGCTTGCCTTGAGTGCTTCAATGTGTGTGTTGGTTTTTTGTGTGTCGTCGTCTCACT
TGGATCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAA
GTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGGGAAATGTGTGCAGCTCTGGCCC

	GTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAAT AAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGG AAACGTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGG GCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGA TGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATG AGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTT ATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATT CCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGT TCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTA TTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTT TGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTT TGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATT TTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCG ATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGA AACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCAT TTGATGCTCGATGAGTTTTTCTAACTGTCAGACCAAGTTTACTCATATATACTTTAGAT TGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATC TCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAA AAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAC AAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTT TTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAG CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCT AATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACT CAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACA CAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATG AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGG TCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGT CCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGG GCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCT GGCCTTTTGCTC	
102	ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTTGGCGAGAGGAA CGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAATGATGGAGAGGGGACCTC AGACACTGAAGGAGACCTCCTTCAACCAGGCCTATGGCAGAGACCTGATGGAGGCCCAG GAGTGGTGCAGGAAGTACATGAAGTCTGGCAATGTGAAGGACCTGCTGCAGGCCTGGGA TCTGTACTATCACGTGTTTCGGAGAATCAGCAAGGCTAGCAGAAGAAAGAGAGGCAGCG GCGAGGGCCGAGGCAGCCTGCTGACCTGCGGTGATGTGGAAGAAAACCCGGGCCCCATG CCCTTGCCCGTGACCGCGTTGCTCCTGCCCTTGGCTCTACTGCTGCACGCCGCTAGACC CATCCTGTGGCACGAGATGTGGCACGAGGGCCTGGAGGAGGCTAGCAGACTGTACTTCG GCGAGAGAAACGTGAAGGGCATGTTCGAGGTGCTGGAGCCCCTGCACGCCATGATGGAG AGAGGCCCTCAGACCCTGAAGGAGACAAGCTTCAACCAAGCCTACGGCAGAGACCTGAT GGAGGCCCAAGAGTGGTGCAGAAAGTACATGAAGAGCGGCAACGTGAAGGACCTGCTGC AAGCCTGGGACCTGTACTACCACGTGTTCAGAAGAATCAGCAAGGGCAGCAATACAAGC AAGGAAAACCCCTTCCTGTTCGCCCTGGAGGCCGTGGTGATCAGCGTGGGCAGCATGGG CCTGATCATCAGCCTGCTGTGCGTGTACTTCTGGCTGGAGAGAACCATGCCTAGAATCC CCACCCTGAAGAACCTGGAGGACCTGGTGACCGAGTACCACGGCAACTTCAGCGCCTGG AGCGGCGTGAGCAAGGGCCTGGCCGAGAGCCTGCAGCCCGACTACAGCGAGCGACTGTG CCTGGTGAGCGAGATTCCCCCTAAGGGCGGGGCCTTGGGTGAGGGACCCGGGGCAAGCC CGTGCAATCAGCACAGCCCCTACTGGGCCCCCCCCTGTTACACCCTGAAGCCCGAGACC GGCAGCGGAGCCACCAATTTCAGCCTCCTGAAACAAGCCGGTGACGTTGAAGAGAACCC CGGCCCCATGCCCCTGGGGTTGCTGTGGTTGGGACTCGCCCTCCTCGGCGCCCTGCACG CTCAAGCCGGCGTTCAAGTGGAGACTATCTCCCCCGGCGACGGCAGAACCTTCCCTAAG AGAGGGCAGACCTGCGTGGTGCACTACACCGGCATGCTGGAGGACGGCAAGAAGTTCGA CAGCAGCAGAGACAGAAACAAGCCCTTCAAGTTCATGCTGGGCAAGCAAGAGGTGATCA GAGGCTGGGAGGAGGGTGTGGCCCAAATGAGCGTGGGGCAGAGAGCCAAATTGACCATC	Construct C.3U nucleotide

	TCACCCGACTACGCTTACGGGGCCACCGGCCATCCCGGCATCATCCCCCCCCACGCCAC CCTGGTGTTCGACGTGGAGCTGCTGAAGCTGGGCGAGGGCAAGGACACCATCCCCTGGC TGGGCCACCTGCTGGTGGGCCTGAGCGGCGCCTTCGGCTTCATCATCCTGGTGTACCTG CTGATCAACTGCAGAAACACCGGCCCCTGGCTGAAGAAGGTGCTGAAGTGCAACACCCC CGACCCTAGCAAGTTCTTCTCTCAGCTGAGCAGCGAGCACGGCGGCGACGTGCAGAAGT GGCTGAGCAGCCCATTTCCTAGCAGCAGCTTTAGCCCCGGCGGCCTAGCTCCCGAGATC AGCCCCCTGGAAGTACTGGAGAGAGATAAGGTGACACAGCTGCTGCTGCAGCAAGACAA GGTGCCCGAGCCCGCTAGCCTGAGCAGCAACCACAGCCTGACAAGCTGCTTCACCAACC AAGGCTACTTCTTCTTCCACCTGCCCGACGCCCTGGAGATCGAGGCCTGCCAAGTGTAC TTCACCTACGATCCGTACAGCGAGGAAGACCCCGACGAGGGAGTGGCCGGCGCCCCTAC CGGCAGCTCGCCACAACCGCTGCAACCTCTGAGCGGCGAGGACGACGCCTACTGCACCT TCCCTAGCAGAGATGATCTGCTGCTCTTTTCCCCTAGCTTGCTGGGGGGCCCTTCGCCT CCAAGTACTGCCCCCGGAGGTAGCGGGGCCGGCGAAGAAAGAATGCCCCCAAGCCTGCA AGAAAGAGTGCCTAGAGACTGGGACCCTCAACCACTGGGCCCACCCACTCCGGGCGTGC CCGACCTGGTAGATTTTCAGCCCCCTCCCGAGCTGGTGCTACGCGAGGCCGGCGAAGAG GTGCCCGACGCTGGGCCAAGAGAGGGTGTGTCATTCCCCTGGAGCCGACCCCCCGGTCA AGGCGAGTTCAGAGCCCTGAACGCTAGACTGCCCCTGAACACCGACGCCTACCTGAGCC TGCAAGAGCTGCAAGGCCAAGACCCCACCCACCTGGTGGGATCCGGAGCAACAAACTTT TCTCTGCTGAAGCAGGCCGGCGATGTGGAAGAAAACCCTGGACCTCTGCTGCTGGTGAC CTCCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGATCCCCGACATCCAGA TGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGCGACAGAGTGACCATCTCCTGT AGGGCCTCTCAGGATATCAGCAAGTACCTGAACTGGTATCAGCAGAAGCCAGATGGCAC AGTGAAGCTGCTGATCTACCACACCTCCAGGCTGCACTCTGGAGTGCCAAGCCGGTTCT CCGGATCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACCTGGAGCAGGAGGAT ATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATATACATTCGGCGGAGGAAC CAAGCTGGAGATCACCGGATCCACATCTGGAAGCGGCAAGCCAGGAAGCGGAGAGGGAT CCACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGGTGGCACCATCCCAG TCTCTGAGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACTACGGCGTGTCCTG GATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGTGATCTGGGGCTCTGAGA CCACATACTATAATTCTGCCCTGAAGAGCCGCCTGACCATCATCAAGGACAACTCCAAG TCTCAGGTGTTTCTGAAGATGAATAGCCTGCAGACCGACGATACAGCCATCTACTATTG CGCCAAGCACTACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGCCAGGGCACCT CCGTGACAGTGTCTAGCGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCGACTTC TGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGCTGGTGACCGTGGC CTTTATCATCTTCTGGGTGAAGAGAGGCAGGAAGAAGCTGCTGTATATCTTTAAGCAGC CCTTCATGCGCCCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGTCGGTTTCCA GAGGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCAGCCGGTCCGCCGATGCCCC TGCCTACCAGCAGGGCCAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGG AGTACGACGTGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGGGAGGCAAGCCTCGG AGAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGC CTATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGT ACCAGGGCCTGAGCACCGCCACAAAGGACACATATGATGCCCTGCACATGCAGGCCCTG CCACCTAGG	
103	MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQ EWCRKYMKSGNVKDLLQAWDLYYHVFRRISKASRRKRGSGEGRGSLLTCGDVEENPGPM PLPVTALLLPLALLLHAARPILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMME RGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSNTS KENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKNLEDLVTEYHGNFSAW SGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET GSGATNFSLLKQAGDVEENPGPMPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPK RGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLGEGKDTIPWLGHLLVGLSGAFGFIILVYL LINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEI SPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVY	Construct C.3U polypeptide

	FTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSP PSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEE VPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLVGSGATNF SLLKQAGDVEENPGPLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISC RASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQED IATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQ SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSK SQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSGAVHTRGLDFACDF WVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR	
104	CTGCTGCTGGTGACCTCCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGAT CCCCGACATCCAGATGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGCGACAGAG TGACCATCTCCTGTAGGGCCTCTCAGGATATCAGCAAGTACCTGAACTGGTATCAGCAG AAGCCAGATGGCACAGTGAAGCTGCTGATCTACCACACCTCCAGGCTGCACTCTGGAGT GCCAAGCCGGTTCTCCGGATCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACC TGGAGCAGGAGGATATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATATACA TTCGGCGGAGGAACCAAGCTGGAGATCACCGGATCCACATCTGGAAGCGGCAAGCCAGG AAGCGGAGAGGGATCCACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGG TGGCACCATCCCAGTCTCTGAGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGAC TACGGCGTGTCCTGGATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGTGAT CTGGGGCTCTGAGACCACATACTATAATTCTGCCCTGAAGAGCCGCCTGACCATCATCA AGGACAACTCCAAGTCTCAGGTGTTTCTGAAGATGAATAGCCTGCAGACCGACGATACA GCCATCTACTATTGCGCCAAGCACTACTATTACGGCGGCTCCTACGCCATGGATTATTG GGGCCAGGGCACCTCCGTGACAGTGTCTAGCGGCGCTGTGCACACCAGAGGACTGGATT TCGCCTGCGACTTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTG CTGGTGACCGTGGCCTTTATCATCTTCTGGGTGAAGAGAGGCAGGAAGAAGCTGCTGTA TATCTTTAAGCAGCCCTTCATGCGCCCTGTGCAGACCACACAGGAGGAGGACGGCTGCA GCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCAGCCGG TCCGCCGATGCCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTATAACGAGCTGAATCT GGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGG GAGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGAC AAGATGGCCGAGGCCTATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGG ACACGATGGCCTGTACCAGGGCCTGAGCACCGCCACAAAGGACACATATGATGCCCTGC ACATGCAGGCCCTGCCACCTAGG	CD19 CAR nucleotide
105	LLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDT AIYYCAKHYYYGGSYAMDYWGQGTSVTVSSGAVHTRGLDFACDFWVLVVVGGVLACYSL LVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	CD19 CAR polypeptide
106	GACATCCAGATGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGCGACAGAGTGAC CATCTCCTGTAGGGCCTCTCAGGATATCAGCAAGTACCTGAACTGGTATCAGCAGAAGC CAGATGGCACAGTGAAGCTGCTGATCTACCACACCTCCAGGCTGCACTCTGGAGTGCCA AGCCGGTTCTCCGGATCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACCTGGA GCAGGAGGATATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATATACATTCG GCGGAGGAACCAAGCTGGAGATCACCGGATCCACATCTGGAAGCGGCAAGCCAGGAAGC GGAGAGGGATCCACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGGTGGC ACCATCCCAGTCTCTGAGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACTACG GCGTGTCCTGGATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGTGATCTGG GGCTCTGAGACCACATACTATAATTCTGCCCTGAAGAGCCGCCTGACCATCATCAAGGA	CD19 scFv nucleotide

	CAACTCCAAGTCTCAGGTGTTTCTGAAGATGAATAGCCTGCAGACCGACGATACAGCCA TCTACTATTGCGCCAAGCACTACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGC CAGGGCACCTCCGTGACAGTGTCTAGC	
107	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVP SRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGS GEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIW GSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWG QGTSVTVSS	CD19 scFv polypeptide
108	GACATCCAGATGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGCGACAGAGTGAC CATCTCCTGTAGGGCCTCTCAGGATATCAGCAAGTACCTGAACTGGTATCAGCAGAAGC CAGATGGCACAGTGAAGCTGCTGATCTACCACACCTCCAGGCTGCACTCTGGAGTGCCA AGCCGGTTCTCCGGATCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACCTGGA GCAGGAGGATATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATATACATTCG GCGGAGGAACCAAGCTGGAGATC	CD19 scFv VL nucleotide
109	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVP SRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI	CD19 scFv VL polypeptide
110	ACCGGATCCACATCTGGAAGCGGCAAGCCAGGAAGCGGAGAGGGATCCACAAAGGGA	CD19 scFv linker nucleotide
111	TGSTSGSGKPGSGEGSTKG	CD19 scFv linker polypeptide
112	GAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGGTGGCACCATCCCAGTCTCTGAGCGT GACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACTACGGCGTGTCCTGGATCAGGCAGC CACCTAGGAAGGGACTGGAGTGGCTGGGCGTGATCTGGGGCTCTGAGACCACATACTAT AATTCTGCCCTGAAGAGCCGCCTGACCATCATCAAGGACAACTCCAAGTCTCAGGTGTT TCTGAAGATGAATAGCCTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGCACT ACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGCCAGGGCACCTCCGTGACAGTG TCTAGC	CD19 scFv VH nucleotide
113	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYY NSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTV SS	CD19 scFv VH polypeptide
114	GGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCGAC	CD8 hinge nucleotide
115	GAVHTRGLDFACD	CD8 hinge polypeptide
116	TTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGCTGGTGACCGT GGCCTTTATCATCTTCTGGGTG	CD28 transmembrane domain nucleotide
117	FWVLVVVGGVLACYSLLVTVAFIIFWV	CD28 transmembrane domain polypeptide
118	CGCGTGAAGTTCAGCCGGTCCGCCGATGCCCCTGCCTACCAGCAGGGCCAGAACCAGCT GTATAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGG GAAGGGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTAC AATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTATTCTGAGATCGGCATGAAGGGAGA GAGGCGCCGGGGCAAGGGACACGATGGCCTGTACCAGGGCCTGAGCACCGCCACAAAGG ACACATATGATGCCCTGCACATGCAGGCCCTGCCACCTAGG	CD3ζ domain nucleotide
119	ESKYGPPSPPSPA	Modified IgG4 Hinge

120	ESKYGPPSPPSP	Modified IgG4 Hinge
121	PSPRPAGQFQTLV	PD1 Hinge
122	EPKSCDKTHTCP	IgG1 Hinge
123	GAVHTRGLDFACD	CD8 Hinge
124	LCPSPLFPGPSKP	CD28 Hinge
125	ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTTGGCGAGAGGAA CGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAATGATGGAGAGGGGACCTC AGACACTGAAGGAGACCTCCTTCAACCAGGCCTATGGCAGAGACCTGATGGAGGCCCAG GAGTGGTGCAGGAAGTACATGAAGTCTGGCAATGTGAAGGACCTGCTGCAGGCCTGGGA TCTGTACTATCACGTGTTTCGGAGAATCAGCAAGGCTAGCAGAAGAAAGAGAGGCAGCG GCGAGGGCCGAGGCAGCCTGCTGACCTGCGGTGATGTGGAAGAAAACCCGGGCCCCATG CCCTTGCCCGTGACCGCGTTGCTCCTGCCCTTGGCTCTACTGCTGCACGCCGCTAGACC CATCCTGTGGCACGAGATGTGGCACGAGGGCCTGGAGGAGGCTAGCAGACTGTACTTCG GCGAGAGAAACGTGAAGGGCATGTTCGAGGTGCTGGAGCCCCTGCACGCCATGATGGAG AGAGGCCCTCAGACCCTGAAGGAGACAAGCTTCAACCAAGCCTACGGCAGAGACCTGAT GGAGGCCCAAGAGTGGTGCAGAAAGTACATGAAGAGCGGCAACGTGAAGGACCTGCTGC AAGCCTGGGACCTGTACTACCACGTGTTCAGAAGAATCAGCAAGGGCAGCAATACAAGC AAGGAAAACCCCTTCCTGTTCGCCCTGGAGGCCGTGGTGATCAGCGTGGGCAGCATGGG CCTGATCATCAGCCTGCTGTGCGTGTACTTCTGGCTGGAGAGAACCATGCCTAGAATCC CCACCCTGAAGAACCTGGAGGACCTGGTGACCGAGTACCACGGCAACTTCAGCGCCTGG AGCGGCGTGAGCAAGGGCCTGGCCGAGAGCCTGCAGCCCGACTACAGCGAGCGACTGTG CCTGGTGAGCGAGATTCCCCCTAAGGGCGGGGCCTTGGGTGAGGGACCCGGGGCAAGCC CGTGCAATCAGCACAGCCCCTACTGGGCCCCCCCCTGTTACACCCTGAAGCCCGAGACC GGCAGCGGAGCCACCAATTTCAGCCTCCTGAAACAAGCCGGTGACGTTGAAGAGAACCC CGGCCCCATGCCCCTGGGGTTGCTGTGGTTGGGACTCGCCCTCCTCGGCGCCCTGCACG CTCAAGCCGGCGTTCAAGTGGAGACTATCTCCCCCGGCGACGGCAGAACCTTCCCTAAG AGAGGGCAGACCTGCGTGGTGCACTACACCGGCATGCTGGAGGACGGCAAGAAGTTCGA CAGCAGCAGAGACAGAAACAAGCCCTTCAAGTTCATGCTGGGCAAGCAAGAGGTGATCA GAGGCTGGGAGGAGGGTGTGGCCCAAATGAGCGTGGGGCAGAGAGCCAAATTGACCATC TCACCCGACTACGCTTACGGGGCCACCGGCCATCCCGGCATCATCCCCCCCCACGCCAC CCTGGTGTTCGACGTGGAGCTGCTGAAGCTGGGCGAGGGCAAGGACACCATCCCCTGGC TGGGCCACCTGCTGGTGGGCCTGAGCGGCGCCTTCGGCTTCATCATCCTGGTGTACCTG CTGATCAACTGCAGAAACACCGGCCCCTGGCTGAAGAAGGTGCTGAAGTGCAACACCCC CGACCCTAGCAAGTTCTTCTCTCAGCTGAGCAGCGAGCACGGCGGCGACGTGCAGAAGT GGCTGAGCAGCCCATTTCCTAGCAGCAGCTTTAGCCCCGGCGGCCTAGCTCCCGAGATC AGCCCCCTGGAAGTACTGGAGAGAGATAAGGTGACACAGCTGCTGCTGCAGCAAGACAA GGTGCCCGAGCCCGCTAGCCTGAGCAGCAACCACAGCCTGACAAGCTGCTTCACCAACC AAGGCTACTTCTTCTTCCACCTGCCCGACGCCCTGGAGATCGAGGCCTGCCAAGTGTAC TTCACCTACGATCCGTACAGCGAGGAAGACCCCGACGAGGGAGTGGCCGGCGCCCCTAC CGGCAGCTCGCCACAACCGCTGCAACCTCTGAGCGGCGAGGACGACGCCTACTGCACCT TCCCTAGCAGAGATGATCTGCTGCTCTTTTCCCCTAGCTTGCTGGGGGGCCCTTCGCCT CCAAGTACTGCCCCCGGAGGTAGCGGGGCCGGCGAAGAAAGAATGCCCCCAAGCCTGCA AGAAAGAGTGCCTAGAGACTGGGACCCTCAACCACTGGGCCCACCCACTCCGGGCGTGC CCGACCTGGTAGATTTTCAGCCCCCTCCCGAGCTGGTGCTACGCGAGGCCGGCGAAGAG GTGCCCGACGCTGGGCCAAGAGAGGGTGTGTCATTCCCCTGGAGCCGACCCCCCGGTCA AGGCGAGTTCAGAGCCCTGAACGCTAGACTGCCCCTGAACACCGACGCCTACCTGAGCC TGCAAGAGCTGCAAGGCCAAGACCCCACCCACCTGGTGGGATCCGGAGCAACAAACTTT TCTCTGCTGAAGCAGGCCGGCGATGTGGAAGAAAACCCTGGACCTCTGCTGCTGGTGAC AAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTTTCTGCTGATCCCCTCCGTGCTGA CCCAGCCTAGCTCCGTGTCTGCCGCACCAGGACAGAAGGTGACAATCAGCTGTTCCGGC TCTACCAGCAACATCGGCAACAATTACGTGAGCTGGTACCAGCAGCACCCTGGCAAGGC CCCAAAGCTGATGATCTACGACGTGTCCAAGAGGCCATCTGGAGTGCCTGATCGGTTCT CCGGCTCTAAGAGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCAGTCCGAGGAC	Construct C.2U nucleotide

	GAGGCAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTCTGTTCGGCAC CGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCAGGATCTGGAG AGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAGGAAACCTGGTGCAGCCA GGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGCTTTACCTTCGGCTCTTTTAGCAT GTCCTGGGTGCGCCAGGCACCTGGAGGAGGACTGGAGTGGGTGGCCGGCCTGAGCGCCC GGTCTAGCCTGACACACTATGCCGACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGAT AACGCCAAGAATAGCGTGTACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGT GTACTATTGCGCCAGGCGCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTT ATATGGACGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCCACCACCACACCTGCT CCTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCTGAGACCTGA GGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCG ACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTGAGCCTGGTG ATCACACTGTACTGCAAGCGCGGCCGGAAGAAGCTGCTcTACATCTTTAAGCAGCCATT CATGCGCCCCGTGCAGACCACACAGGAGGAGGACGGCTGCTCCTGTCGGTTTCCAGAGG AGGAGGAGGGAGGATGTGAGCTGAGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCC TATCAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTA CGACGTGCTGGATAAGAGGAGGGGAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAA AGAACCCACAGGAGGGCCTGTATAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTAC TCCGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTATCA GGGCCTGAGCACCGCCACAAAGGACACATACGATGCCCTGCACATGCAGGCCCTGCCTC CAAGG	
126	ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTTGGCGAGAGGAA CGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAATGATGGAGAGGGGACCTC AGACACTGAAGGAGACCTCCTTCAACCAGGCCTATGGCAGAGACCTGATGGAGGCCCAG GAGTGGTGCAGGAAGTACATGAAGTCTGGCAATGTGAAGGACCTGCTGCAGGCCTGGGA TCTGTACTATCACGTGTTTCGGAGAATCAGCAAGGGCTCCGGCGCCACCAACTTCAGCC TGCTGAAGCAGGCAGGCGATGTGGAGGAGAATCCAGGACCTATGCCACTGGGACTGCTG TGGCTGGGACTGGCCCTGCTGGGCGCCCTGCACGCCCAGGCAGGAGTGCAGGTGGAGAC CATCTCCCCAGGCGACGGACGCACATTTCCAAAGAGGGGACAGACCTGCGTGGTGCACT ACACAGGCATGCTGGAGGATGGCAAGAAGTTCGACAGCTCCCGCGATCGGAATAAGCCC TTTAAGTTCATGCTGGGCAAGCAGGAAGTGATCAGAGGATGGGAGGAGGGAGTGGCACA GATGTCCGTGGGACAGAGGGCCAAGCTGACCATCTCTCCTGACTACGCCTATGGAGCAA CAGGACACCCAGGAATCATCCCACCTCACGCCACCCTGGTGTTTGATGTGGAGCTGCTG AAGCTGGGCGAGGGCTCTAACACAAGCAAGGAGAATCCTTTTCTGTTCGCACTGGAGGC AGTGGTCATCTCCGTGGGCTCTATGGGCCTGATCATCAGCCTGCTGTGCGTGTACTTTT GGCTGGAGAGAACCATGCCAAGGATCCCCACACTGAAGAACCTGGAGGACCTGGTGACC GAGTATCACGGCAATTTCAGCGCCTGGTCCGGCGTGTCTAAGGGACTGGCAGAGTCCCT GCAGCCAGATTACTCTGAGCGGCTGTGCCTGGTGTCCGAGATCCCACCCAAGGGAGGCG CCCTGGGAGAGGGACCAGGAGCCAGCCCTTGCAACCAGCACTCCCCATATTGGGCCCCT CCATGTTACACACTGAAGCCCGAGACCGGCTCTGGCGCCACAAACTTCAGCCTGCTGAA GCAAGCAGGCGACGTGGAAGAAAATCCAGGACCTATGGCACTGCCTGTGACCGCCCTGC TGCTGCCACTGGCCCTGCTGCTGCACGCAGCAAGGCCTATCCTGTGGCACGAAATGTGG CATGAAGGCCTGGAGGAGGCAAGCAGGCTGTATTTTGGCGAGCGGAATGTGAAAGGAAT GTTTGAAGTCCTGGAACCTCTGCATGCCATGATGGAAAGGGGACCACAGACACTGAAGG AGACCAGCTTTAACCAGGCCTATGGAAGGGACCTGATGGAGGCACAGGAGTGGTGCCGG AAGTACATGAAGTCCGGAAATGTGAAAGACCTGCTGCAGGCCTGGGATCTGTATTATCA CGTGTTCAGGCGCATCTCTAAGGGCAAGGATACCATCCCCTGGCTGGGACACCTGCTGG TGGGACTGAGCGGAGCCTTTGGCTTCATCATCCTGGTGTACCTGCTGATCAACTGCAGA AATACCGGCCCTTGGCTGAAGAAGGTGCTGAAGTGTAACACACCAGACCCCTCCAAGTT CTTTTCTCAGCTGTCTAGCGAGCACGGCGGCGATGTGCAGAAGTGGCTGTCCTCTCCTT TTCCAAGCTCCTCTTTCAGCCCAGGAGGACTGGCACCAGAGATCTCCCCCCTGGAGGTG CTGGAGAGGGACAAGGTGACCCAGCTGCTGCTGCAGCAGGATAAGGTGCCCGAGCCTGC CAGCCTGAGCTCCAACCACAGCCTGACATCCTGCTTTACCAATCAGGGCTATTTCTTTT TCCACCTGCCAGACGCCCTGGAGATCGAGGCCTGTCAGGTGTACTTCACCTATGATCCA	Construct C.2 nucleotide

	TACTCCGAAGAGGACCCAGATGAGGGAGTGGCCGGCGCCCCCACAGGCTCTAGCCCACA GCCACTGCAGCCTCTGTCTGGAGAGGACGATGCCTACTGTACCTTTCCCAGCCGCGACG ATCTGCTGCTGTTCAGCCCTTCCCTGCTGGGAGGACCATCTCCACCTAGCACAGCACCA GGAGGAAGCGGGGCAGGGGAGGAGCGGATGCCACCATCTCTGCAGGAGAGGGTGCCTAG GGACTGGGATCCTCAGCCACTGGGACCTCCAACCCCTGGAGTGCCAGACCTGGTGGATT TTCAGCCCCCTCCAGAGCTGGTGCTGCGGGAGGCAGGAGAGGAGGTGCCTGACGCAGGA CCACGCGAGGGCGTGAGCTTTCCATGGTCCAGGCCACCTGGACAGGGAGAGTTCAGAGC CCTGAACGCCAGGCTGCCTCTGAATACAGACGCCTATCTGTCTCTGCAGGAGCTGCAGG GCCAGGATCCAACCCACCTGGTGGGATCCGGAGCAACAAACTTTTCTCTGCTGAAGCAG GCCGGCGATGTGGAAGAAAACCCTGGACCTCTGCTGCTGGTGACAAGCCTGCTGCTGTG CGAGCTGCCTCACCCAGCCTTTCTGCTGATCCCCTCCGTGCTGACCCAGCCTAGCTCCG TGTCTGCCGCACCAGGACAGAAGGTGACAATCAGCTGTTCCGGCTCTACCAGCAACATC GGCAACAATTACGTGAGCTGGTACCAGCAGCACCCTGGCAAGGCCCCAAAGCTGATGAT CTACGACGTGTCCAAGAGGCCATCTGGAGTGCCTGATCGGTTCTCCGGCTCTAAGAGCG GCAATTCCGCCTCTCTGGACATCAGCGGACTGCAGTCCGAGGACGAGGCAGATTACTAT TGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTCTGTTCGGCACCGGCACAAAGCTGAC CGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCAGGATCTGGAGAGGGCAGCACAAAGG GACAGGTGCAGCTGGTGGAGAGCGGAGGAAACCTGGTGCAGCCAGGAGGCTCCCTGCGC CTGTCTTGTGCCGCCAGCGGCTTTACCTTCGGCTCTTTTAGCATGTCCTGGGTGCGCCA GGCACCTGGAGGAGGACTGGAGTGGGTGGCCGGCCTGAGCGCCCGGTCTAGCCTGACAC ACTATGCCGACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGATAACGCCAAGAATAGC GTGTACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGTGTACTATTGCGCCAG GCGCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTTATATGGACGTGTGGG GACAGGGCACCCTGGTGACAGTGAGCTCCACCACCACACCTGCTCCTAGACCACCTACA CCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCTGAGACCTGAGGCCTGTAGACCTGC CGCTGGAGGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGG CTCCTCTGGCTGGAACATGCGGCGTGCTGCTCCTGAGCCTGGTGATCACACTGTACTGC AAGCGCGGCCGGAAGAAGCTGCTcTACATCTTTAAGCAGCCATTCATGCGCCCCGTGCA GACCACACAGGAGGAGGACGGCTGCTCCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGAT GTGAGCTGAGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCCTATCAGCAGGGACAG AACCAGCTGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAA GAGGAGGGGAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGG GCCTGTATAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACTCCGAGATCGGCATG AAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTATCAGGGCCTGAGCACCGC CACAAAGGACACATACGATGCCCTGCACATGCAGGCCCTGCCTCCAAGG	
127	MLLLVTSLLLCELPHPAFLLIPSVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWYQ QHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSEDEADYYCAAWDDSLS EFLFGTGTKLTVLGSTSGSGKPGSGEGSTKGQVQLVESGGNLVQPGGSLRLSCAASGFT FGSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRDNAKNSVYLQMNSLR VEDTAVYYCARRSYDSSGYWGHFYSYMDVWGQGTLVTVSSTTTPAPRPPTPAPTIASQP LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLY IFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR	TagCAR polypeptide
128	GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGW EEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLGEGSNTSKENPF LFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSK GLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET	FKBP12:IL2RG polypeptide without signal peptide
129	ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLM EAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGKDTIPWLGHLLVGLSGAFGFIILV YLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAP EISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQ	FRB:IL2RB polypeptide without

	VYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGP SPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAG EEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	signal peptide

CLAIMS

1. A polycistronic construct comprising in 5' to 3' order (a) a first nucleotide sequence encoding FKBP12-rapamycin binding (FRB), (b) a second nucleotide sequence encoding a synthetic cytokine gamma chain polypeptide, (c) a third nucleotide sequence encoding a synthetic cytokine beta chain polypeptide, and (d) a fourth nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein each of the nucleotide sequences are separated by a nucleotide sequence encoding a cleavage site sequence.

2. The polycistronic construct of claim 1, wherein the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:3, 13, or 50 and/or the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:4, 14 or 51.

3. The polycistronic construct of claim 1 or claim 2, wherein the synthetic cytokine gamma chain polypeptide comprises a first dimerization domain, a first transmembrane domain and an interleukin 2 receptor subunit γ (IL2RG) intracellular domain and the synthetic cytokine beta chain polypeptide comprises a second dimerization domain, a second transmembrane domain and an intracellular domain selected from an interleukin-2 receptor subunit β (IL-2RB) intracellular domain, an interleukin-7 receptor subunit β (IL-7RB) intracellular domain, and an interleukin-21 receptor subunit β (IL-21RB) intracellular domain.

4. The polycistronic construct of claim 3, wherein the first dimerization domain is a FKBP12-rapamycin binding (FRB) domain and the second dimerization domain is a FK506-Binding Protein of size 12 kD (FKBP12) domain.

5. The polycistronic construct of claim 3, wherein the first dimerization domain is a FK506-Binding Protein of size 12 kD (FKBP12) domain and the second dimerization domain is a FKBP12-rapamycin binding (FRB) domain.

6. The polycistronic construct of claim 4 or claim 5, wherein the FRB dimerization domain is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%

identical to the nucleotide sequence of SEQ ID NO: 3, 13 or 50 and/or the FRB dimerization domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:4, 14, or 51.

7. The polycistronic construct of any one of claims 3 to 6, wherein the IL2RG is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:15 and/or the IL2RG comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:16.

8. The polycistronic construct of any one of claims 1 to 4, 6 and 7, wherein the synthetic cytokine gamma chain polypeptide is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:11 and/or the synthetic cytokine gamma chain polypeptide comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:12.

9. The polycistronic construct of any one of claims 4 to 8, wherein the FKBP12 dimerization domain is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:21 or 55 and/or the FKBP12 dimerization domain comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:22.

10. The polycistronic construct of any one of claims 1 to 9, wherein the synthetic cytokine beta chain polypeptide comprises a second dimerization domain, a second transmembrane domain and an interleukin 2 receptor subunit β (IL2RB) intracellular domain.

11. The polycistronic construct of any one of claims 3 to 10, wherein the IL2RB is encoded by a nucleotide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs:23 or 61 and/or the IL2RB comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs:24 or 62.

12. The polycistronic construct of any one of claims 1 to 3 and 5 to 11, wherein the synthetic cytokine beta chain polypeptide is encoded by a nucleotide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO:19 and/or the synthetic cytokine beta chain polypeptide comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:20.

13. The polycistronic construct of any one of claims 1-12, wherein the CAR comprises a light chain variable domain (VL) and a heavy chain variable domain (VH).

14. The polycistronic construct of any one of claims 1 to 13, wherein the CAR comprises an scFv domain.

15. The polycistronic construct of any one of claims 1-14, wherein the CAR comprises an endodomain comprising a signaling domain of a costimulatory molecule and a CD3ζ signaling domain.

16. The polycistronic construct of claim 15, wherein the costimulatory molecule is 4-1BB, or CD28.

17. The polycistronic construct of any one of claims 1 to 16, wherein each of the cleavage site sequences comprises a 2A cleavable linker sequence.

18. The polycistronic construct of claim 17, wherein the 2A cleavable linker is independently a T2A, P2A, E2A or F2A cleavage site.

19. The polycistronic construct of any one of claims 1 to 18, wherein at least one of the cleavage site sequences comprises a furin cleavage site sequence.

20. The polycistronic construct of claim 19, wherein the cleavage site sequence comprises a furin cleavage site sequence and a T2A cleavage sequence (furinT2A).

21. The polycistronic construct of any one of claims 1 to 20, wherein the first nucleotide sequence and second nucleotide sequence are separated by a furinT2A, the second nucleotide

sequence and third nucleotide sequence are separated by a P2A, and the third nucleotide sequence and fourth nucleotide sequence are separated by a P2A.

22. A viral vector comprising the polycistronic construct of any one of claims 1 to 21.

23. The viral vector of claim 22, wherein the viral vector is a lentiviral vector.

24. The viral vector of claim 22 or claim 23 that is a pseudotyped viral vector.

25. The viral vector of any one of claims 22-24, wherein the viral vector further comprises one or more surface T cell activating agents.

26. The viral vector of claim 25, wherein the one or more surface T cell activating agents comprise CD58, an anti-CD3 antibody or antigen binding fragment thereof, or CD80.

27. The viral vector of any one of claims 22-26, comprising a Cocal G-protein or functional variant thereof.

28. A cell comprising the polycistronic construct of any one of claims 1-21.

29. A method of transducing a cell comprising contacting a target cell with the viral vector of any one of claims 22-27.

30. A method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell, comprising contacting the target cell with the viral vector of any one of claims 22-27.

31. The method of claim 30, wherein the target cell comprises a peripheral blood mononuclear cell (PBMC) or a T cell.

32. A method of transducing a T cell, comprising contacting the T cell with a viral vector of any one of claims 22-27.

33. A method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a T cell, comprising contacting the T cell with a viral vector of any one of claims 22-27.

34. A method of delivering a payload to a T cell, comprising contacting the T cell with a viral vector of any one of claims 22-27.

35. The method of any one of claims 30-34, wherein the one or more T cell activating agents bind a receptor on the T cell.

36. The method of any one of claims 30-35, wherein the T cell comprises a CD4+ or CD8+ T cell.

37. The method of any one of claims 30-35, wherein the method is performed *ex vivo* or *in vitro*.

38. The method of any one of claims 30-35, wherein the method is performed *in vivo*.

39. A cell produced by the method of any one of claims 30-38.

40. Use of a cell of claim 39 in the manufacture of a medicament for treating a disease or condition in a subject.

41. Use of a viral vector of any one of claims 22-27 in the manufacture of a medicament for treating a disease or condition in a subject.

42. A cell of claim 39 for use in a method of treating a subject having a disease or condition.

43. A viral vector of any one of claims 22-27 for use in a method of treating a subject having a disease or condition.

44. The use of claim 40 or claim 41, cell of claim 42 or viral vector of claim 43, further comprising administering to the subject a non-physiological ligand, optionally wherein the non-physiological ligand binds to a synthetic cytokine receptor composed of the synthetic gamma chain polypeptide and the synthetic cytokine beta chain polypeptide encoded by the polycistronic construct.

45. The use of claim 44, wherein the non-physiological ligand comprises rapamycin or a rapamycin analog.