CLAIMS

1. A lentiviral vector, comprising:

(a) a viral envelope comprising:

a heterologous lymphocyte targeting protein, the lymphocyte targeting protein comprising:

an extracellular domain comprising a lymphocyte targeting domain; and

a transmembrane domain; and

(b) a mutated VSV-G envelope protein that exhibits inhibited binding to an LDL-R but mediates membrane fusion; and

(c) an expression cassette that comprises a heterologous transgene.

2. The lentiviral vector according to claim 1, wherein the heterologous lymphocyte targeting protein is:

(i) a heterologous T cell targeting protein comprising an extracellular domain comprising a T cell targeting domain,

(ii) a heterologous natural killer (NK) cell targeting protein comprising an extracellular domain comprising a NK cell targeting domain or

(iii) heterologous B cell targeting protein comprising an extracellular domain comprising a B cell targeting domain.

3. The lentiviral vector according to claim 1 or 2, wherein:

(a) the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein that specifically binds to:

(i) a T cell marker selected from CD3, CD28, CD80, 4-1BB, AhR, CD3, CD2, CD7, CD4, CD8, CD25, CD44, CD45RA, CD47, CD62L, CD69, CD94, CD95, CD127, CD161, CD183 (CXCR3), CD184 (CXCR4), CD185 (CXCR5), CD193 (CCR3), CD194 (CCR4), CD195 (CCR5), CD196 (CCR6), CD197 (CCR7), CCR10, PD-1, TCRa/b, CD5, CD27, CD45RO, CD45RB, CD57, CD103, CD122, P2RX7, TIGIT, LAG-3, TIM-3, and IL6ST, or any combination thereof;

(ii) a γδ T cell marker selected from γδ TCR, Vdelta1, Vdelta2, and NKG2D (KLRK1, CD314);
(iii) an NK T cell marker selected from Invariant TCR (Va24-Ja18), CD185 (CXCR5), CXCR6, and IL-21R, or any combination thereof; or
(iv) a MAIT cell marker selected from Va7.2, Ja33, CXCR6, IL-18R, KLRB1 (CD161), and VLA4 (alpha4beta1 integrin), or any combination thereof;

(b) the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein comprising an extracellular domain comprising a T cell targeting domain that specifically binds to a CD3 protein or a CD28 protein;

(c) the heterologous lymphocyte targeting protein is a heterologous NK cell targeting protein that specifically binds to a NK cell marker selected from CD56, NKp46, CD16, KIR(s), NKG2 proteins (e.g., NKG2D (KLRK1, CD314)), KLRB1 (CD161), KLRD1 (cd94), IL2Rb (CD122), IL-21R, SLAMF6 (CD352), SLAMF7 (CD319), and IL-18R, or any combination thereof;

(d) the heterologous lymphocyte targeting protein is a B cell targeting protein that specifically binds to a B cell marker selected from CD19, CD20, CD21, CD22, CD24, CD38, CD40, CD72, CD32b, CD268, CD269, CD267, CD86, CD80, CD52, CD138, CD27, CD28, CD23, CD84, CD257, CD270, CD37, CD74, and CD269, or any combination thereof;

(e) the targeting protein comprises a CD80 protein comprising an extracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 6;

(f) the targeting protein comprises a CD80 protein comprising a transmembrane and intracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 8;

(g) the targeting protein comprises a CD80 protein comprising SEQ ID NO: 2 or SEQ ID NO: 2 absent the signal peptide of SEQ ID NO: 4; or

(h) the targeting protein comprises an anti-CD3 scFv having a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 14, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 18, optionally wherein the targeting protein comprises an anti-CD3 targeting protein comprising: (i) SEQ ID NO: 10 or SEQ ID NO: 10 absent the signal peptide of SEQ ID NO: 12; or (ii) SEQ ID NO: 117 or SEQ ID NO: 117 absent the signal peptide of SEQ ID NO: 12.

4. The lentiviral vector according to any one of the preceding claims, wherein:

(a) the targeting protein as expressed in a host cell comprises a signal sequence; and/or

(b) the mature targeting protein lacks a signal sequence or the mature targeting protein includes at least one amino acid from a signal sequence.

5. The lentiviral vector according to any one of the preceding claims, wherein:

(a) the mutated VSV-G envelope protein comprises a mutation selected from one or more, or two or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354;

an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; and

a deletion of residues 1-8;

(b) the mutated VSV-G envelope protein comprises a mutation selected from one or more of H8A, N9A, Q10A, K47A, K47Q, N180A, I182A, Y209A, T352A, T352W, E353A, R354A, and R354Q;

(c) the mutated VSV-G envelope protein comprises a mutation selected from one or more of H8A, K47A, K47Q, Y209A, R354A, and R354Q;

(d) the mutated VSV-G envelope protein comprises:

(i) a K47 mutation and a R354 mutation;
(ii) a N180 mutation, a I182 mutation, a T352 mutation, and a E353 mutation;
(iii) a T352 mutation and E353 mutation;
(iv) a N9 mutation, a Q10 mutation, and a N180 mutation; or
(v) an insertion of GGS between H8 and N9 and an insertion of GGS between N9 and Q10;
(vii) an insertion of TT between N9 and Q10; or
(viii) an insertion of GGS between P46 and K47

optionally wherein the mutated VSV-G envelope protein comprises:
(i) a K47Q mutation and a R354A mutation;
(ii) a N180A mutation, a I182A mutation, a T352A mutation and a E353A mutation;
(iii) a T352W mutation and a E353A mutation; or
(iv) a N9A mutation, a Q10A mutation, and a N180A mutation; and/or

(e) the mutated VSV-G envelope protein comprises the amino acid sequence of any one of SEQ ID NOS: 74, 93, 95, 97, 99 and 103.

6. The lentiviral vector according to any one of the preceding claims, wherein the heterologous transgene encodes a chimeric antigen receptor (CAR) comprising an extracellular domain comprising a binding domain that specifically binds to a target molecule; an intracellular signaling domain, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) and optionally, a costimulatory signaling domain; and a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

7. The lentiviral vector according to claim 6, wherein the CAR encoded by the transgene comprises:

(a) a binding domain that specifically binds one or more of CD19, BCMA, alpha folate receptor, 5T4, Ab integrin, B7-H3, B7-H6, CAIX, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD52, CD70, CD79a, CD79b, CD80, CD123, CD138, CD171, CEA, CSPG4, EGFR, ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EpCAM, FAP, fetal AchR, FLT3, Fra, GD2, GD3, Glypican-3 (GPC3), HLA-A1 + MAGE1, HLA-A2 + MAGE1, HLA-A3 + MAGE1, HLA-A1 + NY-ESO-1,

HLA-A2 + NY-ESO-1, HLA-A3 + NY-ESO-1, HLADR, IL-11Ralpha, IL-13 Ralpha2, Lambda, Lewis-Y, Kappa, mesothelin, Muc1, Muc16, NCAM, NKG2d ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, VEGFR2, BAFF-R, Claudin18.2, CD86, FcRL5, GPRC5, and TACI;

(b) a transmembrane domain selected from a CD28, CD2, CD4, CD8a, CD5, CD3ε, CD3δ, CD3ζ, CD9, CD16, CD22, CD25, CD27, CD33, CD37, CD40, CD45, CD64, CD79A, CD79B, CD80, CD86, CD95 (Fas), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD154 (CD40L), CD200R, CD223 (LAG3), CD270 (HVEM), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), CD279 (PD-1), CD300, CD357 (GITR), A2aR, DAP10, FcRα, FcRβ, FcRγ, Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRPα, pTα, TCRα, TCRβ, TIM3, TRIM, LPA5, and Zap70 transmembrane domain;

(c) an intracellular signaling domain selected from a CD3γ, CD3δ, CD3ε, CD3ζ, CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcRγ, and CD66d domain;

(d) a costimulatory domain selected from one or more of a CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, LFA-1, LIGHT, NKG2C, NKD2C, SLP76, TRIM, and ZAP70 domain; and

(e) optionally, an extracellular, non-signaling linker domain between the binding domain and the transmembrane domain, wherein the non-signaling linker domain is selected from:

(i) an immunoglobulin hinge region selected from a wild type or modified IgG1, IgG2, IgG3, IgG4, IgA, and IgD hinge region;

(ii) a hinge region selected from a wild type or modified CD8a, CD4, CD28 and CD7 hinge region;

(iii) all or a portion of an Fc domain selected from one or more of a CH1 domain, a CH2 domain, and a CH3 domain; and

(iv) a stalk region of a type II C-lectin derived from a stalk region of CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

8. The lentiviral vector according to claim 6 or claim 7, wherein:

(a) the CAR encoded by the transgene is an anti-CD19 CAR comprising:

a binding domain comprising an anti-CD19 scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 46, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 50;

a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56; and

a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54,

optionally wherein the CAR encoded by the transgene comprises

(i) SEQ ID NO: 42 or SEQ ID NO: 42 absent the signal peptide of SEQ ID NO: 44 or

(ii) SEQ ID NO: 58 or SEQ ID NO: 58 absent the signal peptide of SEQ ID NO: 60;

(b) the CAR encoded by the transgene is an anti-BCMA CAR comprising:

a binding domain comprising an anti-BCMA scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 62, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 66;

a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 122; and

a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54.

9. A method for producing a lymphocyte targeted lentiviral vector according to any one of the preceding claims, the method comprising:

(a) transfecting a producer cell with a a GagPol plasmid, a Rev plasmid, a transgene plasmid, and a VSV-G env plasmid, wherein;

(i) the GagPol plasmid comprises a one or more polynucleotides encoding a lentiviral gag gene and a lentiviral pol gene and is capable of expressing a lentiviral Gag polyprotein and a lentiviral Pol polyprotein within the producer cell;

(ii) the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral Rev protein within the producer cell;

(iii) the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and

(iv) the VSV-G env plasmid comprises a tandem expression cassette, wherein the tandem expression cassette comprises a polynucleotide encoding a mutated VSV-G envelope protein and a polynucleotide encoding a lymphocyte targeting protein and the VSV-G env plasmid is capable of expressing the mutated VSV-G envelope protein and the lymphocyte targeting protein within the producer cell;

(b) culturing the producer cell in a culture medium; and

(c) harvesting lentiviral vector from the culture medium.

10. A method for producing a lymphocyte targeted lentiviral vector according to any one of claims 1-8, the method comprising:

(a) transfecting a producer cell with a a GagPol plasmid, a Rev plasmid, a transgene plasmid, and a VSV-G env plasmid, wherein;

(i) the GagPol plasmid comprises a tandem expression cassette that encodes the Gag polyprotein precursor and Pol polyprotein precursor and a lymphocyte targeting protein and the GagPol plasmid is capable of expressing a lentiviral Gag polyprotein, a lentiviral Pol polyprotein, and the lymphocyte targeting protein within the producer cell;

(ii) the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral Rev protein within the producer cell;

(iii) the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and

(iv) the VSV-G env plasmid comprises a a polynucleotide encoding a mutated VSV-G envelope protein and the VSV-G env plasmid is capable of expressing the mutated VSV-G envelope protein within the producer cell;

(b) culturing the producer cell in a culture medium; and

(c) harvesting lentiviral vector from the culture medium.

11. A method for producing a lymphocyte targeted lentiviral vector according to any one of claims 1-8, the method comprising:

(a) transfecting a producer cell with a a GagPol plasmid, a Rev plasmid, a transgene plasmid, and a VSV-G env plasmid, wherein;

(i) the GagPol plasmid comprises a one or more polynucleotides encoding a lentiviral gag gene and a lentiviral pol gene and is capable of expressing a lentiviral Gag polyprotein and a lentiviral Pol polyprotein within the producer cell;

(ii) the Rev plasmid comprises a tandem expression cassette, wherein the tandem expression cassette comprises a polynucleotide encoding a lentiviral rev gene and a polynucleotide encoding a lymphocyte targeting protein, and the Rev plasmid is capable expressing a lentiviral Rev protein and the lymphocyte targeting protein within the producer cell;

(iii) the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and

(iv) the VSV-G env plasmid comprises a polynucleotide encoding a mutated VSV-G envelope protein and the VSV-G env plasmid is capable of expressing the mutated VSV-G envelope protein within the producer cell;

(b) culturing the producer cell in a culture medium; and

(c) harvesting lentiviral vector from the culture medium.

12. A method for producing a lymphocyte targeted lentiviral vector according to any one of claims 1-8, the method comprising:

(a) transfecting a producer cell with a GagPol plasmid, a Rev plasmid, a transgene plasmid, a VSV-G env plasmid, and a lymphocyte targeting protein plasmid;

(i) the GagPol plasmid comprises a one or more polynucleotides encoding a lentiviral gag gene and a lentiviral pol gene and is capable of expressing a lentiviral Gag polyprotein and a lentiviral Pol polyprotein within the producer cell;

(ii) the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral Rev protein within the producer cell;

(iii) the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and

(iv) the VSV-G env plasmid comprises a polynucleotide encoding a mutated VSV-G envelope protein and is capable of expressing the mutated VSV-G envelope protein within the producer cell; and

(v) the lymphocyte targeting protein plasmid comprises a polynucleotide encoding a lymphocyte targeting protein and is capable of expressing the lymphocyte targeting protein within the producer cell;

(b) culturing the producer cell in a culture medium; and

(c) harvesting lentiviral vector from the culture medium.

13. The method according to any one of claims 9-12, wherein:

(a) the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a T cell targeting protein;

(b) the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a NK cell targeting protein; or

(c) the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a B cell targeting protein.

14. The method according to any one of claims 9-13, wherein:

(a) the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a T cell targeting protein that specifically binds to:

(i) a T cell marker selected from CD3, CD28, CD80, 4-1BB, AhR, CD3, CD2, CD7, CD4, CD8, CD25, CD44, CD45RA, CD47, CD62L, CD69, CD94, CD95, CD127, CD161, CD183 (CXCR3), CD184 (CXCR4), CD185 (CXCR5), CD193 (CCR3), CD194 (CCR4), CD195 (CCR5), CD196 (CCR6), CD197 (CCR7), CCR10, PD-1, TCRa/b, CD5, CD27, CD45RO, CD45RB, CD57, CD103, CD122, P2RX7, TIGIT, LAG-3, TIM-3, and IL6ST, or any combination thereof;

(ii) a γδ T cell marker selected from γδ TCR, Vdelta1, Vdelta2, and NKG2D (KLRK1, CD314, or any combination thereof);

(iii) an NK T cell marker selected from Invariant TCR (Va24-Ja18), CD185 (CXCR5), CXCR6, and IL-21R, or any combination thereof; or

(iv) a MAIT cell marker selected from Va7.2, Ja33, CXCR6, IL-18R, KLRB1 (CD161), and VLA4 (alpha4beta1 integrin), or any combination thereof;

(b) the targeting protein comprises a CD80 protein comprising an extracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 6;

(c) the targeting protein comprises a CD80 protein comprising a transmembrane and intracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 8;

(d) the targeting protein comprises a CD80 protein comprising SEQ ID NO: 2 or SEQ ID NO: 2 absent the signal peptide of SEQ ID NO: 4;

(e) the targeting protein comprises: (i) an anti-CD3 scFv having a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 14, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 18; or (ii) an anti-CD3 scFv having a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 115, and a heavy chain variable region that has at least about 75%, 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 113;

(f) the targeting protein comprises an anti-CD3 targeting protein comprising: (i) SEQ ID NO: 10 or SEQ ID NO: 10 absent the signal peptide of SEQ ID NO: 12; or (ii) SEQ ID NO: 117 or SEQ ID NO: 117 absent the signal peptide of SEQ ID NO: 12;

(g) the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a NK cell targeting protein that specifically binds to an NK cell marker selected from CD56, NKp46, CD16, KIR(s), NKG2 proteins (e.g., NKG2D (KLRK1, CD314)), KLRB1 (CD161), KLRD1 (cd94), IL2Rb (CD122), IL-21R, SLAMF6 (CD352), SLAMF7 (CD319), and IL-18R, or any combination thereof; or

(h) the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a B cell targeting protein that specifically binds to a B cell marker selected from selected from CD19, CD20, CD21, CD22, CD24, CD38, CD40, CD72, CD32b, CD268, CD269, CD267, CD86, CD80, CD52, CD138, CD27, CD28, CD23, CD84, CD257, CD270, CD37, CD74, and CD269, or any combination thereof.

15. The method according to any of claims 9-14, wherein:
(a) the mutated VSV-G envelope protein encoded by the VSV-G env plasmid comprises a mutation selected from one or more or two or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354;

an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; and

a deletion of residues 1-8;

(b) the mutated VSV-G envelope protein comprises a mutation selected from one or more of H8A, N9A, Q10A, K47A, K47Q, N180A, I182A, Y209A, T352A, T352W, E353A, R354A, and R354Q;

(c) the mutated VSV-G envelope protein encoded by the VSV-G env plasmid comprises a mutation selected from one or more of H8A, K47A, K47Q, Y209A, R354A, and R354Q;

(d) the mutated VSV-G envelope protein comprises:

(i) a K47 mutation and a R354 mutation;

(ii) a N180 mutation, a I182 mutation, a T352 mutation, and a E353 mutation;

(iii) a T352 mutation and E353 mutation;

(iv) a N9 mutation, a Q10 mutation, and a N180 mutation; or

(v) an insertion of GGS between H8 and N9 and an insertion of GGS between N9 and Q10;

(vi) an insertion of TT between N9 and Q10; or

(vii) an insertion of GGS between P46 and K47,

optionally wherein the mutated VSV-G envelope protein comprises:

(a) a K47Q mutation and a R354A mutation;

(b) a N180A mutation, a I182A mutation, a T352A mutation and a E353A mutation;

(c) a T352W mutation and a E353A mutation; or

(d) a N9A mutation, a Q10A mutation, and a N180A mutation;

(e) the mutated VSV-G envelope protein comprises the amino acid sequence of any one of SEQ ID NOS: 74, 93, 95, 97, 99, and 103; and/or

(f) the CAR encoded by the transgene plasmid comprises an extracellular domain comprising a binding domain that specifically binds to a target molecule; an intracellular signaling domain, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) and optionally a costimulatory signaling domain; and a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

16. The method according to any of claims 9-15, wherein the CAR encoded by the transgene plasmid comprises:

(a) a binding domain that specifically binds one or more of CD19, BCMA, alpha folate receptor, 5T4, Ab integrin, B7-H3, B7-H6, CAIX, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD52, CD70, CD79a, CD79b, CD80, CD123, CD138, CD171, CEA, CSPG4, EGFR, ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EpCAM, FAP, fetal AchR, FLT3, Fra, GD2, GD3, Glypican-3 (GPC3), HLA-A1 + MAGE1, HLA-A2 + MAGE1, HLA-A3 + MAGE1, HLA-A1 + NY-ESO-1, HLA-A2 + NY-ESO-1, HLA-A3 + NY-ESO-1, HLADR, IL-11Ralpha, IL-13 Ralpha2, Lambda, Lewis-Y, Kappa, mesothelin, Muc1, Muc16, NCAM, NKG2d ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, VEGFR2, BAFF-R, Claudin18.2, CD86, FcRL5, GPRC5, and TACI;

(b) a transmembrane domain selected from a CD28, CD2, CD4, CD8a, CD5, CD3ε, CD3δ, CD3ζ, CD9, CD16, CD22, CD25, CD27, CD33, CD37, CD40, CD45, CD64, CD79A, CD79B, CD80, CD86, CD95 (Fas), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD154 (CD40L), CD200R, CD223 (LAG3), CD270 (HVEM), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), CD279 (PD-1), CD300, CD357 (GITR), A2aR, DAP10, FcRα, FcRβ, FcRγ, Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRPα, pTα, TCRα, TCRβ, TIM3, TRIM, LPA5, and Zap70 transmembrane domain;

(c) an intracellular signaling domain selected from a CD3γ, CD3δ, CD3ε, CD3ζ, CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcRγ, and CD66d domain;

(d) a costimulatory domain selected from one or more of a CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, LFA-1, LIGHT, NKG2C, NKD2C, SLP76, TRIM, and ZAP70 domain; and

(e) optionally, an extracellular, non-signaling linker domain between the binding domain and the transmembrane domain, with the non-signaling linker domain being selected from:

(i) an immunoglobulin hinge region selected from a wild type or modified IgG1, IgG2, IgG3, IgG4, IgA, and IgD hinge region;

(ii) a hinge region selected from a wild type or modified CD8a, CD4, CD28 and CD7 hinge region;

(iii) all or a portion of an Fc domain selected from one or more of a CH1 domain, a CH2 domain, and a CH3 domain; and

(iv) a stalk region of a type II C-lectin derived from a stalk region of CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

17. The method according to any of claims 9-16, wherein

(i) the CAR encoded by the transgene plasmid is an anti-CD19 CAR comprising:

(a) a binding domain comprising an anti-CD19 scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 46, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 50;

(b) a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

(c) a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56; and

(d) a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54.

optionally wherein the CAR encoded by the transgene comprises SEQ ID NO: 42 or SEQ ID NO: 42 absent the signal peptide of SEQ ID NO: 44; or

(ii) the CAR encoded by the transgene plasmid is an anti-BCMA CAR comprising:

(a) a binding domain comprising an anti-BCMA scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 62, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 66;

(b) a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

(c) a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 122; and

(d) a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54

optionally wherein the CAR encoded by the transgene comprises SEQ ID NO: 58 or SEQ ID NO: 58 absent the signal peptide of SEQ ID NO: 60.

18. The method of any one of claims 9-17, wherein:

(a) the producer cell is transduced with a defined ratio of a transgene plasmid, GagPol plasmid, and a VSV-G env plasmid, and Rev plasmid, optionally wherein the defined ratio of transgene plasmid, GagPol plasmid, and a VSV-G env plasmid, and Rev plasmid is about about 1:1:1:1 to about 5:4:1:1, respectively; optionally wherein the producer cell is transduced with a defined ratio of a transgene plasmid, GagPol plasmid, and a VSV-G env plasmid, and Rev plasmid of about 4:2:1:1 or the producer cell is transduced with a defined ratio of a transgene plasmid, GagPol plasmid, and a VSV-G env plasmid, and Rev plasmid of about 3.125:3.125:2.5:1.25;

(b) harvesting lentiviral vector from the culture medium comprises centrifugation, anion exchange, chromatography, or any combination thereof; and/or

(c) a LVV product harvested from the culture medium has a viral titer of about 1e7 TU/mL to about 1e12 TU/mL in concentrated LVV product.

19. A method for producing a T cell, a NK cell or a B cell expressing a heterologous transgene or a CAR, the method comprising:

transducing the T cell, the NK cell or the B cell with one or more LVVs according to any one of claims 1-8, wherein transduction of the T cell, the NK cell or the B cell results in expression of the heterologous transgene or the CAR transgene by the T cell, the NK cell or the B cell.

20. The method according to claim 19, wherein:

(a) the T cell is a resting T cell, the NK cell is a resting NK cell or the B cell is a resting B cell;

(b) transducing the T cell, the NK cell or the B cell is performed in vivo or ex vivo;

(c) the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorly, intraosseously, or intranodally; or transducing the T cell, the NK cell or the B cell comprises contacting T cell, NK cells or B cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject with the one or more LVVs; and/or

(d) transduction is accomplished without use of an exogenous T cell, NK cell or B cell stimulating agent, optionally wherein the exogenous T cell stimulating agent comprises an anti-CD3 antibody or antigen binding fragment thereof, an anti-CD28 antibody or antigen binding fragment thereof, or both.