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(54) Title: LYMPHOCYTE TARGETED LENTIVIRAL VECTORS

(57) Abstract: Provided herein are lentiviral vectors comprising a mutated, heterologous envelope protein, a targeting protein, and at least one transgene for delivery to and expression by a cell characterized by the targeting protein. Also provided are methods and materials for producing the lentiviral vectors described herein, methods for transducing target cells, and cells transduced by lentiviral vectors according to the present disclosure.



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LYMPHOCYTE TARGETED LENTIVIRAL VECTORS

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format
5 in lieu of a paper copy, and is hereby incorporated by reference into the
specification. The name of the text file containing the Sequence Listing is
930207_401WO_SEQUENCE_LISTING.txt. The text file is 158 KB, was created on
February 25, 2022, and is being submitted electronically via EFS-Web.

10 BACKGROUND

Lentiviral vectors play a critical role in gene-modified cell therapies,
particularly T cell therapies. Recently approved T cell therapies rely on retroviral
vectors to transduce the therapeutic molecule (e.g., chimeric antigen receptor (CAR))
into T lymphocytes. An associated risk to CAR T cell production is the transduction of
15 other cell types with the transgene. The use of integrating vectors with broad cell
tropism, e.g., lentiviral vectors pseudotyped with a VSV-G envelope protein, can
represent a serious, though rare, safety concern.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

20 **FIG. 1** is a schematic representation of helper plasmids suitable for use in a
third generation LVV production system.

FIGS. 2A-2B depict graphs (FIG. 2A) and FACS plots (FIG. 2B) showing on-
target and off-target entry of Jurkat T cells and Raji B cells by lentiviral vectors bearing
mutated VSV-G envelope to abolish LDL receptor binding (Trop-002, Trop-051, Trop-
25 052, Trop-055, and Trop-061) and T cell targeting protein CD80. In FIG. 2A, on-target
entry is the left bar and off-target is the right bar of each sample. Binding of the T cell
targeting protein to its cognate ligand on T cells leads to entry of the lentiviral vector,
and subsequent expression of reporter green fluorescent protein (GFP) is measured.

FIGS. 3A-3B depicts graphs showing: (FIG. 3A) T cell targeting protein CD80 expressed from the VSV-G packaging plasmid is expressed at relatively equivalent levels as the mutated VSV-G on the surface of HEK293 producer cells; and (FIG. 3B) LVV generated with this approach can transduce targeted Jurkat T cells but do not transduce Raji B cells.

FIG. 4 depicts graphs showing: (top row) expression levels of CD80 and mutated VSV-G on the surface of HEK293T producer cells using LVV generated by cloning CD80 targeting protein into the Rev packaging plasmid or into the mutated VSV-G packaging plasmid; and (bottom) LVV generated by cloning CD80 targeting protein into the Rev packaging plasmid or into the mutated VSV-G packaging plasmid transduce targeted Jurkat T cells.

FIG. 5 depicts (left) CD80 targeting protein expression on surface of HEK293T producer cells using a five plasmid packaging system as a function of CD80 plasmid concentration; and (right) LVV generated with the five plasmid packaging system transduce targeted Jurkat T cells and transduction efficiency was associated with CD80 packaging plasmid concentration.

FIGS. 6A-6D depict titers of lymphocyte targeting LVV produced in adherent HEK293 or suspension HEK293 producer cells. LVV harvested from HEK293 adherent cell culture medium by centrifugation (FIG. 6A) or by anion exchange chromatography followed by tangential flow filtration (FIG. 6B). LVV harvested from HEK293 suspension cell culture medium by anion exchange chromatography (FIG. 6C). Concentration by AEX/TFF resulted in LVV preparations with a high level of purity and recovery (FIG. 6D).

FIG. 7 depicts a schematic for testing T cell transduction in PBMCs from healthy human donors with LVV comprising a BCMA CAR transgene or T cell targeting LVV (anti-CD3 and CD80) comprising a BCMA CAR transgene. Graphs shown on lower right indicate that even at low MOI, the T cell targeting LVV transduced T cells at a higher level than standard LVV and that T cell targeting LVV is capable of transducing T cells without the presence of IL-2 and exogenous activating antibodies (anti-CD3 and anti-CD28) in contrast to standard LVV.

FIG. 8 depicts graphs showing T cell expansion from PBMCs obtained from three different donors and transduced using standard LVV or T cell redirected LVV (anti-CD3 and CD80) in the presence or absence of exogenous activating anti-CD3 and anti-CD28 antibodies

5 **FIG. 9** depicts a schematic for testing T cell transduction in PBMCs from healthy human donors with LVV comprising a CD19 CAR transgene or T cell targeting LVV (anti-CD3 and CD80) comprising a CD19 CAR transgene. Graphs shown on lower right show that the T cell targeting LVV transduced T cells at a higher level than standard LVV and that T cell targeting LVV is capable of transducing T cells without
10 the presence of exogenous activating antibodies (anti-CD3 and anti-CD28) in contrast to standard LVV.

FIG. 10 depicts graphs showing levels of T cell transduction efficiency and T cell activation with anti-CD3 targeting proteins (12F6 in VH-VL orientation and VL-VH orientation) used to generate T cell targeting LVV.

15 **FIG. 11** depicts graphs showing that BCMA CAR T cells exhibited increased expression of T cell effector cytokines (TNF α – left; TNF α and IFN γ – right) after culture with BCMA-positive cell lines that is not observed with BCMA-negative cell lines whether generated by standard LVV or T cell-redirection LVV (anti-CD3 and CD80).

20 **FIGS. 12A-12B** depict graphs showing *in vivo* delivery of transgene using T cell targeting LVV: (FIG. 12A) T cell targeting LVV (anti-CD3 and CD80) specifically transduce human T cells (CD3+) and not human B cells (CD20+) in humanized mouse model (n=5); and (FIG. 12B) T cell targeting LVV transduce both CD8+ and CD8- (CD4) T cells compared to standard LVV which did not.

25 **FIG. 13** depicts graphs showing that CD80 targeting LVVs enhance transduction of CD4 T cells compared to standard LVVs.

FIG. 14 depicts graphs showing that T cell targeting (anti-CD3 and CD80) LVVs transduce target Jurkat T cells but do not transduce off-target tumor cells (Raji, Ramos, Jeko-1, and NALM-6) compared to standard LVV.

30

DETAILED DESCRIPTION

Engineered lentiviral vectors are described herein. The lentiviral vectors include a mutated, heterologous envelope protein, a targeting protein, and at least one transgene for delivery to and expression by a cell characterized by the targeting protein.

5 In some embodiments, the targeting protein is selected to target an immune cell, including, for example a lymphocyte or a T cell. In certain such embodiments, the lentiviral vectors described herein are capable of selectively targeting and efficiently transducing resting lymphocytes, e.g., T cells. In some embodiments, lentiviral vectors described herein are capable of transducing and/or activating T cells in the absence of
10 an exogenous T cell stimulating agent. In some embodiments, lentiviral vectors described herein enhance transduction of CD4 T cells compared to standard lentiviral vectors.

In some embodiments, the lentiviral vectors incorporating a mutated env and a targeting protein as described herein are capable of producing a high titer LVV product,
15 as compared to standard LVV incorporating another fusogenic env protein (e.g., cocal env, paramyxovirus env, truncated VSV-G env).

Also provided are methods and materials for producing the lentiviral vectors described herein, methods for transducing target cells, and cells transduced by lentiviral vectors
20 according to the present disclosure. In some embodiments, a lentiviral vector as described herein and/or cells transduced by such a vector may be used in treating a disease or disorder responsive to the presence of cells expressing the transgene delivered by the vector.

25 Definitions

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the
30 recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited

herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the term "about" means $\pm 20\%$ of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have" and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

Terms understood by those in the art of antibody technology are each given the meaning acquired in the art, unless expressly defined differently herein. The term "antibody" is used in the broadest sense and includes polyclonal and monoclonal antibodies. An "antibody" may refer to an intact antibody comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as an antigen-binding portion (or antigen-binding domain) of an intact antibody that has or retains the capacity to bind a target molecule. An antibody may be naturally occurring, recombinantly produced, genetically engineered, or modified forms of immunoglobulins, for example intrabodies, peptibodies, nanobodies, single domain antibodies, SMIPs, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, triabodies, tetrabodies, tandem di-scFv, tandem tri-scFv, ADAPTIR). A monoclonal antibody or antigen-binding portion thereof may be non-human, chimeric, humanized, or human, preferably humanized or human. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1988). "Antigen-binding portion" or "antigen-binding domain" of an intact antibody is meant to encompass an "antibody fragment," which indicates a portion of an intact antibody and refers to the antigenic determining variable regions or complementary determining regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, Fab'-SH, F(ab')₂, diabodies, linear antibodies, scFv antibodies, VH, and multispecific antibodies formed from antibody fragments. A "Fab" (fragment antigen binding) is a portion of an antibody that binds to antigens and includes the variable region and CH1 of the heavy chain linked to the light

chain via an inter-chain disulfide bond. An antibody may be of any class or subclass, including IgG and subclasses thereof (IgG₁, IgG₂, IgG₃, IgG₄), IgM, IgE, IgA, and IgD.

The term "variable region" or "variable domain" in the context of an antibody refers to the domain of an antibody heavy or light chain that is involved in binding of the antibody to antigen. The variable domains (or regions) of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three complementary determining regions (CDRs). (*See, e.g.,* Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007)). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.,* Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The terms "complementarity determining region" and "CDR," which are synonymous with "hypervariable region" or "HVR," are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

As used herein, the terms "binding domain", "binding region", and "binding moiety" refer to a molecule, such as a peptide, oligopeptide, polypeptide, or protein that possesses the ability to specifically and non-covalently bind, associate, unite, recognize, or combine with a target molecule (*e.g.,* tumor antigen). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or other target of interest. In some embodiments, the binding domain is an antigen-binding domain, such as an antibody or functional binding domain or antigen-binding portion thereof. Exemplary binding domains include single chain antibody variable regions (*e.g.,* domain antibodies, sFv, scFv, Fab), receptor ectodomains (*e.g.,* TNF- α), ligands (*e.g.,* cytokines, chemokines), or synthetic polypeptides selected for the specific ability to bind to a biological molecule.

"Major histocompatibility complex molecule" (MHC molecule) refers to a glycoprotein that delivers a peptide antigen to a cell surface. MHC class I molecules

are heterodimers composed of a membrane spanning α chain (with three α domains) and a non-covalently associated β 2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, α and β , both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides
5 originating in the cytosol to the cell surface, where peptide:MHC complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by $CD4^+$ T cells. An MHC molecule may be from various animal species, including human, mouse, rat, or other mammals.

10 "Chimeric antigen receptor" (CAR) refers to a chimeric fusion protein comprising two or more distinct domains linked together in a way that does not occur naturally in a host cell and can function as a receptor when expressed on the surface of a cell. CARs are generally composed of an extracellular domain comprising a binding domain that binds a target antigen, an optional extracellular spacer domain, a
15 transmembrane domain, and an intracellular signaling domain (*e.g.*, comprising an immunoreceptor tyrosine-based activation motif (ITAM)), and optionally an intracellular costimulatory domain). In certain embodiments, an intracellular signaling domain of a CAR has an ITAM (*e.g.*, CD3 ζ) containing intracellular signaling domain and an intracellular costimulatory domain (*e.g.*, 4-1BB). In certain embodiments, a
20 CAR is synthesized as a single polypeptide chain or is encoded by a nucleic acid molecule as a single chain polypeptide.

A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain affinities, such as Western blot, ELISA, analytical ultracentrifugation,
25 spectroscopy, surface plasmon resonance (BIACORE®) analysis, and MHC tetramer analysis (*see also, e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci.* 51:660, 1949; Wilson, *Science* 295:2103, 2002; Wolff *et al.*, *Cancer Res.* 53:2560, 1993; Altman *et al.*, *Science* 274:94-96, 1996; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent). As used herein, "specifically binds" refers to an association or union of a
30 binding domain, or a fusion protein thereof, to a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of $1/M$) equal to or greater than $10^5 M^{-1}$, while not significantly associating or uniting with any other molecules or components in a sample.

The terms “antigen” and “Ag” refer to a molecule that is capable of inducing an immune response. The immune response that is induced may involve antibody production, the activation of specific immunologically-competent cells, or both. Macromolecules, including proteins, glycoproteins, and glycolipids, can serve as an antigen. Antigens can be derived from recombinant or genomic DNA. As contemplated herein, an antigen need not be encoded (i) solely by a full-length nucleotide sequence of a gene or (ii) by a “gene” at all. An antigen can be generated or synthesized, or an antigen can be derived from a biological sample. Such a biological sample can include, but is not limited, to a tissue sample, a tumor sample, a cell, or a biological fluid.

The term “epitope” or “antigenic epitope” includes any molecule, structure, amino acid sequence or protein determinant within an antigen that is specifically bound by a cognate immune binding molecule, such as an antibody or fragment thereof (*e.g.*, scFv), T cell receptor (TCR), CAR, or other binding molecule, domain or protein. Epitopic determinants generally contain chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be a linear epitope or a conformational epitope.

As used herein, an “effector domain” is an intracellular portion of a fusion protein or chimeric receptor that can directly or indirectly promote a biological or physiological response in a cell expressing the effector domain when receiving the appropriate signal. In certain embodiments, an effector domain is part of a protein or protein complex that receives a signal when bound. In other embodiments, the effector domain is part of a protein or protein complex that binds directly to a target molecule, which triggers a signal from the effector domain. For example, in response to binding of a CAR to a target molecule, the effector domain may transduce a signal to the interior of the host cell, eliciting an effector function. An effector domain may directly promote a cellular response when it contains one or more signaling domains or motifs. In other embodiments, an effector domain will indirectly promote a cellular response by associating with one or more other proteins that directly promote a cellular response.

“Junction amino acids” or “junction amino acid residues” refer to one or more (*e.g.*, about 2-20) amino acid residues between two adjacent motifs, regions or domains of a polypeptide. Junction amino acids may result from the construct design of a

chimeric protein (*e.g.*, amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a fusion protein).

A “disease” is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein, if the disease is not ameliorated, then the subject’s health continues to deteriorate. In contrast, a “disorder” or “undesirable condition” in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject’s state of health is less favorable than it would be in the absence of the disorder or undesirable condition. Left untreated, a disorder or undesirable condition does not necessarily result in a further decrease in the subject’s state of health.

“Nucleic acid molecule” and “polynucleotide” can be in the form of RNA or DNA, which includes cDNA, genomic DNA, and synthetic DNA. A nucleic acid molecule may be composed of naturally occurring nucleotides (such as deoxyribonucleotides and ribonucleotides), analogs of naturally occurring nucleotides (*e.g.*, α -enantiomeric forms of naturally occurring nucleotides), or a combination of both. Modified nucleotides can have modifications in or replacement of sugar moieties, or pyrimidine or purine base moieties. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. A nucleic acid molecule may be double stranded or single stranded, and if single stranded, may be the coding strand or non-coding (anti-sense strand). A coding molecule may have a coding sequence identical to a coding sequence known in the art or may have a different coding sequence, which, as the result of the redundancy or degeneracy of the genetic code, or by splicing, can encode the same polypeptide.

“Encoding” refers to the inherent property of specific polynucleotide sequences, such as DNA, cDNA, and mRNA sequences, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a polynucleotide encodes a protein if transcription and translation of mRNA corresponding to that polynucleotide produces the protein in a cell or other biological system. Both a coding strand and a

non-coding strand can be referred to as encoding a protein or other product of the polynucleotide. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence.

5 As used herein, the term "endogenous" or "native" refers to a gene, protein, compound, molecule or activity that is normally present in a host or host cell, including naturally occurring variants of the gene, protein, compound, molecule, or activity.

 As used herein, "homologous" or "homolog" refers to a molecule or activity from a host cell that is related by ancestry to a second gene or activity, *e.g.*, from the
10 same host cell, from a different host cell, from a different organism, from a different strain, from a different species. For example, a heterologous molecule or heterologous gene encoding the molecule may be homologous to a native host cell molecule or gene that encodes the molecule, respectively, and may optionally have an altered structure, sequence, expression level or any combination thereof.

15 As used herein, "heterologous" nucleic acid molecule, construct or sequence refers to a nucleic acid molecule or portion of a nucleic acid molecule that is not native to a host cell, but can be homologous to a nucleic acid molecule or portion of a nucleic acid molecule from the host cell. The source of the heterologous nucleic acid molecule, construct or sequence can be from a different genus or species. In some embodiments,
20 the heterologous nucleic acid molecules are not naturally occurring. In certain embodiments, a heterologous nucleic acid molecule is added (*i.e.*, not endogenous or native) into a host cell or host genome by, for example, conjugation, transformation, transfection, transduction, electroporation, or the like, wherein the added molecule can integrate into the host cell genome or exist as extra-chromosomal genetic material (*e.g.*,
25 as a plasmid or other form of self-replicating vector), and can be present in multiple copies. In addition, "heterologous" refers to a non-native enzyme, protein or other activity encoded by a non-endogenous nucleic acid molecule introduced into the host cell, even if the host cell encodes a homologous protein or activity.

 As used herein, the term "engineered," "recombinant," “mutant,” “modified” or
30 "non-natural" refers to an organism, microorganism, cell, nucleic acid molecule, or vector that has been modified by introduction of a heterologous nucleic acid molecule, or refers to a cell or microorganism that has been genetically engineered by human intervention—that is, modified by introduction of a heterologous nucleic acid

molecule, or refers to a cell or microorganism that has been altered such that expression of an endogenous nucleic acid molecule or gene is controlled, deregulated or constitutive, where such alterations or modifications can be introduced by genetic engineering. Human-generated genetic alterations can include, for example, modifications introducing nucleic acid molecules (which may include an expression control element, such as a promoter) encoding one or more proteins, chimeric receptors, or enzymes, or other nucleic acid molecule additions, deletions, substitutions, or other functional disruption of or addition to a cell's genetic material. Exemplary modifications include those in coding regions or functional fragments thereof heterologous or homologous polypeptides from a reference or parent molecule. Additional exemplary modifications include, for example, modifications in non-coding regulatory regions in which the modifications alter expression of a gene or operon.

As used here, the term “transgene” refers to a gene or polynucleotide encoding a protein of interest (*e.g.*, a CAR) whose expression is desired in a host cell and that has been transferred by genetic engineering techniques into a cell. A transgene may encode proteins of therapeutic interest as well as proteins that are reporters, tags, markers, suicide proteins, etc. A transgene may be from a natural source, modification of a natural gene, or a recombinant or synthetic molecule. In certain embodiments, a transgene is a component of a vector.

The term “overexpressed” or “overexpression” of an antigen refers to an abnormally high level of antigen expression in a cell. Overexpressed antigen or overexpression of antigen is often associated with a disease state, such as in hematological malignancies and cells forming a solid tumor within a specific tissue or organ of a subject. Solid tumors or hematological malignancies characterized by overexpression of a tumor antigen can be determined by standard assays known in the art.

As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as

peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types.

“Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among
5 others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

As used herein, the term “mature polypeptide” or “mature protein” refers to a protein or polypeptide that is secreted or localized in the cell membrane or inside
10 certain cell organelles (*e.g.*, the endoplasmic reticulum, golgi, or endosome) and includes a partially cleaved N-terminal signal sequence (*e.g.*, one or more amino acids of the signal sequence remaining but less than the whole signal sequence) or does not include an N-terminal signal sequence (*i.e.*, the N-terminal signal sequence has been entirely removed, such as by an endogenous cleavage process, from the protein or
15 polypeptide).

A “signal sequence”, also referred to as “signal peptide”, “leader sequence”, “leader peptide”, “localization signal” or “localization sequence”, is a short peptide (usually 13-36 amino acids in length) present at the N-terminus of newly synthesized proteins that are destined for the plasma membrane or a secretory pathway. A signal
20 sequence typically comprises a short stretch of hydrophilic, positively charged amino acids at the N-terminus, a central hydrophobic domain of 5-15 residues, and a C-terminal region with a cleavage site for a signal sequence. In eukaryotes, a signal sequence prompts translocation of the newly synthesized protein to the endoplasmic reticulum where it is cleaved by the signal peptidase, creating a mature protein that then
25 proceeds to its appropriate destination. The diversity of signal sequence length and amino acid composition makes it difficult to precisely predict the cleavage site. For polypeptide sequences disclosures herein, where a signal sequence is noted, the polypeptide sequence absent the signal sequence or having a partial signal sequence is also contemplated.

30 The “percent identity” between two or more nucleic acid or amino acid sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical positions/total number of positions x 100), taking into account the number of gaps, and the length of each gap that needs to be introduced

to optimize alignment of two or more sequences. The comparison of sequences and determination of percent identity between two or more sequences can be accomplished using a mathematical algorithm, such as BLAST and Gapped BLAST programs at their default parameters (*e.g.*, Altschul *et al.*, *J. Mol. Biol.* 215:403, 1990; see also BLASTN
5 at www.ncbi.nlm.nih.gov/BLAST).

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (*see, e.g.*, WO 97/09433, page 10, published March 13, 1997; Lehninger, *Biochemistry*, Second Edition; Worth Publishers, Inc.
10 NY:NY (1975), pp.71-77; Lewin, *Genes IV*, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8).

The term "chimeric" refers to any nucleic acid molecule or protein that is not endogenous and comprises a combination of sequences joined or linked together that are not naturally found joined or linked together in nature. For example, a chimeric
15 nucleic acid molecule may comprise nucleic acids encoding various domains from multiple different genes. In another example, a chimeric nucleic acid molecule may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences that are derived from the same source but arranged in a manner different than that found in nature.

20 The term "promoter" as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the
25 promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may include an enhancer sequence and other regulatory elements that are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one that expresses the gene product in a tissue specific manner.

30 A "constitutive" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

An “inducible” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

5 A “tissue-specific” promoter is a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

10 The phrase “under transcriptional control” or “operatively linked” as used herein means that a promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.

15 A “vector” is a nucleic acid molecule that is capable of transporting another nucleic acid. Vectors may be, for example, plasmids, cosmids, viruses, or phage. The term should also be construed to include non-plasmid and non-viral compounds that facilitate transfer of nucleic acid into cells. An “expression vector” is a vector that is capable of directing the expression of a protein encoded by one or more transgenes carried by the vector when it is present in the appropriate environment. The one or more transgenes to be expressed by a vector as described herein are encoded in an
20 expression cassette.

25 A “lentiviral vector” is a vector derived from a lentivirus and includes one or more lentiviral packaging proteins and/or one or more lentiviral proteins necessary for expression of the one or more genes carried by the vector. The abbreviation “LVV” is used herein to refer to a lentiviral vector (singular), as well as multiple lentiviral vectors (plural).

 “Lentivirus” refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Examples of lentiviruses include, but are not limited to HIV (human immunodeficiency virus, including HIV type 1 and HIV type 2, equine infectious anemia virus, feline immunodeficiency virus (FIV), bovine immune
30 deficiency virus (BIV), and simian immunodeficiency virus (SIV).

 “Retrovirus” refers to are RNA viruses with a single strand positive-sense RNA molecule. Retroviruses comprise a reverse transcriptase enzyme and an integrase enzyme. Upon entry into a target cell, retroviruses utilize their reverse transcriptase to

transcribe their RNA molecule into a DNA molecule. Subsequently, the integrase enzyme is used to integrate the DNA molecule into the host cell genome. Upon integration into the host cell genome, the sequence from the retrovirus is referred to as a provirus (e.g., proviral sequence or provims sequence).

5 As used herein, the term “expression cassette” refers to a distinct component of a vector nucleic acid comprising at least one transgene and regulatory sequences controlling its expression (*e.g.*, promoter, 3’UTR) in a host cell. A tandem expression cassette refers to a component of a vector nucleic acid comprising at least two transgenes under the control of the same set of regulatory sequences for tandem
10 expression of the at least two transgenes. In certain embodiments, the tandem expression cassette comprises at least two transgenes under the control of the same promoter. In certain embodiments, the first transgene and second transgene are separated by an internal ribosome entry site (IRES), furin cleavage site, or self-cleaving viral 2A peptide to allow for co-expression of two proteins from a single mRNA.

15 The term “immune system cell” or “immune cell” means any cell of the immune system that originates from a hematopoietic stem cell in the bone marrow. Hematopoietic stem cells give rise to two major lineages: myeloid progenitor cells (which give rise to myeloid cells such as monocytes, macrophages, dendritic cells, megakaryocytes and granulocytes) and lymphoid progenitor cells (which give rise to
20 lymphoid cells such as T cells, B cells and natural killer (NK) cells). Exemplary immune system cells include a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a regulatory T cell, a natural killer cell, and a dendritic cell. Macrophages and dendritic cells may also be referred to as “antigen presenting cells” or “APCs,” which are specialized cells that can activate T cells when a major
25 histocompatibility complex (MHC) receptor on the surface of the APC complexed with a peptide interacts with a TCR on the surface of a T cell.

 The term “lymphocyte” refers to immune cells of lymphoid origin, which are cells that show at least one phenotype characteristic of a lymphocyte or a precursor or progenitor thereof that distinguishes the cells from cells of erythroid or myeloid
30 lineages. The term “lymphocytes” encompasses T cells, B cells, and natural killer (NK) cells.

 The term “T cells” refers to cells of T cell lineage. “Cells of T cell lineage” refer to cells that show at least one phenotypic characteristic of a T cell or a precursor or

progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for T cells (*e.g.*, CD3⁺, CD4⁺, CD8⁺), or a physiological, morphological, functional, or immunological feature specific for a T cell.

5 For example, cells of the T cell lineage may be progenitor or precursor cells committed to the T cell lineage; CD25⁺ immature and inactivated T cells; cells that have undergone CD4 or CD8 lineage commitment; thymocyte progenitor cells that are CD4⁺CD8⁺ double positive; single positive CD4⁺ or CD8⁺; TCRαβ or TCR γδ; or mature and functional or activated T cells. The term “T cells” encompasses naïve T cells (CD45RA⁺, CCR7⁺,
 10 CD62L⁺, CD27⁺, CD45RO⁻), central memory T cells (CD45RA⁻, CD45RO⁺, CD62L⁺, CCR7⁺, CD27⁺), effector memory T cells (CD45RA⁻, CD45RO⁺, CCR7⁻, CD62L⁻, CD27⁻), mucosal-associated invariant T (MAIT) cells, γδ T cells, Tregs, natural killer T cells, and tissue resident T cells.

The term “natural killer cells” or “NK cells” refers to large granular
 15 lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation. NK cells differ
 from natural killer T cells (NKTs) phenotypically, by origin and by respective effector
 20 functions; often, NKT cell activity promotes NK cell activity by secreting IFNγ. In contrast to NKT cells, NK cells do not express T-cell antigen receptors (TCR) or pan T marker CD3 or surface immunoglobulins (Ig) B cell receptors, but they usually express the surface markers CD16 (FcγRIII) and CD56 in humans, NK1.1 or NK1.2 in C57BL/6 mice. Up to 80% of human NK cells also express CD8.

25 The term “B cells” refers to cells of the B cell lineage. “Cells of B cell lineage” refers to cells that show at least one phenotypic characteristic of a B cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for B cells (*e.g.*, cells positive for one or
 30 more of CD19⁺, CD72⁺, CD24⁺, CD20⁺, CD21⁺, CD22⁺, CD38⁺, CD40⁺, CD72⁺, CD32b⁺, CD268⁺, CD269⁺, CD267⁺, CD86⁺, CD80⁺, CD40⁺, CD52⁺, CD138⁺, CD27⁺, CD28⁺, CD21⁺, CD23⁺, CD84⁺, CD257⁺, CD270⁺, CD37⁺, and CD74⁺), or a physiological, morphological, functional, or immunological feature specific for a B

cell. For example, cells of the B cell lineage may be progenitor or precursor cells committed to the B cell lineage (e.g., pre-pro-B cells, pro-B cells, and pre-B cells); immature and inactivated B cells or mature and functional or activated B cells. Thus, “B cells” encompass naïve B cells, plasma cells, regulatory B cells, marginal zone B cells, follicular B cells, lymphoplasmacytoid cells, plasmablast cells, and memory B cells (e.g., CD27⁺, IgD⁻).

The term “cytotoxic activity,” also referred to as “cytolytic activity,” with respect to a cell (e.g., T cell) that expresses an immune receptor (e.g., a CAR) on its surface, means that upon antigen-specific signaling (e.g., via the CAR) the cell induces a target cell to undergo apoptosis. In some embodiments, a cytotoxic cell may induce apoptosis in a target cell via the release of cytotoxins, such as perforin, granzyme, and granulysin, from granules. Perforins insert into the target cell membrane and form pores that allow water and salts to rapidly enter the target cell. Granzymes are serine proteases that induce apoptosis in the target cell. Granulysin is also capable of forming pores in the target cell membrane and is a proinflammatory molecule. In some embodiments, a cytotoxic cell may induce apoptosis in a target cell via interaction of Fas ligand, which is upregulated on T cell following antigen-specific signaling, with Fas molecules expressed on the target cell. Fas is an apoptosis-signaling receptor molecule on the surface of a number of different cells.

A “disease” is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein, if the disease is not ameliorated, then the subject’s health continues to deteriorate. In contrast, a “disorder” or “undesirable condition” in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject’s state of health is less favorable than it would be in the absence of the disorder or undesirable condition. Left untreated, a disorder or undesirable condition does not necessarily result in a further decrease in the subject’s state of health.

The term “cancer” as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. The aberrant cells may form solid tumors or constitute a hematological malignancy. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include, but are not limited to, breast cancer, prostate cancer, ovarian cancer,

cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

The term “subject,” “patient” and “individual” are used interchangeably herein and are intended to include living organisms in which an immune response can be
5 elicited (*e.g.*, mammals). Examples of subjects include humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, and transgenic species thereof.

“Adoptive cellular immunotherapy” or “adoptive immunotherapy” refers to the administration of naturally occurring or genetically engineered, disease antigen-specific
10 immune cells (*e.g.*, T cells). Adoptive cellular immunotherapy may be autologous (immune cells are from the recipient), allogeneic (immune cells are from a donor of the same species) or syngeneic (immune cells are from a donor genetically identical to the recipient).

“Autologous” refers to a graft (*e.g.*, organ, tissue, cells) derived from the same
15 subject to which it is later to be re-introduced.

“Allogeneic” refers to a graft derived from a different subject of the same species.

A “therapeutically effective amount” or “effective amount” of a lentiviral vector or a cell transduced by a lentiviral vector as described herein (*e.g.*, a T cell expressing a
20 CAR as encoded by the transgene of the lentiviral vector) refers to that amount of lentiviral particles or cells sufficient to result in amelioration of one or more symptoms of the disease, disorder, or undesired condition being treated.

“Treat” or “treatment” or “ameliorate” refers to medical management of a disease, disorder, or undesired condition of a subject. In general, an appropriate dose or
25 treatment regimen comprising a lentiviral vector or a cell expressing a CAR of this disclosure is administered in an amount sufficient to elicit a therapeutic or prophylactic benefit. Therapeutic or prophylactic/preventive benefit includes improved clinical outcome; lessening or alleviation of symptoms associated with a disease, disorder, or undesired condition; decreased occurrence of symptoms; improved quality of life;
30 longer disease-free status; diminishment of extent of disease, disorder, or undesired condition; stabilization of disease state; delay of disease progression; remission; survival; prolonged survival; or any combination thereof.

The term “anti-tumor effect” refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with a cancerous condition. An “anti-tumor effect” can also be manifested by prevention of a hematological malignancy or tumor formation.

Additional definitions are provided throughout the present disclosure.

Lentiviral Vector

Lentivirus is a genus of retroviruses that typically gives rise to slowly developing diseases due to their ability to incorporate into a host genome. Modified lentiviral genomes are useful as viral vectors for the delivery of a nucleic acids to a host cell.

The present disclosure provides self-inactivating lentiviral vectors (“LVV”) that include a viral envelope comprising a mutated, heterologous envelope protein and a tropism defining molecule (also referred to as a “targeting protein”). The LVV described herein further includes a transgene, with the LVV being capable of specifically binding to a target immune cell and transducing the target immune cell such that the transgene is expressed by the immune cell. In some embodiments, the LVV may carry more than one transgene. In further specific embodiments, the transgene encodes a chimeric antigen receptor (CAR).

Mutated Viral Envelope Protein

Lentiviral vectors as described herein are pseudotyped with a mutated heterologous viral envelope protein that, in the absence of mutation, mediates both cellular attachment and membrane fusion. In particular embodiments, the mutated envelope protein includes at least one mutation that inhibits the envelope protein’s ability to bind its native target, while preserving the envelope protein’s fusogenic properties. In specific embodiments, the heterologous envelope protein is a vesicular stomatitis virus G protein (“VSV-G”, “VSV-G envelope protein” or “VSV-G protein”) that includes one or more mutations that inhibit binding of VSV-G to the low-density

lipoprotein receptor (“LDL-R”), while preserving the VSV-G protein’s fusogenic function.

When referring to the envelope protein’s ability to bind its native target, the terms “inhibit” and “inhibits” encompass both complete elimination of binding by the envelope protein to its native target, as well as a significant reduction in binding by the envelope protein to its native target. In particular embodiments, “significant reduction” refers to a reduction selected from a reduction of at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, and at least 10% in binding to the native target.

The nucleic acid and amino acid sequences set forth in Table 10 provide reference sequences for a wild type VSV-G envelope protein and examples of mutated VSV-G envelope proteins according to the present disclosure. SEQ ID NO: 77 is a reference nucleic acid sequence encoding the VSV-G envelope protein, and SEQ ID NO: 78 is an amino acid sequence of a reference VSV-G envelope protein. SEQ ID NO: 87 is a nucleic acid sequence encoding a cleavable signal peptide of the reference VSV-G envelope protein. SEQ ID NO: 88 is an amino acid sequence of the cleavable signal peptide of the reference VSV-G envelope protein. SEQ ID NO: 89 is a reference nucleic acid sequence encoding the VSV-G envelope protein, absent the signal sequence. SEQ ID NO: 90 is an amino acid sequence of a reference VSV-G envelope protein absent the sequence. The position and nature of VSV-G mutations disclosed herein are described in reference to the nucleic acid sequence and the amino acid sequence provided by SEQ ID NO: 77 absent the N-terminal signal sequence of SEQ ID NO: 87 and SEQ ID NO: 78 absent the N-terminal signal sequence of SEQ ID NO: 88, respectively. Thus, reference to a position 1 of the VSV-G env amino acid sequence for the purpose of identifying mutation position refers to position 17 of the wildtype VSV-G amino acid sequence of SEQ ID NO: 78, which is a lysine (K), or position 1 of the wildtype VSV-G amino acid sequence of SEQ ID NO: 90.

In some embodiments, the mutated VSV-G envelope protein includes a mutation at amino acid position H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, 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, an insertion of GGS between N208 and Y209, and/or a deletion of residues 1-8. In certain embodiments, the VSV-G envelope protein includes two or more mutations at amino acid positions selected from H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, 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, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In other embodiments, the VSV-G envelope protein includes three or more mutations at amino acid positions selected from H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, 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, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In other embodiments, the mutated VSV-G envelope protein comprises a H8A, K47A, K47Q, Y209A, R354A, and/or R354Q mutation. In still other embodiments, the VSV-G envelope protein includes one or more mutations selected from N9, Q10, K50, A51, S183, S179, N180, I182, M184, I347, T350, T352, and E353, 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, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In yet other embodiments, the VSV-G envelope protein includes two or more mutations selected from a mutation at one or more of N9, Q10, K50, A51, S183, S179, N180, I182, M184, I347, T350, T352, and E353, 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, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In some embodiments, a mutated VSV-G envelope protein is selected from one or more of an H8A, N9A, Q10A, K47A, K47Q, N180A, I182A, Y209A, T352A, T352W, E353A, R354A, and R354Q mutation. In some embodiments, a mutated VSV-G envelope protein is selected from one or more of an H8A, K47A, K47Q, Y209A, R354A, and R354Q mutation. In some embodiments, the mutated VSV-G envelope protein comprises a K47 mutation and a R354 mutation. In some embodiments, the

mutated VSV-G envelope protein comprises a K47Q mutation and a R354A mutation. In some embodiments, the mutated VSV-G envelope protein comprises a N180 mutation, a I182 mutation, a T352 mutation, and a E353 mutation. In some
5 embodiments, the mutated VSV-G envelope protein comprises a N180A mutation, a I182A mutation, a T352A mutation, and a E353A mutation. In some embodiments, the mutated VSV-G envelope protein comprises a T352 mutation and E353 mutation. In further embodiments, the mutated VSV-G envelope protein comprises a T352W mutation and E353A mutation. In some embodiments, the mutated VSV-G envelope protein comprises a N9 mutation, a Q10 mutation, and a N180 mutation. In some
10 embodiments, the mutated VSV-G envelope protein comprises a N9A mutation, a Q10A mutation, and a N180A mutation. In some embodiments, the mutated VSV-G envelope protein comprises an insertion of GGS between H8 and N9 and an insertion of GGS between N9 and Q10. In some embodiments, the mutated VSV-G envelope protein comprises an insertion of TT between N9 and Q10. In some embodiments, the
15 mutated VSV-G envelope protein comprises an insertion of GGS between P46 and K47. In other embodiments, the mutated VSV-G envelope protein is as described in Nikolic et al., “Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein.” *Nature Comm.*, 2018, 9:1029, the relevant disclosures of which are incorporated by reference herein.

20 In specific embodiments, the mutant VSV envelope protein comprises a K47Q mutation and R354A mutation. In specific embodiments, the mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 74.

 In specific embodiments, the mutant VSV envelope protein comprises a N180A mutation, an I182A mutation, a T352A mutation, and an E353A mutation. In specific
25 embodiments, the mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 93.

 In specific embodiments, the mutant VSV envelope protein comprises a T352W mutation and an E353A mutation. In specific embodiments, the mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 95.

30 In specific embodiments, the mutant VSV envelope protein comprises a N9A mutation, a Q10A mutation, and a N180A mutation. In specific embodiments, the

mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 97.

In specific embodiments, the mutant VSV envelope protein comprises an insertion of TT between N9 and Q10 mutation. In specific embodiments, the mutant
5 VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 99.

In specific embodiments, the mutant VSV envelope protein comprises an insertion of GGS between P46 and K47 mutation. In specific embodiments, the mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 101.

In specific embodiments, the mutant VSV envelope protein comprises an
10 insertion of GGS between H8 and N9 and an insertion of GGS between N9 and Q10. In specific embodiments, the mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 103.

Targeting Protein

15 The targeting protein is a membrane-bound protein displayed on the viral envelope and includes an extracellular domain that includes a lymphocyte targeting domain and a transmembrane domain. A lymphocyte targeting domain is any protein or peptide that has an amino acid sequence and is a binding partner for a target molecule or ligand (*e.g.*, a cognate protein or ligand) on a target lymphocyte cell surface. In some
20 embodiments, a lymphocyte targeting domain is a T cell targeting domain. In some embodiments, a lymphocyte targeting domain is a NK cell targeting domain. In some embodiments, a lymphocyte targeting domain is a B cell targeting domain. For example, in certain embodiments, the extracellular domain of the targeting protein includes a lymphocyte targeting domain that specifically binds a protein or ligand on
25 the surface of a defined population of cells, such as a population of lymphocytes characterized by the presence of the targeted protein or ligand on the cell surface. In some embodiments, a lymphocyte targeting domain targets a specific type of lymphocyte, such as for example, T cells, B cells, or natural killer (NK) cells. In some embodiments, the targeting protein additionally includes an extracellular linker or hinge
30 domain positioned between the transmembrane domain and the extracellular domain.

In some embodiments, the targeting protein is a full-length cell surface protein or receptor, wherein each component (*e.g.*, each of the extracellular domain, transmembrane domain, and intracellular domain) derived from a single protein or receptor. In other embodiments, the targeting protein is a chimeric protein, having at least two components derived from a different protein or receptor. In some

5 embodiments, the targeting protein is obtained from a mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, and pigs. In some embodiments, the targeting protein is a fully human sequence. In some embodiments, the targeting protein is a chimeric sequence comprising human

10 sequence component(s) and component(s) from another species or synthetic component(s). In some embodiments, a chimeric targeting protein may be a fully synthetic protein. In still other embodiments, a chimeric targeting protein may include one or more of an extracellular domain, an extracellular binding domain, a transmembrane domain, an intracellular domain, a hinge domain, or a linker that is a

15 fully synthetic protein.

In some embodiments, the lymphocyte targeting domain includes an antibody. In such embodiments, the extracellular targeting domain may be a full-length antibody, an antibody fragment, a nanobody, or a single chain Fv fragment (scFv).

In particular embodiments, the targeting molecule includes a T cell targeting

20 domain that specifically binds to a T cell surface marker, including, for example, a T cell antigen, a T cell surface receptor, or any other protein present on the surface of a targeted T cell. In some embodiments, the targeting protein specifically binds to 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,

25 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, IL6ST, and any combination thereof.

In further embodiments, the targeting protein specifically binds to a $\gamma\delta$ T cell

30 marker selected from $\gamma\delta$ TCR, Vdelta1, Vdelta2, NKG2D (KLRK1, CD314), and any combination thereof.

In other embodiments, the targeting protein specifically binds to an NK T cell marker selected from Invariant TCR (Va24-Ja18), CD185 (CXCR5), CXCR6, IL-21R, and any combination thereof.

In other embodiments, the targeting protein specifically binds to a MAIT cell marker selected from Va7.2, Ja33, CXCR6, IL-18R, KLRB1 (CD161), VLA4 (alpha4beta1 integrin), and any combination thereof.

In other embodiments, the targeting protein 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), IL-18R, and any combination thereof.

In other embodiments, the targeting protein 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, and any combination thereof.

In embodiments, the targeting protein specifically binds to a lymphocyte marker (e.g., B cell and T cells) selected from CD80, CD27, CD28, and any combination thereof.

In some embodiments of the targeting protein, a linker is positioned between the transmembrane domain and the lymphocyte targeting domain. A linker is an amino acid linker and may be a rigid linker, a flexible linker, or an oligomerized linker. A rigid linker is an amino acid sequence that lacks flexibility (*e.g.*, may comprise at least one proline). In some embodiments, a rigid linker comprises a platelet-derived growth factor receptor (PDGFR) stalk or a CD8a stalk. In some embodiments, a PDGFR stalk comprises an amino acid sequence comprising AVGQDTQEVIVVPHSLPFK (SEQ ID NO: 104). In some embodiments, a PDGFR stalk comprises an amino acid sequence comprising ASAKPTTTPAPRPPTPAPTIASQPLSLRPEAARPAAGGAVHTRGLDFAK (SEQ ID NO: 105).

A flexible linker is an amino acid sequence that has many degrees of freedom (*e.g.*, may comprise a plurality of amino acids with small side chains, *e.g.*, glycine or alanine). In some embodiments, a flexible linker comprises an amino acid sequence

comprising GAPGAS. In some embodiments, a flexible linker comprises an amino acid sequence consisting of GAPGSGGGGSGGGGSAS (SEQ ID NO: 106). In some embodiments, a flexible linker comprises an amino acid sequence comprising GGGGS. In some embodiments, a flexible linker comprises an amino acid sequence comprising GGGG. In some embodiments, a flexible linker comprises an amino acid sequence comprising (GAPGAS)_N, (G₃S)_N, or (G₄S)_N, wherein N is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

An oligomerized linker is an amino acid that can oligomerize to another related amino acid. In some embodiments, an oligomerized linker is an amino acid sequence that can form a dimer, trimer, or tetramer. In some embodiments, an oligomerized linker comprises an IgG4 hinge domain (*e.g.*, ESKYGPPCPPCPAVGQDTQEVIVPHSLPFFK (SEQ ID NO: 107)). In some embodiments, an oligomerized linker comprises an amino acid sequence that can form a tetrameric coiled coil (*e.g.*, ASGGGGSGELAAIKQELAAIKKELAAIKWELAAIKQGAG (SEQ ID NO: 108)). In some embodiments, an oligomerized linker comprises an amino acid sequence that can form a dimeric coiled coil (*e.g.*, ASESKYGPPCPPCP (SEQ ID NO: 109)).

The targeting protein will typically include a signal sequence (also referred to as a signal peptide or localization sequence). The signal sequence can be located at the N- or C-terminal ends of the targeting protein. A signal sequence functions to translocate the targeting protein to the membrane that serves as the envelope of the lentiviral vector. Non-limiting examples of signal sequences that might be included in a targeting protein as described herein include an Ig Kappa leader sequence (*e.g.*, a murine Ig Kappa leader sequence comprising: METDTLLLWVLLLWVPGSTG (SEQ ID NO: 110)), CD8a signal peptide sequence (*e.g.*, a CD8a signal peptide comprising: MALPVTALLLPLALLLHAARP (SEQ ID NO: 12)), and a B2M signal peptide sequence (*e.g.*, a B2M signal peptide sequence comprising: MSRSVALAVLALLSLSGLEA (SEQ ID NO: 123)). In certain embodiments, a mature targeting protein has a partially cleaved signal sequence that retains one or more amino acids of the full-length signal sequence. In other embodiments, cleavage by a signal peptidase results in complete removal of the signal sequence, resulting in a mature targeting protein that lacks a signal sequence entirely.

In some embodiments, the targeting protein is a CD80 protein. Where the targeting protein is a CD80 protein, it may include an extracellular domain according to SEQ ID NO: 6. In such embodiments, a CD80 protein may include a signal peptide according to SEQ ID NO: 4 and a transmembrane and intracellular domain according to SEQ ID NO: 8. In a specific embodiment, a CD80 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence according to SEQ ID NO: 2 or SEQ ID NO: 2 absent the signal peptide of SEQ ID NO: 4.

In some embodiments, where the targeting protein is a CD80 protein, it may include 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. In such embodiments, a CD80 protein may include a signal peptide 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: 4, and/or 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. In a specific embodiment, a CD80 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence 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: 2 or SEQ ID NO: 2 absent the signal peptide of SEQ ID NO: 4.

20

TABLE 1: CD80 T cell targeting molecule

Full molecule	
Nucleotide	Amino acid (signal peptide underlined)
ATGGGTCATACACGCCGCAAGGA	<u>MGHTRRQGTSPSKCPYLNFFQLLVL</u>
ACCTCACCATCTAAGTGCCCATAT	<u>AGLSHFCSGVIHVTKEVKEVATLSC</u>
CTGAATTTCTTTCAACTTCTCGTGC	GHNVSVEELAQTRIWQKEKKMVL
TGGCGGGGCTCAGTCATTTCTGCA	TMMSGDMNIWPEYKNRTIFDITNNL
GTGGGGGTCATTCACGTTACTAAAG	SIVILALRPSDEGTYECVVLKYEKDA
AGGTCAAGGAGGTCGCAACATTGA	FKREHLAEVTL SVKADFP TPSISDFEI
GTTGTGGCCATAACGTATCAGTTG	PTSNIRRIICSTSGGFPEPHLSWLENG
AAGAACTCGCGCAGACACGGATTT	EELNAINTTVSQDPETELYAVSSKLD
ACTGGCAAAGGAAAAGAAGATG	FNMTTNHSFMCLIKYGH LRVNQTFN
GTGTTGACAATGATGAGCGGTGAC	WNTTKQEHFPDNL LPSWAITLISVN
ATGAACATTTGGCCAGAGTACAAA	GIFVICCLTYCFAPRCRE (SEQ ID
AATCGAACGATATTGATATAACC	NO: 2)
AATAACTTGTCATAGTAATACTT	
GCCTTGCGACCTTCTGACGAGGGA	

ACGTATGAATGTGTAGTGCTTAAG TATGAAAAAGATGCCTTTAAGCGG GAACACTTGGCTGAGGTTACACTC TCCGTTAAGGCGGACTTTCCTACG CCGTCTATATCCGACTTCGAGATA CCCACTTCTAACATTCGACGCATC ATTTGCTCAACCTCAGGTGGTTTCC CAGAGCCTCACTTGAGCTGGCTGG AGAATGGCGAAGAACTTAACGCA ATCAATACCACGGTGTCCCAAGAC CCGGAGACAGAGCTGTACGCCGTG TCATCCAAACTGGATTTTAACATG ACGACAAATCATAGTTTCATGTGT CTGATCAAATATGGGCATCTCAGG GTGAATCAGACTTTTAATTGGAAC ACTACCAAACAAGAGCACTTCCCA GATAATCTGTTGCCAAGCTGGGCG ATAACTCTTATCTCCGTCAACGGT ATCTTCGTAATTTGCTGCCTCACCT ATTGTTTCGCGCCTCGATGCCGAG AA (SEQ ID NO: 1)	
CD80 signal peptide	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGA ACCTCACCATCTAAGTGCCCATAT CTGAATTTCTTTCAACTTCTCGTGC TGGCGGGGCTCAGTCATTTCTGCA GTGGGGGTC (SEQ ID NO: 3)	MGHTRRQGTSPSKCPYLNFFQLVL AGLSHFCSGV (SEQ ID NO: 4)
CD80 extracellular domain (ECD)	
Nucleotide	Amino acid
ATTCACGTTACTAAAGAGGTCAAG GAGGTCGCAACATTGAGTTGTGGC CATAACGTATCAGTTGAAGAACTC GCGCAGACACGGATTTACTGGCAA AAGGAAAAGAAGATGGTGTGAC AATGATGAGCGGTGACATGAACAT TTGGCCAGAGTACAAAAATCGAAC GATATTCGATATAACCAATAACTT GTCCATAGTAATACTTGCTTGCG ACCTTCTGACGAGGGAACGTATGA ATGTGTAGTGCTTAAGTATGAAAA AGATGCCTTTAAGCGGGAACACTT GGCTGAGGTTACACTCTCCGTAA GGCGGACTTTCCTACGCCGTCTAT ATCCGACTTCGAGATACCACTTC TAACATTTCGACGCATCATTTGCTC AACCTCAGGTGGTTTCCCAGAGCC TCACTTGAGCTGGCTGGAGAATGG	IHVTKEVKEVATLSCGHNVSVLELA QTRIYWQKEKKMVLTMMSGDMNI WPEYKNRTIFDITNNLSIVILALRPSD EGTYECVVLKYEKDAFKREHLAEV TLSVKADFPSPSISDFEIPTSNIRRIICS TSGGFPEPHLSWLENGEELNAINTTV SQDPETELYAVSSKLDFNMTTNHSF MCLIKYGHRLRVNQTFNWNTTKQEH FPDNLLPS (SEQ ID NO: 6)

CGAAGAACTTAACGCAATCAATAC CACGGTGTCCCAAGACCCGGAGAC AGAGCTGTACGCCGTGTCATCCAA ACTGGATTTTAAACATGACGACAAA TCATAGTTTCATGTGTCTGATCAAA TATGGGCATCTCAGGGTGAATCAG ACTTTTAATTGGAACACTACCAAA CAAGAGCACTTCCCAGATAATCTG TTGCCAAGC (SEQ ID NO:5)	
CD80 transmembrane and intracellular domain	
Nucleotide	Amino acid
TGGGCGATAACTCTTATCTCCGTC AACGGTATCTTCGTAATTTGCTGCC TCACCTATTGTTTCGCGCCTCGATG CCGAGAA (SEQ ID NO: 7)	WAITLISVNGIFVICCLTYCFAPRCRE (SEQ ID NO: 8)

In some embodiments, lentiviral vectors comprising a CD80 targeting protein exhibit enhanced transduction CD4 T cells over CD8 T cells as a relative percentage of total CD4 and CD8 T cells compared to the transduction by standard LVV. As used herein, “standard lentiviral vector” or “standard LVV” refers to a lentiviral vector

5 which does not contain a mutated VSV-G envelope protein and a lymphocyte targeting protein of the present disclosure. In some embodiments, a standard LVV is generated using a 3rd generation LVV packaging system. In some embodiments, lentiviral vectors comprising a CD80 targeting protein enhance transduction of CD4 T cells over CD8 T cells as a relative percentage of total CD4 and CD8 T cells at least 5%, 10%, 15%,

10 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more compared to standard LVV.

In some embodiments, the targeting protein is an anti-CD3 targeting protein. In some embodiments, an anti-CD3 targeting protein is an antibody or binding fragment thereof, including for example, a scFv. Where the targeting protein is an anti-CD3

15 targeting protein, it may include an anti-CD3 scFv having a light chain variable region according to SEQ ID NO: 14, a G₃S linker according to SEQ ID NO: 16, and a heavy chain variable region according to SEQ ID NO: 18. In some embodiments, an anti-CD3 targeting protein may comprise an anti-CD3 scFv having a light chain variable region according to SEQ ID NO: 115, a G₃S linker according to SEQ ID NO: 16, and a heavy

20 chain variable region according to SEQ ID NO: 113. An anti-CD3 targeting protein as described may include a signal peptide according to SEQ ID NO: 12 and a hinge and

transmembrane domain according to SEQ ID NO: 20. In a specific embodiment, an anti-CD3 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence according to SEQ ID NO: 10 or SEQ ID NO: 10 absent the signal peptide of SEQ ID NO: 12. In another specific embodiment, an anti-CD3 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence according to SEQ ID NO: 117 or SEQ ID NO: 117 absent the signal peptide of SEQ ID NO: 12.

In some embodiments, where the targeting protein is an anti-CD3 targeting protein, it may include 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. An anti-CD3 targeting protein as described may include a signal peptide 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: 12 and a 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: 20. In a specific embodiment, an anti-CD3 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence 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: 10 or SEQ ID NO: 10 absent the signal peptide of SEQ ID NO: 12.

In some embodiments, where the targeting protein is an anti-CD3 targeting protein, it may include 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. An anti-CD3 targeting protein as described may include a signal peptide 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: 12 and a 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: 20. In a specific embodiment, an anti-CD3 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence 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: 117 or SEQ ID NO: 117 absent the signal peptide of SEQ ID NO: 12.

TABLE 2: Anti-CD3 T cell targeting molecules

Anti-CD3 (UCTH1) Full molecule	
Nucleotide	Amino acid (signal peptide underlined)
ATGGCCTTACCAGTGACCGCCTTG	<u>MALPVTALLLPLALLLHAARPD</u> IQM
CTCCTGCCGCTGGCCTTGCTGCTCC	TQTTSSLASLGDRVTISCRASQDIR
ACGCCGCCAGGCCGGACATCCAGA	NYLNWYQQKPDGTVKLLIYYTSRL
TGACCCAGACCACCTCCTCCCTGT	HSGVPSKFSGSGSGTDYSLTISNLEQ
CTGCCTCTCTGGGAGACAGAGTCA	EDIATYFCQQGNTLPWTFAGGTKLE
CCATCAGTTGCAGGGCAAGTCAGG	IKRAGGSGGGSGGGSGGGSEVQLQ
ACATTAGAAATTATTTAAACTGGT	QSGPELVKPGASMKISCKASGYSFT
ATCAACAGAAACCAGATGGAACCTG	GYTMNWVKQSHGKNLEWMGLINP
TTAAACTCCTGATCTACTACACATC	YKGVSTYNQKFKDKATLTVDKSSST
AAGATTACACTCAGGAGTCCCATC	AYMELLSLTSEDSAVYYCARSGYY
AAAGTTCAGTGGCAGTGGGTCTGG	GDSDWYFDVWGAGTTVTVSSTTTP
AACAGATTATTCTCTCACCATTAG	APRPPTPAPTIASQPLSLRPEACRPAA
CAACCTGGAGCAAGAGGATATTGC	GGAVHTRGLDFACDIYIWAPLAGTC
CACTTACTTTTGCCAACAGGGTAA	GVLLLSLVITLYC (SEQ ID NO: 10)
TACGCTTCCGTGGACGTTTCGCTGG	
AGGCACCAAGCTGGAAATCAAAC	
GGGCTGGAGGCGGTAGTGGCGGTG	
GATCAGGTGGAGGCAGCGGTGGC	
GGATCTGAGGTGCAGCTCCAGCAG	
TCTGGACCTGAGCTGGTGAAGCCT	
GGAGCTTCAATGAAGATATCCTGC	
AAGGCTTCTGGTTACTCATTCACTG	
GCTACACCATGAACTGGGTGAAGC	
AGAGTCATGGAAAGAACCTTGAGT	
GGATGGGACTTATTAATCCTTACA	
AAGGTGTTAGTACCTACAACCAGA	
AGTTCAAGGACAAGGCCACATTAA	
CTGTAGACAAGTCATCCAGCACAG	
CCTACATGGAACCTCAGTCTGA	
CATCTGAGGACTCTGCAGTCTATT	
ACTGTGCAAGATCGGGGTACTACG	
GTGATAGTGACTGGTACTTCGATG	
TCTGGGGCGCAGGGACCACGGTCA	
CCGTCTCCTCAACCACtACaCCAGCa	
CCtaGACCACCAACACCTGCGCCaAC	
CATCGCaTCGCAGCCaCTGTCTCTGC	

GCCCAGAGGCaTGCCGGCCAGCaG CtGGGGGCGCAGTGCACACaAGGG GGCTGGACTTCGCaTGTGATATCTA CATCTGGGCaCCaTTGGCaGGGACT TGTGGGGTCCTTCTCCTGTCACTGG TTATCACCCCTTTACTGC (SEQ ID NO: 9)	
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCC ACGCCGCCAGGCCG (SEQ ID NO: 11)	MALPVTALLPLALLLHAARP (SEQ ID NO:12)
Anti-CD3 (UCTH1) Light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACCCAGACCACC TCCTCCCTGTCTGCCTCTCTGGGAG ACAGAGTCACCATCAGTTGCAGGG CAAGTCAGGACATTAGAAATTATT TAAACTGGTATCAACAGAAACCAG ATGGAACGTGTTAACTCCTGATCT ACTACACATCAAGATTACACTCAG GAGTCCCATCAAAGTTCAGTGGCA GTGGGTCTGGAACAGATTATTCTC TCACCATTAGCAACCTGGAGCAAG AGGATATTGCCACTTACTTTTGCCA ACAGGGTAATACGCTTCCGTGGAC GTTCGCTGGAGGCACCAAGCTGGA AATCAAACGGGCT (SEQ ID NO: 13)	DIQMTQTTSSLSASLGDRVITISCRAS QDIRNYLNWYQQKPDGTVKLLIYYT SRLHSGVPSKFSGSGSGTDYSLTISN LEQEDIATYFCQQGNTLPWTFAGGT KLEIKRA (SEQ ID NO:14)
G₃S linker	
Nucleotide	Amino acid
GGAGGCGGTAGTGGCGGTGGATCA GGTGGAGGCAGCGGTGGCGGATCT (SEQ ID NO: 15)	GGGSGGGSGGGSGGGS (SEQ ID NO:16)
Anti-CD3 (UCTH1) heavy chain variable region	
Nucleotide	Amino acid
GAGGTGCAGCTCCAGCAGTCTGGA CCTGAGCTGGTGAAGCCTGGAGCT TCAATGAAGATATCCTGCAAGGCT TCTGGTACTCATTCACTGGCTACA CCATGAACTGGGTGAAGCAGAGTC ATGGAAGAAGACCTTGAGTGGATGG GACTTATTAATCCTTACAAAGGTG TtagTACCTACAACCAGAAAGTTCA AGGACAAGGCCACATTAAGTGTAG ACAAGTCATCCAGCACAGCCTACA TGGAACCTCCTCAGTCTGACATCTG AGGACTCTGCAGTCTATTACTGTG	EVQLQQSGPELVKPGASMKISCKAS GYSFTGYTMNWVKQSHGKNLEWM GLINPYKGVSTYNQKFKDKATLTVD KSSSTAYMELLSLTSEDSAVYYCAR SGYYGDSDWYFDVWGAGTTVTVSS (SEQ ID NO: 18)

CAAGATCGGGGTACTACGGTGATA GTGACTGGTACTTCGATGTCTGGG GCGCAGGGACCACGGTCACCGTCT CCTCA (SEQ ID NO: 17)	
CD8a hinge and transmembrane	
Nucleotide	Amino acid
ACCACTACACCAGCACCTAGACCA CCAACACCTGCGCCAACCATCGCA TCGCAGCCACTGTCTCTGCGCCA GAGGCATGCCGGCCAGCAGCTGGG GGCGCAGTGCACACAAGGGGGCT GGACTTCGCATGTGATATCTACAT CTGGGCACCATTGGCAGGGACTTG TGGGGTCCTTCTCCTGTCACTGGTT ATCACCCCTTACTGC (SEQ ID NO: 19)	TTTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYWAPL AGTCGVLLLSLVITLYC (SEQ ID NO: 20)
Anti-CD3 (12F6) heavy chain variable region	
Nucleotide	Amino Acid
CAAGTGCAGCTCCAGCAGAGCGGC GCTGAGCTGGCCCGGCCGCGCC AGCGTGAAGATGAGCTGTAAAGCC AGCGGCTATACATTTACCAGCTAC ACCATGCACTGGGTCAAGCAGCGG CCTGGCCAGGGCCTGGAATGGATT GGATATATCAACCCCAGCAGCGGC TACACCAAGTACAACCAGAAATTC AAGGACAAGGCCACCCTGACCGCC GACAAGAGCTCCTCAACAGCCTAC ATGCAACTGAGCAGCCTGACCAGC GAGGATAGCGCCGTGTACTACTGC GCCAGATGGCAGGACTACGACGTG TACTTCGACTACTGGGGCCAAGGC ACAACACTGACCGTGTCCAGC (SEQ ID NO: 112)	QVQLQQSGAELARPGASVKMSCKA SGYTFTSYTMHWVKQRPGQGLEWI GYINPSSGYTKYNQKFKDKATLTAD KSSSTAYMQLSSLTSEDSAVYYCAR WQDYDVYFDYWGGQTTLTVSS (SEQ ID NO: 113)
Anti-CD3 (12F6) light chain variable region	
Nucleotide	Amino Acid
CAGATCGTGCTGAGCCAGTCCCCA GCCATCCTGTCTGCCAGCCCTGGC GAGAAGGTGACCATGACCTGCAGA GCCTCTTCTTCTGTTTCCTACATGC ACTGGTATCAGCAAAAGCCCGGCA GCTCTCCTAAGCCTTGGATCTACG CCACAAGCAACCTGGCTAGCGGCG TGCCTGCTCGCTTCAGCGGCAGCG GCAGCGGCACCAGCTACAGCCTGA CCATCAGCAGAGTGGAAGCCGAG GACGCCGCCACCTACTACTGCCAG CAGTGGTCCTCTAATCCTCCAACA	QIVLSQSPAILSASPGEKVTMTCRAS SSVSYMHWYQQKPGSSPKPWIYATS NLASGVPARFSGSGSGTSYSLTISRV EAEDAATYYCQQWSSNPPTFGGGT KLETKR (SEQ ID NO: 115)

TTCGGCGGGCGGCACCAAGCTGGAA ACCAAAAGA (SEQ ID NO: 114)	
Anti-CD3 (12F6) Full molecule	
Nucleotide	Amino Acid (signal peptide underlined)
ATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCC ACGCCGCCAGGCCGCAAGTGCAGC TCCAGCAGAGCGGCGCTGAGCTGG CCCGGCCCGGCCGCGCAGCGTGAAGA TGAGCTGTAAAGCCAGCGGCTATA CATTTACCAGCTACACCATGCACT GGGTCAAGCAGCGGCCTGGCCAGG GCCTGGAATGGATTGGATATATCA ACCCCAGCAGCGGCTACACCAAGT ACAACCAGAAATTCAAGGACAAG GCCACCCTGACCGCCGACAAGAGC TCCTCAACAGCCTACATGCAACTG AGCAGCCTGACCAGCGAGGATAGC GCCGTGTACTACTGCGCCAGATGG CAGGACTACGACGTGTACTTCGAC TACTGGGGCCAAGGCACAACACTG ACCGTGTCCAGCGGAGGCGGTAGT GGCGGTGGATCAGGTGGAGGCAG CGGTGGCGGATCTCAGATCGTGCT GAGCCAGTCCCCAGCCATCCTGTC TGCCAGCCCTGGCGAGAAGGTGAC CATGACCTGCAGAGCCTCTTCTTCT GTTTCCTACATGCACTGGTATCAG CAAAAGCCCGGCAGCTCTCCTAAG CCTTGGATCTACGCCACAAGCAAC CTGGCTAGCGGCGTGCCTGCTCGC TTCAGCGGCAGCGGCAGCGGCACC AGCTACAGCCTGACCATCAGCAGA GTGGAAGCCGAGGACGCCGCCACC TACTACTGCCAGCAGTGGTCCTCT AATCCTCCAACATTCGGCGGCGGC ACCAAGCTGGAAACCAAAAGAAC CACTACACCAGCACCTAGACCACC AACACCTGCGCCAACCATCGCATC GCAGCCACTGTCTCTGCGCCCAGA GGCATGCCGGCCAGCAGCTGGGGG CGCAGTGACACAAGGGGGGCTGG ACTTCGCATGTGATATCTACATCTG GGCACCATTGGCAGGGACTTGTGG GGTCCTTCTCCTGTCACTGGTTATC ACCCTTTACTGC (SEQ ID NO: 116)	<u>MALPVTALLLPLALLLHAARP</u> QVQLQQSGAELARPGASVKMSCKA SGYTFTSYTMHWVKQRPGQGLEWI GYINPSSGYTKYNQKFKDKATLTAD KSSSTAYMQLSSLTSEDSAVYYCAR WQDYDVYFDYWGQGTTTLTVSSGG GSGGGSGGGSGGGG QIVLSQSPAILSASPGEKVTMTCRAS SSVSYMHWYQQKPGSSPKPWIYATS NLASGVPARFSGSGSGTSYSLTISR EAEDAATYYCQQWSSNPPTFGGGT KLETKRRTTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGLDFACDI YIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 117)

In still further embodiments, a lentiviral vector according to the present description may include multiple targeting proteins or a single targeting protein having multiple different lymphocyte targeting domains. For example, the lentiviral vectors described herein can include at least two targeting proteins, with each of the targeting proteins having a NK cell targeting domain that specifically binds a different target. In another example, the lentiviral vectors described herein can include at least two targeting proteins, with each of the targeting proteins having a T cell targeting domain that specifically binds a different target. In another example, the lentiviral vectors described herein can include at least two targeting proteins, with each of the targeting proteins having a B cell targeting domain that specifically binds a different target. In yet another example, the lentiviral vectors described herein can include at least two targeting proteins, with a first targeting protein having a T cell targeting domain and a second targeting protein having a NK cell targeting domain. In yet another example, the lentiviral vectors described herein can include at least two targeting proteins, with a first targeting protein having a T cell targeting domain and a second targeting protein having a B cell targeting domain. In yet another example, the lentiviral vectors described herein can include at least two targeting proteins, with a first targeting protein having a B cell targeting domain and a second targeting protein having a NK cell targeting domain. In alternative embodiments, the lentiviral vectors described herein include a single targeting protein having two or more different lymphocyte targeting domains, two or more different T cell targeting domains, two or more different NK cell targeting domains, or two or more different B cell targeting domains (*e.g.*, a bi-specific or multi-specific targeting protein). In any such embodiments, the T lymphocyte targeting domains included in the targeting proteins can be selected from any of those described herein. In yet another example, the lentiviral vectors described herein can include at least two targeting proteins, with a first targeting protein having a lymphocyte targeting domain and a second targeting protein having a T cell targeting domain. In yet another example, the lentiviral vectors described herein can include at least two targeting proteins, with a first targeting protein having a lymphocyte targeting domain and a second targeting protein having a B cell targeting domain.

In an exemplary embodiment, a lentiviral vector having multiple targeting proteins includes a CD80 targeting protein and an anti-CD3 targeting protein as detailed

in Table 1 and Table 2, respectively. In some embodiments, a CD80 targeting protein includes an amino acid sequence according to SEQ ID NO: 2 or SEQ ID NO: 2 absent the signal peptide of SEQ ID NO: 4. In some embodiments, an anti-CD3 targeting protein includes an amino acid sequence according to SEQ ID NO: 10 or SEQ ID NO: 10 absent the signal peptide of SEQ ID NO: 12. In some embodiments, an anti-CD3 targeting protein includes an amino acid sequence according to SEQ ID NO: 117 or SEQ ID NO: 117 absent the signal peptide of SEQ ID NO: 12. Where the lentiviral vector includes a CD80 targeting protein and an anti-CD3 targeting protein, the targeting proteins may be encoded on separate expression vectors used in producing the lentiviral vector. Alternatively, both a CD80 targeting protein and an anti-CD3 targeting protein may be encoded on a tandem expression cassette, providing a single expression cassette in a single expression vector that provides expression of both targeting proteins for production of the lentiviral vector. Exemplary embodiments of nucleic acid sequences and the corresponding amino acid sequences for a CD80 targeting protein and an anti-CD3 targeting protein expressed from the same expression cassette are provided in Table 3. In such an embodiment, the two targeting proteins may be linked by a P2A self-cleaving peptide. Table 3 provides an exemplary nucleic acid that may be used in a tandem expression cassette that includes a CD80 binding protein linked to an anti-CD3 binding protein via a P2A sequence. In a particular embodiment, the P2A sequence is encoded by a nucleic acid sequence 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: 29 and the amino acid sequence of the P2A sequence has an amino acid sequence 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: 30. In some embodiments, the tandem expression cassette that includes a CD80 binding protein linked to an anti-CD3 binding protein comprises the nucleic acid sequence of SEQ ID NO: 21 or 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: 21. In some embodiments, the tandem expression cassette that includes a CD80 binding protein linked to an anti-CD3 binding protein encodes an amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 22 absent the signal peptide of SEQ ID NO: 24. In some embodiments, the tandem expression cassette that includes a CD80 binding protein linked to an anti-CD3 binding protein encodes an amino acid sequence having 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: 22 or SEQ ID NO: 22 absent the signal peptide of SEQ ID NO: 24.

TABLE 3: Co-expressed CD80 and anti-CD3 T cell targeting molecules

Full molecule	
Nucleotide	Amino acid (Signal peptide underlined)
ATGGGTCATACACGCCGCCAAGGA ACCTCACCATCTAAGTGCCCATAT CTGAATTTCTTTCAACTTCTCGTGC TGGCGGGGCTCAGTCATTTCTGCA GTGGGGTTCATTACGTTACTAAAG AGGTCAAGGAGGTGCGAACATTGA GTTGTGGCCATAACGTATCAGTTG AAGAAGCTCGCGCAGACACGGATT ACTGGCAAAAGGAAAAGAAGATG GTGTTGACAATGATGAGCGGTGAC ATGAACATTTGGCCAGAGTACAAA AATCGAACGATATTCGATATAACC AATAACTTGTCCATAGTAATACTT GCCTTGCGACCTTCTGACGAGGGA ACGTATGAATGTGTAGTGCTTAAG TATGAAAAAGATGCCTTTAAGCGG GAACACTTGGCTGAGGTTACACTC TCCGTTAAGGCGGACTTTCCTACG CCGTCTATATCCGACTTCGAGATA CCCCTTCTAACATTCGACGCATC ATTTGCTCAACCTCAGGTGGTTTCC CAGAGCCTCACTTGAGCTGGCTGG AGAATGGCGAAGAACTTAACGCA ATCAATACCACGGTGTCCCAAGAC CCGGAGACAGAGCTGTACGCCGTG TCATCCAACTGGATTTTAACATG ACGACAAATCATAGTTTCATGTGT CTGATCAAATATGGGCATCTCAGG GTGAATCAGACTTTTAATTGGAAC ACTACCAAACAAGAGCACTTCCCA GATAATCTGTTGCCAAGCTGGGCG ATAACTCTTATCTCCGTCAACGGT ATCTTCGTAATTTGCTGCCTCACCT ATTGTTTCGCGCCTCGATGCCGAG AAGGCAGCGGCGCCACCAACTTCT CCCTGCTGAAGCAGGCCGGCGACG TGGAAGAAAACCCTGGCCCCATGG CCTTACCAGTGACCGCCTTGCTCCT GCCGCTGGCCTTGCTGCTCCACGC	MGHTRRQGTSPSKCPYLNFFQLLV <u>AGLSHFCSGV</u> IHVTKEVKEVATLSC GHNVSVEELAQTRIYWQKEKKMVL TMMSGDMNIWPEYKNRTIFDITNNL SIVILALRPSDEGTYECVVLKYEKDA FKREHLAEVTLVSKADFPSPISDFEI PTSNIRRIICSTSGGFPEPHLSWLENG EELNAINTTVSQDPETELYAVSSKLD FNMTTNHSFMCLIKYGHRLRVNQTFN WNTTKQEHFPDNLPSWAITLISVN GIFVICCLTYCFAPRCREGSGATNFS <u>LLKQAGDVEENPGPMALPVTALLLP</u> <u>LALLLHAARPDIQMTQTTSSL</u> SASLG DRV TISCRASQDIRNYLNWYQQKPD GTVKLLIYYTSRLHSGVPSKFSGSGS GTDYSLTISNLEQEDIATYFCQQGNT LPWTFAGGTKLEIKRAGGSGSGGSG GGSGGGSEVQLQQSGPELVKPGAS MKISCKASGYSFTGYTMNWVKQSH GKNLEWMGLINPYKGVSTYNQKFK DKATLTVDKSSSTAYMELLSLTSED SAVYYCARSGYYGDS DWYFDVWG AGTTVTVSSTTPAPRPPTPAPTIA SQ PLSLRPEACRPAAGGAVHTRGLDFA CDIYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 22)

CGCCAGGCCCGGACATCCAGATGAC CCAGACCACCTCCTCCCTGTCTGCC TCTCTGGGAGACAGAGTCACCATC AGTTGCAGGGCAAGTCAGGACATT AGAAATTATTTAAACTGGTATCAA CAGAAACCAGATGGAAGTGTAAA CTCCTGATCTACTACACATCAAGA TTACACTCAGGAGTCCCATCAAAG TTCAGTGGCAGTGGGTCTGGAACA GATTATTCTCTCACCATTAGCAACC TGGAGCAAGAGGATATTGCCACTT ACTTTTGCCAACAGGGTAATACGC TTCCGTGGACGTTTCGCTGGAGGCA CCAAGCTGGAAATCAAACGGGCTG GAGGCGGTAGTGGCGGTGGATCAG GTGGAGGCAGCGGTGGCGGATCTG AGGTGCAGCTCCAGCAGTCTGGAC CTGAGCTGGTGAAGCCTGGAGCTT CAATGAAGATATCCTGCAAGGCTT CTGGTTACTCATTCCTGGCTACAC CATGAACTGGGTGAAGCAGAGTCA TGGAAAGAACCTTGAGTGGATGGG ACTTATTAATCCTTACAAAGGTGTT AGTACCTACAACCAGAAGTTCAAG GACAAGGCCACATTAAGTGTAGAC AAGTCATCCAGCACAGCCTACATG GAACTCCTCAGTCTGACATCTGAG GACTCTGCAGTCTATTACTGTGCA AGATCGGGGTACTACGGTGATAGT GACTGGTACTTCGATGTCTGGGGC GCAGGGACCACGGTCACCGTCTCC TCAACCACtACaCCAGCaCCtaGACC ACCAACACCTGCGCCaACCATCGCa TCGCAGCCaCTGTCTCTGCGCCCAG AGGCaTGCCGGGCCAGCaGCtGGGGG CGCAGTGCACACaAGGGGGCTGGA CTTCGCaTGTGATATCTACATCTGG GCaCCaTTGGCaGGGACTTGTGGGG TCCTTCTCCTGTCACTGGTTATCAC CCTTTACTGC (SEQ ID NO: 21)	
CD80 signal peptide	
Nucleotide ATGGGTCATACACGCCGCCAAGGA ACCTCACCATCTAAGTGCCCATAT CTGAATTTCTTTCAACTTCTCGTGC TGGCGGGGCTCAGTCATTTCTGCA GTGGGGGTC (SEQ ID NO: 23)	Amino acid MGHTRRQGTSPSKCPYLNFFQLVL AGLSHFCSGV (SEQ ID NO: 24)
CD80 extracellular domain (ECD)	

Nucleotide	Amino acid
ATTCACGTTACTAAAGAGGTCAAG GAGGTCGCAACATTGAGTTGTGGC CATAACGTATCAGTTGAAGAACTC GCGCAGACACGGATTTACTGGCAA AAGGAAAAGAAGATGGTGTGAC AATGATGAGCGGTGACATGAACAT TTGGCCAGAGTACAAAAATCGAAC GATATTCGATATAACCAATAACTT GTCCATAGTAATACTTGCCTTGCG ACCTTCTGACGAGGGAACGTATGA ATGTGTAGTGCTTAAGTATGAAAA AGATGCCTTTAAGCGGGAACACTT GGCTGAGGTTACACTCTCCGTAA GGCGGACTTTCCTACGCCGTCTAT ATCCGACTTCGAGATACCCACTTC TAACATTTCGACGCATCATTTGCTC AACCTCAGGTGGTTTCCCAGAGCC TCACTTGAGCTGGCTGGAGAATGG CGAAGAACTTAACGCAATCAATAC CACGGTGTCCCAAGACCCGGAGAC AGAGCTGTACGCCGTGTCATCCAA ACTGGATTTTAACATGACGACAAA TCATAGTTTCATGTGTCTGATCAAA TATGGGCATCTCAGGGTGAATCAG ACTTTTAATTGGAACACTACCAAA CAAGAGCACTTCCCAGATAATCTG TTGCCAAGC (SEQ ID NO: 25)	IHVTKEVKEVATLSCGHNVSVEELA QTRIYWQKEKKMVLTMMSGDMNI WPEYKNRTIFDITNNLSIVILALRPSD EGTYESCVVLKYEKDAFKREHLAEV TLSVKADFPTPSISDFEIPTSNIRRIICS TSGGFPEPHLSWLENGEELNAINTTV SQDPETELYAVSSKLDFNMTTNHSF MCLIKYGHRLRVNQTFNWNTTKQEH FPDNLLPS (SEQ ID NO: 26)
CD80 transmembrane and intracellular domain	
Nucleotide	Amino acid
TGGGCGATAACTCTTATCTCCGTC AACGGTATCTTCGTAATTTGCTGCC TCACCTATTGTTTCGCGCCTCGATG CCGAGAA (SEQ ID NO: 27)	WAITLISVNGIFVICCLTYCFAPRCRE (SEQ ID NO: 28)
P2A self-cleaving peptide	
Nucleotide	Amino acid
GGCAGCGGCGCCACCAACTTCTCC CTGCTGAAGCAGGCCGGCGACGTG GAAGAAAACCCTGGCCCC (SEQ ID NO: 29)	GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 30)
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCC ACGCCGCCAGGCCG (SEQ ID NO: 31)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 32)
Anti-CD3 Light chain variable region	
Nucleotide	Amino acid

GACATCCAGATGACCCAGACCACC TCCTCCCTGTCTGCCTCTCTGGGAG ACAGAGTCACCATCAGTTGCAGGG CAAGTCAGGACATTAGAAATTATT TAAACTGGTATCAACAGAAACCAG ATGGAACCTGTTAAACTCCTGATCT ACTACACATCAAGATTACACTCAG GAGTCCCATCAAAGTTCAGTGGCA GTGGGTCTGGAACAGATTATTCTC TCACCATTAGCAACCTGGAGCAAG AGGATATTGCCACTTACTTTTGCCA ACAGGGTAATACGCTTCCGTGGAC GTTCGCTGGAGGCACCAAGCTGGA AATCAAACGGGCT (SEQ ID NO:33)	DIQMTQTTSSLSASLGDRVITISCRAS QDIRNYLNWYQQKPDGTVKLLIYYT SRLHSGVPSKFSGSGSGTDYSLTISN LEQEDIATYFCQQGNTLPWTFAGGT KLEIKRA (SEQ ID NO: 34)
G₃S linker	
Nucleotide	Amino acid
GGAGGCGGTAGTGGCGGTGGATCA GGTGGAGGCAGCGGTGGCGGATCT (SEQ ID NO: 35)	GGGSGGGSGGGSGGGS (SEQ ID NO: 36)
Anti-CD3 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGCAGCTCCAGCAGTCTGGA CCTGAGCTGGTGAAGCCTGGAGCT TCAATGAAGATATCCTGCAAGGCT TCTGGTTACTCATTCAGTGGCTACA CCATGAACTGGGTGAAGCAGAGTC ATGGAAGAACCTTGAGTGGATGG GACTTATTAATCCTTACAAAGGTG TTAGTACCTACAACCAGAAGTTCA AGGACAAGGCCACATTAAGTGTAG ACAAGTCATCCAGCACAGCCTACA TGGAACCTCAGTCTGACATCTG AGGACTCTGCAGTCTATTACTGTG CAAGATCGGGGTACTACGGTGATA GTGACTGGTACTTCGATGTCTGGG GCGCAGGGACCACGGTCACCGTCT CCTCA (SEQ ID NO: 37)	EVQLQQSGPELVKPGASMKISCKAS GYSFTGYTMNWVKQSHGKNLEWM GLINPYKGVSTYNQKFKDKATLTVD KSSSTAYMELLSLTSEDSAVYYCAR SGYYGDSDWYFDVWGAGTTVTVSS (SEQ ID NO: 38)
CD8a hinge and transmembrane	
Nucleotide	Amino acid
ACCACTACACCAGCACCTAGACCA CCAACACCTGCGCCAACCATCGCA TCGCAGCCACTGTCTCTGCGCCA GAGGCATGCCGCCAGCAGCTGGG GGCGCAGTGCACACAAGGGGGCT GGACTTCGCATGTGATATCTACAT CTGGGCACCATTGGCAGGGACTTG TGGGGTCCTTCTCCTGTCAGTGGTT	TTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYC (SEQ ID NO: 40)

ATCACCCCTTTACTGC (SEQ ID NO: 39)	
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Transgene

5 The lentiviral vectors described herein include a transgene encoding one or more proteins that is delivered to and expressed by a lymphocyte (e.g., T cell, B cell, or NK cell) targeted by the vector. In particular embodiments, the transgene encodes a chimeric antigen receptor (CAR) that includes: 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 a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

Binding domains suitable for use in CARs of the present disclosure include any antigen-binding polypeptide. A binding domain may comprise an antibody or antigen binding fragment thereof, including for example, a full length heavy chain, Fab fragment, Fab', F(ab')₂, sFv, VH domain, VL domain, dAb, VHH, CDR, and scFv, specific for a target disease antigen. In certain embodiments, a CAR binding domain is murine, chimeric, human, or humanized. In further embodiments, CAR binding domain is an scFv having V_H and V_L regions that are human or humanized. In further embodiments, the CAR binding domain is an scFv having a (GGGS)_N linker or (GGGGS)_N linker, wherein N=1-10, joining the V_H and V_L regions. In other embodiments, the CAR binding domain is an scFv having a linker comprising SEQ ID NO: 64 joining the V_H and V_L regions.

A target molecule that is bound by an extracellular domain of a CAR of the present disclosure may be found on or in association with a target cell. Exemplary target cells include a cancer cell, a cell associated with an autoimmune disease or disorder, a neurodegenerative disease, or with an inflammatory disease or disorder, an infectious microbe (e.g., bacteria, virus, or fungi), and an infected cell (e.g., virus-infected cell). A cell of an infectious organism, such as a mammalian parasite, is also contemplated as a target cell.

The extracellular domain included in a CAR as described herein can include a binding domain that targets one or more of a variety of target molecules. In certain embodiments, the CAR includes a binding domain that specifically binds a tumor antigen. In some such embodiments, the CAR binding domain 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-13Ralpha2, 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.

In certain embodiments, the extracellular domain of a CAR encoded by the transgene of the lentiviral vectors described the present disclosure optionally comprises an extracellular, non-signaling spacer or linker domain between the binding domain and the transmembrane domain. Where included, such a spacer or linker domain may position the binding domain away from the host cell surface to further enable proper cell-to-cell contact, binding, and activation. An extracellular spacer domain is generally located between the extracellular binding domain and the transmembrane domain of the CAR. The length of the extracellular spacer may be varied to optimize target molecule binding based on the selected target molecule, selected binding epitope, binding domain size and affinity (*see, e.g., Guest et al., J. Immunother. 28:203-11, 2005; PCT Publication No. WO 2014/031687*). In certain embodiments, an extracellular spacer domain is an immunoglobulin hinge region (*e.g., IgG1, IgG2, IgG3, IgG4, IgA, IgD*). An immunoglobulin hinge region may be a wild type immunoglobulin hinge region or an altered wild type immunoglobulin hinge region. An altered IgG4 hinge region is described in PCT Publication No. WO 2014/031687, which hinge region is incorporated herein by reference in its entirety. In some embodiments, an extracellular spacer domain comprises a modified IgG4 hinge region having an amino acid sequence of ESKYGPPCPPCP (SEQ ID NO: 118).

Other examples of hinge regions that may be used in the CARs described herein include the hinge region from the extracellular regions of type 1 membrane proteins, such as CD8a, CD4, CD28 and CD7, which may be wild-type or variants thereof. In some embodiments, an extracellular spacer domain comprises all or a portion of an immunoglobulin Fc domain selected from: a CH1 domain, a CH2 domain, a CH3 domain, or combinations thereof (*see, e.g.*, PCT Publication WO2014/031687, which spacers are incorporated herein by reference in their entirety). In yet further embodiments, an extracellular spacer domain may comprise a stalk region of a type II C-lectin (the extracellular domain located between the C-type lectin domain and the transmembrane domain). Type II C-lectins include CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

CARs of the present disclosure comprise a transmembrane domain that connects and is positioned between the extracellular domain and the intracellular signaling domain. The transmembrane domain typically ranges in length from about 15 amino acids to about 30 amino acids. The transmembrane domain is a hydrophobic alpha helix that transverse the host cell membrane and anchors the CAR in the host cell membrane. The transmembrane domain may be directly fused to the binding domain or to the extracellular spacer domain if present. In certain embodiments, the transmembrane domain is derived from an integral membrane protein (*e.g.*, receptor, cluster of differentiation (CD) molecule, enzyme, transporter, cell adhesion molecule, or the like). The transmembrane domain can be selected from the same molecule as the extracellular domain or the intracellular signaling domain. In some embodiments, the transmembrane domain and the extracellular domain are each selected from different molecules. In some embodiments, the transmembrane domain and the intracellular signaling domain are each selected from different molecules. In yet other embodiments, the transmembrane domain, the extracellular domain, and the intracellular signaling domain are each selected from different molecules.

Exemplary transmembrane domains for use in CARs of the present disclosure include 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, FcR α , FcR β , FcR γ , Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRP α , pT α , TCR α , TCR β , TIM3, TRIM, LPA5, and Zap70 transmembrane domain. An exemplary
 5 CD8a transmembrane domain with CD8a hinge region comprises an amino acid sequence of SEQ ID NO: 52.

The intracellular signaling domain of a CAR is an intracellular effector domain and is capable of transmitting functional signals to a cell in response to binding of the extracellular domain of the CAR to a target molecule and activates at least one of the
 10 normal effector functions or responses of the immune cell, *e.g.*, a T cell engineered to express the CAR. In some embodiments, the CAR induces a function of a T cell such as cytolytic activity or T helper activity, such as secretion of cytokines or other factors. The intracellular signaling domain may be any portion of an intracellular signaling molecule that retains sufficient signaling activity. In some embodiments, the
 15 intracellular signaling domain is obtained from an antigen receptor component (*e.g.*, TCR) or costimulatory molecule. In some embodiments, a full-length intracellular signaling domain of an antigen receptor or costimulatory molecule is used. In some embodiments, a truncated portion of an intracellular signaling domain of an antigen receptor or costimulatory molecule is used, provided that the truncated portion retains
 20 sufficient signal transduction activity. In further embodiments, an intracellular signaling domain is a variant of a full length or truncated portion of an intracellular signaling domain of an antigen receptor co stimulatory molecule, provided that the variant retains sufficient signal transduction activity (*i.e.*, is a functional variant).

In certain embodiments, the intracellular signaling domain of a CAR comprises
 25 an immunoreceptor tyrosine-based activation motif (ITAM) containing signaling domain. An ITAM containing signaling domain generally contains at least one (one, two, three, four, or more) ITAMs, which refer to a conserved motif of YXXL/I-X₆₋₈-YXXL/I. An ITAM containing signaling domain may initiate T cell activation signaling following antigen binding or ligand engagement. ITAM-signaling domains
 30 include, for example, intracellular signaling domains of CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcR γ , and CD66d. Exemplary

CD3 ζ signaling domains that may be used in CARs of the present disclosure comprise an amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 122.

CAR intracellular signaling domains optionally comprise a costimulatory signaling domain, which, when activated in conjunction with a primary or classic (*e.g.*,
 5 ITAM-driven) activation signal, promotes or enhances T cell response, such as T cell activation, cytokine production, proliferation, differentiation, survival, effector function, or combinations thereof. Costimulatory signaling domains for use in CARs include, for example, CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150
 10 (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, ZAP70, or any combination thereof. In a particular embodiment, the costimulatory signaling domain comprises a OX40, CD2, CD27, CD28, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), or 4-1BB (CD137) signaling
 15 domain. An exemplary 4-1BB costimulatory signaling domain comprises an amino acid sequence of SEQ ID NO: 54. In certain embodiments, a CAR comprises one, two, or more costimulatory signaling domains.

In some embodiments, a CAR of the present disclosure is a first generation CAR, a second generation CAR, or a third generation CAR. A first generation CAR generally
 20 has an intracellular signaling domain comprising an intracellular signaling domain of CD3 ζ , Fc γ RI, or other ITAM-containing activating domain to provide a T cell activation signal. Second generation CARs further comprise a costimulatory signaling domain (*e.g.*, a costimulatory signaling domain from an endogenous T cell costimulatory receptor, such as CD28, 4-1BB, or ICOS). Third generation CARs
 25 comprise an ITAM-containing activating domain, a first costimulatory signaling domain and a second costimulatory signaling domain.

In some embodiments, one or more of the extracellular domain, the binding domain, the linker, the transmembrane domain, the intracellular signaling domain, or the costimulatory domain comprises junction amino acids. "Junction amino acids" or
 30 "junction amino acid residues" refer to one or more (*e.g.*, about 2-20) amino acid residues between two adjacent domains, motifs, regions, modules, or fragments of a protein, such as between a binding domain and an adjacent linker, between a

transmembrane domain and an adjacent extracellular or intracellular domain, or on one or both ends of a linker that links two domains, motifs, regions, modules, or fragments (e.g., between a linker and an adjacent binding domain or between a linker and an adjacent hinge). Junction amino acids may result from the construct design of a fusion protein (e.g., amino acid residues resulting from the use of a restriction enzyme site or self-cleaving peptide sequences during the construction of a polynucleotide encoding a fusion protein). For example, a transmembrane domain of a fusion protein may have one or more junction amino acids at the amino-terminal end, carboxy -terminal end, or both.

- 10 In specific embodiments, the transgene encodes an anti-CD19 CAR molecule. In such embodiments, the extracellular binding domain may include an scFv as detailed in Table 4, having an anti-CD19 light chain variable region of SEQ ID NO: 46, a G4S linker of SEQ ID NO: 48, and an anti-CD19 heavy chain variable region of SEQ ID NO: 50. An anti-CD19 CAR useful in the context of the present description may include a CD8a hinge and transmembrane domain of SEQ ID NO: 52. In certain
- 15 embodiments, the anti-CD19 CAR includes a 4-1BB costimulatory domain according to SEQ ID NO: 54 and a CD3 ζ effector domain according to SEQ ID NO: 56. In a specific embodiment, an anti-CD19 CAR encoded by the transgene has an amino acid sequence according to SEQ ID NO: 42 or SEQ ID NO: 42 absent the signal peptide of
- 20 SEQ ID NO: 44.

- In other embodiments, the transgene encodes an anti-CD19 CAR molecule and extracellular binding domain may include an scFv having an anti-CD19 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 an anti-
- 25 CD19 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. An anti-CD19 CAR useful in the context of the present description may include 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
- 30 NO: 52. In certain embodiments, the anti-CD19 CAR includes 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 and a CD3 ζ 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. In a specific embodiment, an anti-CD19 CAR encoded by the transgene has an amino acid sequence 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: 42 or SEQ ID NO: 42 absent the signal peptide of SEQ ID NO: 44.

TABLE 4: Anti-CD19 CAR molecule

Full molecule	
Nucleotide	Amino acid (signal peptide underlined)
ATGGCCTTACCAGTGACCGCCTTG	<u>MALPVTALLLPLALLLHAARPD</u> IQM
CTCCTGCCGCTGGCCTTGCTGCTCC	TQTTSSLASLGDRVTISCRASQDISK
ACGCCGCCAGGCCGGACATCCAGA	YLNWYQQKPDGTVKLLIYHTSRLHS
TGACACAGACTACATCCTCCCTGT	GVPSRFSGSGSGTDYSLTISNLEQEDI
CTGCCTCTCTGGGAGACAGAGTCA	ATYFCQQGNTLPYTFGGGTKLEITG
CCATCAGTTGCAGGGCAAGTCAGG	GGGSGGGGSGGGGSEVKLQESGPG
ACATTAGTAAATATTTAAATTGGT	LVAPSQSLSVTCTVSGVSLPDYGV
ATCAGCAGAAACCAGATGGAAC	WIRQPPRKGLEWLGVWVWGSETTYYN
TTAAACTCCTGATCTACCATACATC	SALKSRLTIKDNSKSQVFLKMNSLQ
AAGATTACACTCAGGAGTCCCATC	TDDTAIYYCAKHYYYGGSYAMDY
AAGGTTCAAGTGGCAGTGGGTCTGG	WGQGSVTVSSTTTPAPRPPTPAPTI
AACAGATTATTCTCTCACCATTAG	ASQPLSLRPEACRPAAGGAVHTRGL
CAACCTGGAGCAAGAAGATATTGC	DFACDIYIWAPLAGTCGVLLLSLVIT
CACTTACTTTTGCCAACAGGGTAA	LYCKRGRKKLLYIFKQPFMRPVQTT
TACGCTTCCGTACACGTTCGGAGG	QEEDGCSCRFPEEEEGGCELRVKFSR
GGGGACCAAGCTGGAGATCACAG	SADAPAYKQGQNQLYNELNLGRRE
GTGGaGGTGGaTCGGGCGGTGGTG	EYDVLDRRRGRDPEMGGKPRRKNP
GGTCGGGTGGCGGCGGATCTGAGG	QEGLYNELQKDKMAEAYSEIGMKG
TGAAACTGCAGGAGTCAGGACCTG	ERRRGKGHDGLYQGLSTATKDTYD
GCCTGGTGGCGCCCTCACAGAGCC	ALHMQALPPR (SEQ ID NO: 42)
TGTCGTCACATGCACTGTCTCAG	
GGGTCTCATTACCCGACTATGGTG	
TAAGCTGGATTCGCCAGCCTCCAC	
GAAAGGGTCTGGAGTGGCTGGGA	
GTAATATGGGGTAGTGAAACCACA	
TACTATAATTCACTCTCAAATCC	
AGACTGACCATCATCAAGGACAAC	
TCCAAGAGCCAAGTTTTCTTAAAA	
ATGAACAGTCTGCAAACTGATGAC	
ACAGCCATTTACTACTGTGCCAAA	
CATTATTACTACGGTGGTAGCTAT	
GCTATGGACTACTGGGGCCAAGGA	
ACCTCAGTCACCGTCTCCTCAACC	
ACtACaCCAGCaCCtaGACCACCAAC	
ACCtGCGCCaACCATCGCaTCGCAG	
CCaCTGTCTCTGCGCCCAGAGGCaT	

GCCGGCCAGCaGcTGGGGGCGCAG TGCACACaAGGGGGCTGGACTTCG CaTGTGATATCTACATCTGGGCaCC aTTGGCaGGGACTTGTGGGGTCCTT CTCCTGTCACTGGTTATCACCCCTT ACTGCAAACGGGGCAGAAAGAAA CTCCTGTATATATTCAAACAACCA TTTATGAGACCAGTACAACTACT CAAGAGGAAGATGGCTGTAGCTGC CGATTTCCAGAAGAAGAAGAAGG AGGATGTGAACTGAGAGTGAAGTT CAGCAGGAGCGCAGACGCCCCCGC GTACAAGCAGGGCCAGAACCAGCT CTATAACGAGCTCAATCTAGGACG AAGAGAGGAGTACGATGTTTTGGA CAAGAGACGTGGCCGGGACCCTGA GATGGGGGGGAAAGCCGAGAAGGA AGAACCCTCAGGAAGGCCTGTACA ATGAACTGCAGAAAGATAAGATG GCGGAGGCCTACAGTGAGATTGGG ATGAAAGGCGAGCGCCGGAGGGG CAAGGGGCACGATGGCCTTTACCA GGGTCTCAGTACAGCCACCAAGGA CACCTACGACGCCCTTCACATGCA GGCCCTGCCCCCTCGC (SEQ ID NO: 41)	
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCC ACGCCGCCAGGCCG (SEQ ID NO: 43)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 44)
Anti-CD19 light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACACAGACTACA TCCTCCCTGTCTGCCTCTCTGGGAG ACAGAGTCACCATCAGTTGCAGGG CAAGTCAGGACATTAGTAAATATT TAAATTGGTATCAGCAGAAACCAG ATGGAACGTGTTAACTCCTGATCT ACCATACATCAAGATTACACTCAG GAGTCCCATCAAGGTTCACTGGCA GTGGGTCTGGAACAGATTATTCTC TCACCATTAGCAACCTGGAGCAAG AAGATATTGCCACTTACTTTTGCCA ACAGGGTAATACGCTTCCGTACAC	DIQMTQTTSSLASLGDRVITISCRAS QDISKYLNWYQQKPDGTVKLLIYHT SRLHSGVPSRFSGSGSGTDYSLTISN LEQEDIATYFCQQGNTLPYTFGGGT KLEIT (SEQ ID NO: 46)

GTTCGGAGGGGGGACCAAGCTGG AGATCACA (SEQ ID NO: 45)	
G4S Linker	
Nucleotide	Amino acid
GGTGGaGGTGGaTCGGGCGGTGGT GGGTCGGGTGGCGGCGGATCT (SEQ ID NO: 47)	GGGGSGGGGSGGGGS (SEQ ID NO: 48)
Anti-CD19 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGAAACTGCAGGAGTCAGG ACCTGGCCTGGTGGCGCCCTCACA GAGCCTGTCCGTCACATGCACTGT CTCAGGGGTCTCATTACCCGACTA TGGTGTAAGCTGGATTCGCCAGCC TCCACGAAAGGGTCTGGAGTGGCT GGGAGTAATATGGGGTAGTGAAAC CACATACTATAATTCAGCTCTCAA ATCCAGACTGACCATCATCAAGGA CAACTCCAAGAGCCAAGTTTTCTT AAAAATGAACAGTCTGCAAACCTGA TGACACAGCCATTTACTACTGTGC CAAACATTATTACTACGGTGGTAG CTATGCTATGGACTACTGGGGCCA AGGAACCTCAGTCACCGTCTCCTC A (SEQ ID NO: 49)	EVKLQESGPGLVAPSQSLSVTCTVS GVSLPDYGVSWIRQPPRKGLEWLGV IWGSETTYYN SALKSRLTIKDNSKS QVFLKMNSLQTDDTAIYYCAKHYY YGGSYAMDYWGQGTSTVTVSS (SEQ ID NO: 50)
CD8a Hinge and Transmembrane	
Nucleotide	Amino acid
ACCACTACACCAGCACCTAGACCA CCAACACCTGCGCCAACCATCGCA TCGCAGCCACTGTCTCTGCGCCCA GAGGCATGCCGGCCAGCAGCTGGG GGCGCAGTGACACACAAGGGGGCT GGACTTCGCATGTGATATCTACAT CTGGGCACCATTGGCAGGGACTTG TGGGGTCCTTCTCCTGTCAGTGGT ATCACCTTTACTGC (SEQ ID NO: 51)	TTTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYC (SEQ ID NO: 52)
4-1BB costimulatory domain	
Nucleotide	Amino acid
AAACGGGGCAGAAAGAACTCCT GTATATATTCAAACAACCATTTAT GAGACCAGTACAACTACTCAAGA GGAAGATGGCTGTAGCTGCCGATT	KRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCEL (SEQ ID NO: 54)

TCCAGAAGAAGAAGAAGGAGGAT GTGAACTG (SEQ ID NO: 53)	
CD3 zeta effector domain #1	
Nucleotide	Amino acid
AGAGTGAAGTTCAGCAGGAGCGC AGACGCCCCCGCGTACAAGCAGGG CCAGAACCAGCTCTATAACGAGCT CAATCTAGGACGAAGAGAGGAGT ACGATGTTTTGGACAAGAGACGTG GCCGGGACCCTGAGATGGGGGGA AAGCCGAGAAGGAAGAACCCTCA GGAAGGCCTGTACAATGAACTGCA GAAAGATAAGATGGCGGAGGCCT ACAGTGAGATTGGGATGAAAGGC GAGCGCCGGAGGGGCAAGGGGCA CGATGGCCTTTACCAGGGTCTCAG TACAGCCACCAAGGACACCTACGA CGCCCTTCACATGCAGGCCCTGCC CCCTCGC (SEQ ID NO: 55)	RVKFSRSADAPAYKQGQNQLYNEL NLGRREEYDVLDKRRGRDPENMGK PRRKNPQEGLYNELQKDKMAEAYS EIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR (SEQ ID NO: 56)

In other embodiments, the transgene encodes an anti-BCMA CAR molecule. In such embodiments, the extracellular binding domain may include an scFv as detailed in Table 5, having an anti-BCMA light chain variable region of SEQ ID NO: 62, a linker of SEQ ID NO: 64, and an anti-BCMA heavy chain variable region of SEQ ID NO: 66. An anti-BCMA CAR useful in the context of the present description may include a CD8a hinge and transmembrane domain of SEQ ID NO: 52. In certain embodiments, the anti-BCMA CAR includes a 4-1BB costimulatory domain according to SEQ ID NO: 54 and a CD3 ζ effector domain according to SEQ ID NO: 122. In a specific embodiment, an anti-BCMA CAR encoded by the transgene has an amino acid sequence according to SEQ ID NO: 58 or SEQ ID NO: 58 absent the signal peptide of SEQ ID NO: 60.

In some embodiments, the transgene encodes an anti-BCMA CAR molecule. In such embodiments, the extracellular binding domain may include an scFv having an anti-BCMA 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 an anti-BCMA 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. An anti-BCMA CAR useful in the context of the present

description may include 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. In certain embodiments, the anti-BCMA CAR includes 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 and a CD3 ζ 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. In a specific embodiment, an anti-BCMA CAR encoded by the transgene has an amino acid sequence 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: 58 or SEQ ID NO: 58 absent the signal peptide of SEQ ID NO: 60.

TABLE 5: Anti-BCMA CAR molecule

Full molecule	
Nucleotide	Amino acid (signal peptide underlined)
ATGGCACTCCCCGTCACCGCCCTT	<u>MALPVTALLLPLALLLHAARPDIVL</u>
CTCTTGCCCCCTCGCCCTGCTGCTGC	TQSPPSLAMSLGKRATISCRASESVTI
ATGCTGCCAGGCCCCGACATTGTGC	LGSHLIHWYQQKPGQPPTLLIQLASN
TCCTCAGTCACCTCCCAGCCTGG	VQTGVPARFSGSGSRTDFTLTIDPVE
CCATGAGCCTGGGAAAAAGGGCC	EDDVAVYYCLQSRTIPRTFGGGTKL
ACCATCTCCTGTAGAGCCAGTGAG	EIKGSTSGSGKPGSGEGSTKGQIQLV
TCCGTCACAATCTTGGGGAGCCAT	QSGPELKKPGETVKISCKASGYTFTD
CTTATTCAGTGGTATCAGCAGAAG	YSINWVKRAPGKGLKWMGWINTET
CCCGGGCAGCCTCCAACCCTTCTT	REPAYAYDFRGRFAFSLETSASTAY
ATTCAGCTCGCGTCAAACGTCCAG	LQINNLYKYEDTATYFCALDYSYAM
ACGGGTGTACCTGCCAGATTTTCT	DYWGQGTSTVTVSSAAATTTTPAPRPP
GGTAGCGGGTCCCGCACTGATTTT	TPAPTIASQPLSLRPEACRPAAGGAV
ACACTGACCATAGATCCAGTGGAA	HTRGLDFACDIYIWAPLAGTCGVLL
GAAGACGATGTGGCCGTGTATTAT	LSLVITLYCKRGRKKLLYIFKQPFMR
TGTCTGCAGAGCAGAACGATTCCT	PVQTTQEEDGCSCRFPEEEEEGGCEL
CGCACATTTGGTGGGGGTACTAAG	VKFSRSADAPAYQQGQNQLYNELN
CTGGAGATTAAGGGAAGCACGTCC	LGRREEYDVLDKRRGRDPEMGGKP
GGCTCAGGGAAGCCGGGCTCCGGC	RRKNPQEGLYNELQKDKMAEAYSEI
GAGGGAAGCACGAAGGGGCAAAT	GМКGERRRGKГHDGLYQGLSTATK
TCAGCTGGTCCAGAGCGGACCTGA	DTYDALHMQALPPR (SEQ ID NO:
GCTGAAAAAACC CGGCGAGACTGT	58)
TAAGATCAGTTGTAAAGCATCTGG	
CTATACCTTCACCGACTACAGCAT	
AAATTGGGTGAAACGAGCCCCTGG	
AAAGGGCCTCAAATGGATGGGTTG	
GATCAATACCGAAACTAGGGAGCC	
TGCTTATGCATATGACTTCCGCGG	

GAGATTCGCCTTTTCACTCGAGAC ATCTGCCTCTACTGCTTACCTCCAA ATAAACAACCTCAAGTATGAAGAT ACAGCCACTTACTTTTGCGCCCTCG ACTATAGTTACGCCATGGACTACT GGGGACAGGGAACCTCCGTTACCG TCAGTTCCGCGGCCGCAACCACAA CACCTGCTCCAAGGCCCCCACAC CCGCTCCAACCTATAGCCAGCCAAC CATTGAGCCTCAGACCTGAAGCTT GCAGGCCCCGCAGCAGGAGGCGCC GTCCATACGCGAGGCCTGGACTTC GCGTGTGATATTTATATTTGGGCA CCTTTGGCCGGAACATGTGGGGTG TTGCTTCTCTCCCTTGTGATCACTC TGTATTGTAAGCGCGGGAGAAAGA AGCTCCTGTACATCTTCAAGCAGC CTTTTATGCGACCTGTGCAAACCA CTCAGGAAGAAGATGGGTGTTTCAT GCCGCTTCCCCGAGGAGGAAGAAG GAGGGTGTGAACTGAGGGTGAAAT TTTCTAGAAGCGCCGATGCTCCCG CATATCAGCAGGGTCAGAATCAGC TCTACAATGAATTGAATCTCGGCA GGCGAGAAGAGTACGATGTTCTGG ACAAGAGACGGGGCAGGGATCCC GAGATGGGGGGAAAGCCCCGGAG AAAAAATCCTCAGGAGGGGTTGTA CAATGAGCTGCAGAAGGACAAGA TGGCTGAAGCCTATAGCGAGATCG GAATGAAAGGCGAAAGACGCAGA GGCAAGGGGCATGACGGTCTGTAC CAGGGTCTCTCTACAGCCACCAAG GACACTTATGATGCGTTGCATATG CAAGCCTTGCCACCCCGC (SEQ ID NO: 57)	
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCACTCCCCGTCACCGCCCTT CTCTTGCCCCCTCGCCCTGCTGCTGC ATGCTGCCAGGCCC (SEQ ID NO: 59)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 60)
Anti-BCMA light chain variable region	
Nucleotide	Amino acid
GACATTGTGCTCACTCAGTCACCT CCCAGCCTGGCCATGAGCCTGGGA AAAAGGGCCACCATCTCCTGTAGA GCCAGTGAGTCCGTCACAATCTTG	DIVLTQSPPSLAMSLGKRATISCRAS ESVTILGSHLIHWYQQKPGQPPTLLI QLASNVQTGVPARFSGSGSRTDFTL

GGGAGCCATCTTATTCAGTGGTAT CAGCAGAAGCCCGGGCAGCCTCCA ACCCTTCTTATTCAGCTCGCGTCAA ACGTCCAGACGGGTGTACCTGCCA GATTTTCTGGTAGCGGGTCCCGCA CTGATTTTACACTGACCATAGATC CAGTGGAAGAAGACGATGTGGCC GTGTATTATTGTCTGCAGAGCAGA ACGATTCCTCGCACATTTGGTGGG GGTACTAAGCTGGAGATTAAG (SEQ ID NO: 61)	TIDPVEEDDVAVYYCLQSRTIPRTFG GGTKLEIK (SEQ ID NO: 62)
218 Linker	
Nucleotide	Amino acid
GGAAGCACGTCCGGCTCAGGGAA GCCGGGCTCCGGCGAGGGAAGCA CGAAGGGG (SEQ ID NO: 63)	GSTSGSGKPGSGEGSTKG (SEQ ID NO: 64)
Anti-BCMA heavy chain variable region	
Nucleotide	Amino acid
CAAATTCAGCTGGTCCAGAGCGGA CCTGAGCTGAAAAAACCCGGCGAG ACTGTTAAGATCAGTTGTAAAGCA TCTGGCTATACCTTCACCGACTAC AGCATAAATTGGGTGAAACGAGCC CCTGGAAAGGGCCTCAAATGGATG GGTTGGATCAATACCGAAACTAGG GAGCCTGCTTATGCATATGACTTC CGCGGGAGATTTCGCTTTTCACTC GAGACATCTGCCTCTACTGCTTAC CTCCAAATAAACAACCTCAAGTAT GAAGATACAGCCACTTACTTTTGC GCCCTCGACTATAGTTACGCCATG GACTACTGGGGACAGGGAACCTCC GTTACCGTCAGTTCC (SEQ ID NO: 65)	QIQLVQSGPELKKPGETVKISCKASG YTFTDYSINWVKRAPGKGLKWMG WINTETREPAYAYDFRGRFAFSLETS ASTAYLQINNLYEDTATYFCALDY SYAMDYWGQGTSVTVSS (SEQ ID NO: 66)
CD8a Hinge and Transmembrane	
Nucleotide	Amino acid
ACCACAACACCTGCTCCAAGGCCC CCCACACCCGCTCCAACCTATAGCC AGCCAACCATGAGCCTCAGACCT GAAGCTTGCAGGCCCCGCAGCAGGA GGCGCCGTCCATACGCGAGGCCTG GACTTCGCGTGTGATATTTATATTT GGGCACCTTTGGCCGGAACATGTG GGGTGTTGCTTCTCTCCCTTGTGAT CACTCTGTATTGT (SEQ ID NO: 119)	TTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYC (SEQ ID NO: 52)
4-1BB costimulatory domain	
Nucleotide	Amino acid

AAGCGCGGGAGAAAGAAGCTCCT GTACATCTTCAAGCAGCCTTTTATG CGACCTGTGCAAACCACTCAGGAA GAAGATGGGTGTTTCATGCCGCTTC CCCGAGGAGGAAGAAGGAGGGTG TGAAGT (SEQ ID NO: 120)	KRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEEGGCEL (SEQ ID NO: 54)
CD3 zeta effector domain #2	
Nucleotide	Amino acid
AGGGTGAAATTTTCTAGAAGCGCC GATGCTCCCGCATATCAGCAGGGT CAGAATCAGCTCTACAATGAATTG AATCTCGGCAGGCGAGAAGAGTAC GATGTTCTGGACAAGAGACGGGGC AGGGATCCCGAGATGGGGGGAAA GCCCCGGAGAAAAAATCCTCAGGA GGGGTTGTACAATGAGCTGCAGAA GGACAAGATGGCTGAAGCCTATAG CGAGATCGGAATGAAAGGCGAAA GACGCAGAGGCAAGGGGCATGAC GGTCTGTACCAGGGTCTCTCTACA GCCACCAAGGACACTTATGATGCG TTGCATATGCAAGCCTTGCCACCC CGC (SEQ ID NO: 121)	RVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPGEMGGK PRRKNPQEGLYNELQKDKMAEAYS EIGMKGERRRGKGHDGLYQGLSTA TKDITYDALHMQALPPR (SEQ ID NO: 122)

CARs of the present disclosure may comprise polynucleotide sequences derived from any mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, transgenic species thereof, or any combination thereof. In some embodiments, the chimeric antigen receptor is murine, chimeric, human, or humanized.

Methods for Producing Lentiviral Vectors

Methods for producing the LVV disclosed are provided herein. LVV have been developed and based on the retroviral genome by combining its components into recombinant plasmid DNA vectors. The plasmid DNA vectors can then be transfected into producer cell lines to transfer genes required for lentiviral particle production. LVV packaging systems generally comprise a transfer plasmid encoding the transgene of interest, an envelope plasmid (e.g., VSV-G), and packaging plasmid(s). Second generation LVV packaging systems contain a single packaging plasmid encoding Gag, Pol, Rev, and Tat genes and a separate Env plasmid. In some embodiments, the methods described herein utilize a third generation vector system for producing LVV.

Third generation vector production systems improve upon the safety of 2nd generation LVV packaging systems and are typically four plasmid systems that include a transgene plasmid combined with three packaging plasmids, VSV-G, GagPol, and Rev. Thus, Rev and GagPol are separated into two plasmids. Tat is also eliminated from 3rd generation LVV packaging systems by the addition of a chimeric 5' LTR fused to a heterologous promoter (e.g., CMV or RSV promoter) on the transfer plasmid. The *gag* gene encodes a Gag polyprotein precursor comprising structural proteins of the lentivirus, including matrix, capsid, and nucleocapsid. The *pol* gene encodes a Pol polyprotein precursor supplying enzymatic functions of the lentivirus essential for replication, including protease, reverse transcriptase, and integrase. The *rev* gene encodes the Rev protein which binds to the Rev Response Element (RRE) to allow nuclear export of unspliced and singly spliced HIV RNA during viral replication. The Gag and Pol polyprotein precursors are cleaved during production of viral particles.

Producer cells that may be used for making LVV of the present disclosure include human embryonic kidney (HEK) 293 cells and derivatives thereof. Producer cells may be an adherent cell line, such as HEK293T producer cells, or a suspension cell line, such as HEK293T/17 SF producer cells.

The transgene plasmid is combined with the three packaging plasmids, GagPol plasmid, VSV-G env plasmid, and Rev plasmid, and used to transfect producer cells, such as HEK293 producer cells. In some embodiments, the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid. In some embodiments, the ratios of the packaging plasmids are determined by mass. In some embodiments, the mass of each of the transgene plasmid and GagPol plasmid are higher than the mass of each of the VSV-G env plasmid and Rev plasmid. In some embodiments, the defined ratio of transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid is at about 1:1:1:1 to about 5:4:1:1. In some embodiments, the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 2:1:1:1 to about 5:4:1:1. In some embodiments, the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 2:1:1:1. In some embodiments, the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid,

VSV-G env plasmid, and Rev plasmid at about 3:1:1:1. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 3.125:3.125:2.5:1.25. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 4:2:1:1. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 5:4:1:1. Productive lentiviral particles are harvested from the producer cell culture media. Exemplary materials and methods for producing LVV particles are described in *Production of Lentiviral Vectors*, Merten et al., Molecular Therapy – Methods & Clinical Development (2016), 3, 16017, the contents of which are incorporated herein by reference. Third generation production systems are used for research and development and clinical purposes. Schematic representations of helper plasmids suitable for use in a third generation LVV production system are provided in **FIG. 1**.

The transgene plasmid encodes the only genetic materials that is transferred to the lymphocytes (e.g., T cells or NK cells) targeted by the resulting LVV and comprises a lentiviral backbone containing the transgene expression cassette flanked by cis-acting elements for encapsidation, reverse transcription and integration. In specific embodiments, the transgene plasmid includes a deletion in the U3 element of the 3' LTR that causes loss of transcriptional capacity of the viral LTR once transferred into the target cells. The expression cassette included in the transgene plasmid may encode a single heterologous protein (e.g., a single CAR as described herein) or multiple heterologous proteins (e.g., multiple CARs as described herein) for introduction into and expression by the target cell.

In some embodiments of a four plasmid LVV system, the VSV-G env plasmid includes a tandem expression cassette that encodes a mutated VSV-G envelope protein and a targeting protein as disclosed herein. In specific embodiments, the tandem expression cassette included in the VSV-G env plasmid includes a polynucleotide that encodes a first signal peptide, a polynucleotide that encodes a targeting protein, a polynucleotide that encodes one of an internal ribosomal entry site (IRES), a furin cleavage site, or a viral 2A peptide, a polynucleotide that encodes a second signal peptide, and a polynucleotide that encodes a mutated VSV-G envelope protein. In

certain embodiments, the polynucleotide encoding the mutant VSV-G envelope protein is positioned 5' to the polynucleotide encoding the targeting protein. In other embodiments, the polynucleotide encoding the mutant VSV-G envelope protein is positioned 3' to the polynucleotide encoding the targeting protein. The polynucleotide that encodes the targeting protein and the polynucleotide that encodes the mutated VSV-G are separated in the tandem cassette by the polynucleotide that encodes the IRES, furin cleavage site, or viral 2A peptide, which allows for co-expression of the two proteins from a single mRNA. In certain embodiments, a viral 2A peptide is a porcine teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or a variant thereof. Specific examples of amino acid and nucleotide sequences for the viral 2A peptides are provided in Table 7.

Table 7: 2A sequences

T2A peptide from <i>Thosea asigna</i> virus capsid protein	EGRGSLTCDG VEENPGP (SEQ ID NO: 79)	GAGGGCAGGGGAAGTCTTC TAACATGCGGGGACGTGGA GGAAAATCCCGGCCCC (SEQ ID NO: 80)
P2A peptide from porcine teschovirus-1 polyprotein	ATNFSLLKQAG DVEENPGP (SEQ ID NO: 81)	GCCACGAACTTCTCTCTGTT AAAGCAAGCAGGAGACGT GGAAGAAAACCCCGGTCCT (SEQ ID NO: 82)
F2A peptide from foot-and-mouth disease virus polyprotein	VKQTLNFDLLK LAGDVESNPGP (SEQ ID NO: 83)	GTGAAACAGACTTTGAATT TTGACCTTCTCAAGTTGGC GGGAGACGTCGAGTCCAAC CCTGGGCC (SEQ ID NO: 84)
E2A peptide from equine rhinitis A virus polyprotein	QCTNYALLKLA GDVESNPGP (SEQ ID NO: 85)	CAGTGTACTAATTATGCTC TCTTGAAATTGGCTGGAGA TGTTGAGAGCAACCCAGGT CCC (SEQ ID NO: 86)

In certain embodiments, the present disclosure provides a method for producing a lymphocyte targeted lentiviral vector according to any one of the preceding claims, the method comprising: transfecting a producer cell with a GagPol plasmid, a Rev plasmid, a transgene plasmid, and a VSV-G env plasmid, wherein; 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;

the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral rev protein within the producer cell; the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and the VSV-G env plasmid comprises a tandem expression cassette, wherein the
5 tandem expression cassette comprises a polynucleotide encoding a mutated VSV-G envelope protein as disclosed herein and a polynucleotide encoding a lymphocyte targeting protein as disclosed herein 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; culturing the producer cell in a culture medium; and
10 harvesting lentiviral vector from the culture medium.

In some embodiments, the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid. In some embodiments, the defined ratio of transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 1:1:1:1 to about 5:4:1:1. In some embodiments, the
15 the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 2:1:1:1 to about 5:4:1:1. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 2:1:1:1. In some embodiments, the the producer cells are transfected with a defined
20 ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 3:1:1:1. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 3.125:3.125:2.5:1.25. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env
25 plasmid, and Rev plasmid at about 4:2:1:1. In some embodiments, the the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, and Rev plasmid at about 5:4:1:1.

In one embodiment, the tandem expression cassette of the VSV-G env plasmid encodes an anti-CD3 targeting protein and a mutated VSV-G envelope protein. For
30 example, in a specific embodiment, the tandem expression cassette may comprise a polynucleotide 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: 67, which encodes an amino acid sequence 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: 68. Such an embodiment is described in more detail in Table 8. In another embodiment, the tandem expression cassette may comprise a polynucleotide that has about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 67 absent the signal peptide of SEQ ID NO: 31, which encodes an amino acid sequence 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: 68 absent the signal peptide of SEQ ID NO: 32. As illustrated in Table 8, the anti-CD3 targeting protein is separated from the mutated VSV-G envelope protein via a P2A self-cleaving peptide. In certain embodiments, that P2A peptide may be encoded by a nucleic acid sequence 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: 69, and the P2A peptide may have an amino acid sequence 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: 70.

TABLE 8: Anti-CD3 targeting molecule co-expressed with mutant VSV-G

Full molecule	
Nucleotide	Amino acid (signal peptide underlined)
ATGGCCTTACCAGTGACCGCCTTG	<u>MALPVTALLLPLALLLHAARPDIQM</u>
CTCCTGCCGCTGGCCTTGCTGCTCC	TQTTSSLSASLGDRVTISCRASQDIR
ACGCCGCCAGGCCGACATCCAGA	NYLNWYQQKPDGTVKLLIYYTSRL
TGACCCAGACCACCTCCTCCCTGT	HSGVPSKFSGSGSGTDYSLTISNLEQ
CTGCCTCTCTGGGAGACAGAGTCA	EDIATYFCQQGNTLPWTFAGGTKLE
CCATCAGTTGCAGGGCAAGTCAGG	IKRAGGSGGGSGGGSGGGSEVQLQ
ACATTAGAAATTATTTAAACTGGT	QSGPELVKPGASKMKISCKASGYST
ATCAACAGAAACCAGATGGAACCTG	GYTMNWVKQSHGKNLEWMGLINP
TTAAACTCCTGATCTACTACACATC	YKGVSTYNQKFKDKATLTVDKSSST
AAGATTACACTCAGGAGTCCCATC	AYMELLSLTSEDSAVYYCARSGYY
AAAGTTCAGTGGCAGTGGGTCTGG	GDSDWYFDVWGAGTTVTVSSTTTP
AACAGATTATTCTCTCACCATTAG	APRPPTPAPTIASQPLSLRPEACRPAA
CAACCTGGAGCAAGAGGATATTGC	GGAVHTRGLDFACDIYIWAPLAGTC
CACTTACTTTTGCCAACAGGGTAA	GVLLLSLVITLYCGSGATNFSLLKQA
TACGCTTCCGTGGACGTTTCGCTGG	GDVEENPGPMKCLLYLAFLFIGVNC
AGGCACCAAGCTGGAAATCAAAC	KFTIVFPHNQKGNWKNVPSNYHYCP
GGGCTGGAGGCGGTAGTGGCGGTG	SSSDLNWHNDLIGTALQVKMPQSH
GATCAGGTGGAGGCAGCGGTGGC	KAIQADGWMCHASKWVTTCDFRW
GGATCTGAGGTGCAGCTCCAGCAG	YGPKYITHSIRSFTPSVEQCKESIEQT
TCTGGACCTGAGCTGGTGAAGCCT	KQGTWLNPGFPPQSCGYATVTDAE
GGAGCTTCAATGAAGATATCCTGC	AVIVQVTPHHVLVDEYTGEWVDSQ
AAGGCTTCTGGTTACTCATTCACTG	FINGKCSNYICPTVHNSTTWHSYK
GCTACACCATGAACTGGGTGAAGC	VKGLCDSNLISMDITFFSEDGELSSL

AGAGTCATGGAAAGAACCTTGAGT GGATGGGACTTATTAATCCTTACA AAGGTGTTAGTACCTACAACCAGA AGTTCAAGGACAAGGCCACATTAA CTGTAGACAAGTCATCCAGCACAG CCTACATGGAACCTCCTCAGTCTGA CATCTGAGGACTCTGCAGTCTATT ACTGTGCAAGATCGGGGTACTACG GTGATAGTGACTGGTACTTCGATG TCTGGGGCGCAGGGACCACGGTCA CCGTCTCCTCAACCACTACACCAG CACCTAGACCACCAACACCTGCGC CAACCATCGCATCGCAGCCACTGT CTCTGCGCCCAGAGGCATGCCGGC CAGCAGCTGGGGGCGCAGTGCACA CAAGGGGGCTGGACTTCGCATGTG ATATCTACATCTGGGCACCATTGG CAGGGACTTGTGGGGTCCTTCTCC TGTCACTGGTTATCACCCCTTTACTG CGGCAGCGGCGCCACCAACTTCTC CCTGCTGAAGCAGGCCGCGACGT GGAAGAAAACCTGGCCCCATGAA GTGTCTGCTGTACCTGGCGTTCCTG TTTATCGGGGTGAACTGCAAGTTC ACTATCGTGTTTCCGCACAACCAA AAGGGCAACTGGAAAAACGTGCCT TCAAATTACCATTATTGCCCCAGC AGCTCGGACCTGAACTGGCACAAT GACCTCATTGGAACCGCGCTGCAG GTGAAGATGCCACAGAGCCACAA GGCTATCCAGGCTGACGGATGGAT GTGCCACGCGTCAAAATGGGTGAC TACCTGCGATTTCCGCTGGTACGG ACCAAAATACATCACGCACAGCAT CAGATCATTACCCCGTCAGTGGA ACAATGCAAAGAATCCATCGAACA GACTAAGCAGGGAACCTGGCTGAA CCCTGGATTTCCGCCGCGAGTCGTG TGGGTACGCAACCGTGACCGATGC AGAGGCCGTGATCGTGCAAGTCAC GCCGCATCACGTGCTTGTGGACGA GTACACCGGAGAATGGGTTCGATTC CCAGTTCATCAACGGCAAGTGCTC CAACTACATTTGCCCAACCGTGCA CAACAGCACTACTTGGCATAGCGA CTACAAAGTGAAGGGTCTGTGTGA TTCCAACCTGATCTCCATGGATATC ACTTTCTTCTCGGAAGACGGCGAA CTGTCTCACTGGGCAAAGAAGGA	GKEGTGFRSNYFAYETGGKACKMQ YCKHWGVRLPSGVWFEMADKDLF AAARFPECPEGSSISAPSQTSVDVSLI QDVERILDYSLCQETWSKIRAGLPIS PVDLSYLAPKNPGTGPAFTIINGTLK YFETRYIRVDIAAPILSRMVGMISGT TTEAELWDDWAPYEDVEIGPNGVL RTSSGYKFPLYMIGHGMLDSDLHLS SKAQVFEHPHIQDAASQLPDDESLFF GDTGLSKNPIELVEGWFSWKSSIAS FFFIIGLIIGLFLVLRVGIHLCKIKHT KKRQIYTDIEMNRLGK (SEQ ID NO: 68)
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ACTGGGTTTCGCTCAAATTACTTCG CCTACGAAACTGGAGGAAAAGCCT GCAAGATGCAGTACTGCAAGCACT GGGGCGTGAGACTACCCAGCGGTG TCTGGTTCGAGATGGCCGATAAGG ACCTGTTTGCAGCAGCGAGATTCC CGGAATGCCCTGAGGGATCGAGCA TCTCCGCTCCAAGCCAAACTTCAG TGGACGTGAGCCTGATCCAGGACG TGGAACGGATTCTCGACTACTCGC TGTGCCAGGAGACCTGGTCGAAGA TCAGAGCGGGACTGCCCATCTCAC CGGTGGACCTGTCCTACCTGGCGC CAAAGAATCCGGGCACTGGACCGG CGTTCACCATCATCAACGGCACCC TCAAATACTTCGAGACGCGGTACA TCCGGGTGGACATCGCAGCTCCGA TCCTCTCCCGGATGGTGGGAATGA TCTCGGGGACTACTACCGAAGCCG AGCTCTGGGACGACTGGGCACCTT ACGAGGATGTCGAGATCGGACCTA ACGGAGTGCTCCGGACCTCCTCCG GGTACAAGTTCCTCTGTACATGA TCGGCCATGGCATGCTGGACTCGG ATCTGCATCTGTCGTCCAAAGCAC AGGTGTTTGAACACCCACACATTC AAGACGCCGCCAGCCAGCTGCCGG ACGATGAGTCGCTGTTCTTCGGAG ACACGGGCTTGTCAAAGAATCCCA TCGAGCTGGTGGAAGGATGGTTTT CATCCTGGAAAAGCAGCATCGCTT CATTCTTCTTCATCATTGGCCTGAT CATCGGCCTATTTCTAGTCCTGCGG GTGGGAATTCATCTGTGCATCAAG CTCAAGCACACTAAGAAGCGGCAA ATCTACACTGATATCGAGATGAAT CGCCTGGGCAAG (SEQ ID NO: 67)	
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCC ACGCCGCCAGGCCG (SEQ ID NO: 31)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 32)
Anti-CD3 Light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACCCAGACCACC TCCTCCCTGTCTGCCTCTCTGGGAG ACAGAGTCACCATCAGTTGCAGGG	DIQMTQTSSLASLGDRVTISCRAS QDIRNYLNWYQQKPDGTVKLLIYYT SRLHSGVPSKFSGSGSGTDYSLTISN

CAAGTCAGGACATTAGAAATTATT TAAACTGGTATCAACAGAAACCAG ATGGAAGTGTAACTCCTGATCT ACTACACATCAAGATTACACTCAG GAGTCCCATCAAAGTTCAGTGGCA GTGGGTCTGGAACAGATTATTCTC TCACCATTAGCAACCTGGAGCAAG AGGATATTGCCACTTACTTTTGCCA ACAGGGTAATACGCTTCCGTGGAC GTTCGCTGGAGGCACCAAGCTGGA AATCAAACGGGCT (SEQ ID NO: 33)	LEQEDIATYFCQQGNTLPWTFAGGT KLEIKRA (SEQ ID NO: 34)
G₃S linker	
Nucleotide	Amino acid
GGAGGCGGTAGTGGCGGTGGATCA GGTGGAGGCAGCGGTGGCGGATCT (SEQ ID NO: 35)	GGGSGGGSGGGSGGGS (SEQ ID NO: 36)
Anti-CD3 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGCAGCTCCAGCAGTCTGGA CCTGAGCTGGTGAAGCCTGGAGCT TCAATGAAGATATCCTGCAAGGCT TCTGGTACTCATTCACTGGCTACA CCATGAAGTGGGTGAAGCAGAGTC ATGGAAAGAACCTTGAGTGGATGG GACTTATTAATCCTTACAAAGGTG TTAGTACCTACAACCAGAAGTTCA AGGACAAGGCCACATTAAGTGTAG ACAAGTCATCCAGCACAGCCTACA TGGAAGTCTCAGTCTGACATCTG AGGACTCTGCAGTCTATTACTGTG CAAGATCGGGGTACTACGGTGATA GTGACTGGTACTTCGATGTCTGGG GCGCAGGGACCACGGTCACCGTCT CCTCA (SEQ ID NO: 37)	EVQLQQSGPELVKPGASMKISCKAS GYSFTGYTMNWVKQSHGKNLEWM GLINPYKGVSTYNQKFKDKATLTVD KSSSTAYMELLSLTSEDSAVYYCAR SGYYGDSDWYFDVWGAGTTVTVSS (SEQ ID NO: 38)
CD8a hinge and transmembrane	
Nucleotide	Amino acid
ACCACTACACCAGCACCTAGACCA CCAACACCTGCGCCAACCATCGCA TCGCAGCCACTGTCTCTGCGCCA GAGGCATGCCGCCAGCAGCTGGG GGCGCAGTGCACACAAGGGGGCT GGACTTCGCATGTGATATCTACAT CTGGGCACCATTGGCAGGGACTTG TGGGGTCCTTCTCCTGTCAGTGGT ATCACCTTTACTGC (SEQ ID NO: 39)	TTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYC (SEQ ID NO: 40)
P2A self-cleaving peptide	
Nucleotide	Amino acid

GGCAGCGGCGCCACCAACTTCTCC CTGCTGAAGCAGGCCGCGACGTG GAAGAAAACCCTGGCCCC (SEQ ID NO: 69)	GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 70)
VSV-G signal sequence	
Nucleotide	Amino acid
ATGAAGTGTCTGCTGTACCTGGCG TTCCTGTTTATCGGGGTGAACTGC (SEQ ID NO: 71)	MKCLLYLAFLFIGVNC (SEQ ID NO: 72)
Mutant VSV-G (Trop-002)	
Nucleotide	Amino acid
AAGTTCACCTATCGTGTTTCCGCAC AACCAAAAGGGCAACTGGAAAAA CGTGCCTTCAAATTACCATTATTGC CCCAGCAGCTCGGACCTGAACTGG CACAATGACCTCATTGGAACCGCG CTGCAGGTGAAGATGCCACAGAGC CACAAGGCTATCCAGGCTGACGGA TGGATGTGCCACGCGTCAAAATGG GTGACTACCTGCGATTTCCGCTGG TACGGACCAAAATACATCACGCAC AGCATCAGATCATTCACCCCGTCA GTGGAACAATGCAAAGAATCCATC GAACAGACTAAGCAGGGAACCTG GCTGAACCCTGGATTTCCGCCGCA GTCGTGTGGGTACGCAACCGTGAC CGATGCAGAGGCCGTGATCGTGCA AGTCACGCCGCATCACGTGCTTGT GGACGAGTACACCGGAGAATGGG TCGATTCCCAGTTCATCAACGGCA AGTGCTCCAACCTACATTGCCCCAA CCGTGCACAACAGCACTACTTGGC ACAGCGACTACAAAGTGAAGGGTC TGTGTGATTCCAACCTGATCTCCAT GGATATCACTTTCTTCTCGGAAGA CGGCGAACTGTCCTCACTGGGCAA AGAAGGAACTGGGTTCGCTCAAA TTACTTCGCCTACGAACTGGAGG AAAAGCCTGCAAGATGCAGTACTG CAAGCACTGGGGCGTGAGACTACC CAGCGGTGTCTGGTTCGAGATGGC CGATAAGGACCTGTTTGCAGCAGC GAGATTCCCGGAATGCCCTGAGGG ATCGAGCATCTCCGCTCCAAGCCA AACTTCAGTGGACGTGAGCCTGAT CCAGGACGTGGAACGGATTCTCGA CTACTCGCTGTGCCAGGAGACCTG GTCGAAGATCAGAGCGGGACTGCC	KFTIVFPHNQKGNWKNVPSNYHYCP SSSDLNWHNDLIGTALQVKMPQSH KAIQADGWMCHASKWVTTCDFRW YGPKYITHSIRSFTPSVEQCKESIEQT KQGTWLNPGFPPQSCGYATVTDAE AVIVQVTPHHVLVDEYTG EWVDSQ FINGKCSNYICPTVHNSTTWHSYK VKGLCDSNLISMDITFFSEDGELSSL GKEGTGFRSNYFAYETGGKACKMQ YCKHWGVRLPSGVWFEMADKDLF AAARFPECPEGSSISAPSQTSVDVSLI QDVERILDYSLCQETWSKIRAGLPIS PVDLSYLAPKNPGTGPAFTIINGTLK YFETRYIRVDIAAPILSRMVGMISGT TTEAELWDDWAPYEDVEIGPNGVL RTSSGYKFPLYMIGHGMLDSDLHLS SKAQVFEHPHIQDAASQLPDDESLEF GDTGLSKNPIELVEGWFSWKSSIAS FFFIIGLIIGLFLVLRVGIHLCKLKHT KKRQIYTDIEMNRLGK (SEQ ID NO: 74)

CATCTCACCGGTGGACCTGTCCTA CCTGGCGCCAAAGAATCCGGGCAC TGGACCGGCGTTCACCATCATCAA CGGCACCCTCAAATACTTCGAGAC GCGGTACATCCGGGTGGACATCGC AGCTCCGATCCTCTCCCGGATGGT GGGAATGATCTCGGGGACTACTAC CGAAGCCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGAT CGGACCTAACGGAGTGCTCCGGAC CTCCTCCGGGTACAAGTTCCCTCTG TACATGATCGGCCATGGCATGCTG GACTCGGATCTGCATCTGTCGTCC AAAGCACAGGTGTTTGAACACCCA CACATTCAAGACGCCGCCAGCCAG CTGCCGGACGATGAGTCGCTGTTC TTCGGAGACACGGGCTTGTCAAAG AATCCCATCGAGCTGGTGGAAGGA TGGTTTTCATCCTGGAAAAGCAGC ATCGCTTCATTCTTCTTCATCATTG GCCTGATCATCGGCCTATTTCTAGT CCTGCGGGTGGGAATTCATCTGTG CATCAAGCTCAAGCACACTAAGAA GCGGCAAATCTACACTGATATCGA GATGAATCGCCTGGGCAAG (SEQ ID NO: 73)	
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In another embodiment, the tandem expression cassette of the VSV-G env plasmid encodes a CD80 targeting protein and a mutated VSV-G envelope protein. For example, in a specific embodiment, the tandem expression cassette may comprise a

5 polynucleotide according to SEQ ID NO: 75, which encodes an amino acid sequence of SEQ ID NO: 76. Such an embodiment is described in more detail in Table 9. As illustrated in Table 9, the CD80 targeting protein is separated from the mutated VSV-G envelope protein via a P2A self-cleaving peptide encoded by SEQ ID NO: 69 and having an amino acid sequence according to SEQ ID NO: 70.

10 In some embodiments, where the tandem expression cassette of the VSV-G env plasmid encodes a CD80 targeting protein and a mutated VSV-G envelope protein, the tandem expression cassette may include a polynucleotide 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: 75, which encodes an amino acid sequence 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: 76. In certain embodiments, the CD80 targeting protein is separated from the mutated VSV-G envelope protein via a P2A self-cleaving peptide encoded a nucleic acid sequence 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: 69, and the P2A self-cleaving peptide includes an amino acid 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: 70.

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TABLE 9: CD80 T cell targeting molecule co-expressed with mutant VSV-G

Full molecule	
Nucleotide	Amino acid (signal sequence underlined)
ATGGGGTCATACACGCCGCCAAGGA	<u>MGHTRRQGTSPSKCPYLNFFQLLV</u>
ACCTCACCATCTAAGTGCCCATAT	<u>AGLSHFCSGVIHVTKEVKEVATLSC</u>
CTGAATTTCTTTCAACTTCTCGTGC	GHNVSVEELAQTRIYWQKEKKMVL
TGGCGGGGCTCAGTCATTTCTGCA	TMMSGDMNIWPEYKNRTIFDITNNL
GTGGGGGTCATTACGTTACTAAAG	SIVILALRPSDEGTYECVVLKYEKDA
AGGTCAAGGAGGTGCAACATTGA	FKREHLAEVTL SVKADFP TPSISDFEI
GTTGTGGCCATAACGTATCAGTTG	PTSNIRRIICSTSGGFPEPHLSWLENG
AAGAACTCGCGCAGACACGGATTT	EELNAINTTVSQDPETELYAVSSKLD
ACTGGCAAAAGGAAAAGAAGATG	FNMTTNHSFMCLIKYGH LRVNQTFN
GTGTTGACAATGATGAGCGGTGAC	WNTTKQEHFPDNL LPSWAITLISVN
ATGAACATTTGGCCAGAGTACAAA	GIFVICCLTYCFAPRCREGSGATNFS
AATCGAACGATATTCGATATAACC	<u>LLKQAGDVEENPGPMKCLLYLAFLF</u>
AATAACTTGTCCATAGTAATACTT	<u>IGVNCKFTIVFPHNQKGNWKNVPSN</u>
GCCTTGCGACCTTCTGACGAGGGA	YHYCPSSSDLNWHNDLIGTALQVK
ACGTATGAATGTGTAGTGCTTAAG	MPQSHKAIQADGWMCHASKWVTT
TATGAAAAAGATGCCTTTAAGCGG	CDFRWYGPKYITHSIRSFTPSVEQCK
GAACACTTGGCTGAGGTTACACTC	ESIEQTKQGTWLNPGFPPQSCGYAT
TCCGTTAAGGCGGACTTTCCTACG	VTDAEAVIVQVTPHHVLVDEYTGE
CCGTCTATATCCGACTTCGAGATA	WVDSQFINGKCSNYICPTVHNSTTW
CCCACTTCTAACATTCGACGCATC	HSDYKVKGLCDNLISMDITFFSEDG
ATTTGCTCAACCTCAGGTGGTTTCC	ELSSLGKEGTGFRSNYFAYETGGKA
CAGAGCCTCACTTGAGCTGGCTGG	CKMQYCKHWGVRLPSGVWFEMAD
AGAATGGCGAAGAACTTAACGCA	KDLFAAARFPECPEGSSISAPSQTSV
ATCAATACCACGGTGTCCCAAGAC	DVSLIQDVERILDYSLCQETWSKIRA
CCGGAGACAGAGCTGTACGCCGTG	GLPISPVDLSYLAPKNPGTGPAFTIIN
TCATCCAAACTGGATTTTAACATG	GTLKYFETRYIRVDIAAPILSRMVGM
ACGACAAATCATAGTTTCATGTGT	ISGTTTEAELWDDWAPYEDVEIGPN
CTGATCAAATATGGGCATCTCAGG	GVLRTSSGYKFPLYMIGHGMLDSDL
GTGAATCAGACTTTTAATTGGAAC	HLSSKAQVFEHPHIQDAASQLPDDE

ACTACCAAACAAGAGCACTTCCCA GATAATCTGTTGCCAAGCTGGGCG ATAACTCTTATCTCCGTCAACGGT ATCTTCGTAATTTGCTGCCTCACCT ATTGTTTCGCGCCTCGATGCCGAG AAGGCAGCGGCGCCACCAACTTCT CCCTGCTGAAGCAGGCCGGCGACG TGGAAGAAAACCCTGGCCCCATGA AGTGTCTGCTGTACCTGGCGTTCCT GTTTATCGGGGTGAACTGCAAGTT CACTATCGTGTTTCCGCACAACCA AAAGGGCAACTGGAAAAACGTGC CTTCAAATTACCATTATTGCCCCAG CAGCTCGGACCTGAACTGGCACAA TGACCTCATTGGAACCGCGCTGCA GGTGAAGATGCCACAGAGCCACA AGGCTATCCAGGCTGACGGATGGA TGTGCCACGCGTCAAAATGGGTGA CTACCTGCGATTTCCGCTGGTACG GACCAAAATACATCACGCACAGCA TCAGATCATTACCCCCGTCAGTGG AACAATGCAAAGAATCCATCGAAC AGACTAAGCAGGGAACCTGGCTGA ACCCTGGATTTCCGCCGCGAGTCGT GTGGGTACGCAACCGTGACCGATG CAGAGGCCGTGATCGTGCAAGTCA CGCCGCATCACGTGCTTGTGGACG AGTACACCGGAGAATGGGTCGATT CCCAGTTCATCAACGGCAAGTGCT CCAACCTACATTTGCCCAACCGTGC ACAACAGCACTACTTGGCACAGCG ACTACAAAGTGAAGGGTCTGTGTG ATTCCAACCTGATCTCCATGGATA TCACTTTCTTCTCGGAAGACGGCG AACTGTCCTCACTGGGCAAAGAAG GAACTGGGTTTCGCTCAAATTACT TCGCCTACGAAACTGGAGGAAAAG CCTGCAAGATGCAGTACTGCAAGC ACTGGGGCGTGAGACTACCCAGCG GTGTCTGGTTCGAGATGGCCGATA AGGACCTGTTTGCAGCAGCGAGAT TCCCGGAATGCCCTGAGGGATCGA GCATCTCCGCTCCAAGCCAACTT CAGTGGACGTGAGCCTGATCCAGG ACGTGGAACGGATTCTCGACTACT CGCTGTGCCAGGAGACCTGGTCGA AGATCAGAGCGGGACTGCCCATCT CACCGGTGGACCTGTCCTACCTGG CGCCAAAGAATCCGGGCACTGGAC	SLFFGDTGLSKNPIELVEGWFSWKS SIASFFFIIGLIIGLFLVLRVGIHLCKL KHTKKRQIYTDIEMNRLGK (SEQ ID NO: 76)
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CGGCGTTCACCATCATCAACGGCA CCCTCAAATACTTCGAGACGCGGT ACATCCGGGTGGACATCGCAGCTC CGATCCTCTCCCGGATGGTGGGAA TGATCTCGGGGACTACTACCGAAG CCGAGCTCTGGGACGACTGGGCAC CTTACGAGGATGTCGAGATCGGAC CTAACGGAGTGCTCCGGACCTCCT CCGGGTACAAGTTCCTCTGTACA TGATCGGCCATGGCATGCTGGACT CGGATCTGCATCTGTCGTCCAAAG CACAGGTGTTTGAACACCCACACA TTCAAGACGCCGCCAGCCAGCTGC CGGACGATGAGTCGCTGTTCTTCG GAGACACGGGCTTGTCAAAGAATC CCATCGAGCTGGTGGAAGGATGGT TTTCATCCTGGAAAAGCAGCATCG CTTCATTCTTCTTCATCATTGGCCT GATCATCGGCCTATTTCTAGTCCTG CGGGTGGGAATTCATCTGTGCATC AAGCTCAAGCACACTAAGAAGCG GCAAATCTACACTGATATCGAGAT GAATCGCCTGGGCAAG (SEQ ID NO: 75)	
CD80 signal peptide	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGA ACCTCACCATCTAAGTGCCCATAT CTGAATTTCTTTCAACTTCTCGTGC TGGCGGGGCTCAGTCATTTCTGCA GTGGGGGTC (SEQ ID NO: 3)	MGHTRRQGTSPSKCPYLNFFQLLVL AGLSHFCSGV (SEQ ID NO: 4)
CD80 extracellular domain (ECD)	
Nucleotide	Amino acid
ATTCACGTTACTAAAGAGGTCAAG GAGGTCGCAACATTGAGTTGTGGC CATAACGTATCAGTTGAAGAACTC GCGCAGACACGGATTTACTGGCAA AAGGAAAAGAAGATGGTGTGAC AATGATGAGCGGTGACATGAACAT TTGGCCAGAGTACAAAAATCGAAC GATATTCGATATAACCAATAACTT GTCCATAGTAATACTTGCCTTGCG ACCTTCTGACGAGGGAACGTATGA ATGTGTAGTGCTTAAGTATGAAAA AGATGCCTTTAAGCGGGAACACTT GGCTGAGGTTACACTCTCCGTAA GGCGGACTTTCCTACGCCGTCTAT ATCCGACTTCGAGATACCCACTTC	IHVTKEVKEVATLSCGHNVSVEELA QTRIYWQKEKKMVLTMMSGDMNI WPEYKNRTIFDITNNLSIVILALRPSD EGTYECVVLKYEKDAFKREHLAEV TLSVKADFPTPSISDFEIPTSNIRRIICS TSGGFPEPHLSWLENGEELNAINTTV SQDPETELYAVSSKLDFNMTTNHSF MCLIKYGHRLRVNQTFNWNTTKQEH FPDNLLPS (SEQ ID NO: 6)

TAACATTTCGACGCATCATTTGCTC AACCTCAGGTGGTTTCCCAGAGCC TCACTTGAGCTGGCTGGAGAATGG CGAAGAACTTAACGCAATCAATAC CACGGTGTCCCAAGACCCGGAGAC AGAGCTGTACGCCGTGTCATCCAA ACTGGATTTTAAACATGACGACAAA TCATAGTTTCATGTGTCTGATCAAA TATGGGCATCTCAGGGTGAATCAG ACTTTTAATTGGAACACTACCAAA CAAGAGCACTTCCCAGATAATCTG TTGCCAAGC (SEQ ID NO: 5)	
CD80 transmembrane and intracellular domain	
Nucleotide	Amino acid
TGGGCGATAACTCTTATCTCCGTC AACGGTATCTTCGTAATTTGCTGCC TCACCTATTGTTTCGCGCCTCGATG CCGAGAA (SEQ ID NO: 7)	WAITLISVNGIFVICCLTYCFAPRCRE (SEQ ID NO: 8)
P2A self-cleaving	
Nucleotide	Amino acid
GGCAGCGGCGCCACCAACTTCTCC CTGCTGAAGCAGGCCGGCGACGTG GAAGAAAACCCTGGCCCC (SEQ ID NO: 69)	GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 70)
VSV-G signal sequence	
Nucleotide	Amino acid
ATGAAGTGTCTGCTGTACCTGGCG TTCCTGTTTATCGGGGTGAACTGC (SEQ ID NO: 71)	MKCLLYLAFLFIGVNC (SEQ ID NO: 72)
Mutant VSV-G	
Nucleotide	Amino acid
AAGTTCACATATCGTGTTTCCGCAC AACCAAAAGGGCAACTGGAAAAA CGTGCCTTCAAATTACCATTATTGC CCCAGCAGCTCGGACCTGAACTGG CACAATGACCTCATTGGAACCGCG CTGCAGGTGAAGATGCCACAGAGC CACAAGGCTATCCAGGCTGACGGA TGGATGTGCCACGCGTCAAAATGG GTGACTACCTGCGATTTCGCTGG TACGGACCAAAATACATCACGCAC AGCATCAGATCATTCACCCCGTCA GTGGAACAATGCAAAGAATCCATC GAACAGACTAAGCAGGGAACCTG GCTGAACCCTGGATTTCCGCCGCA GTCGTGTGGGTACGCAACCGTGAC CGATGCAGAGGCCGTGATCGTGCA AGTCACGCCGCATCACGTGCTTGT	KFTIVFPHNQKGNWKNVPSNYHYCP SSSDLNWHNDLIGTALQVKMPQSH KAIQADGWMCHASKWVTTCDFRW YGPKYITHSIRSFTPSVEQCKESIEQT KQGTWLNPGFPPQSCGYATVTD AEAVIVQVTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTW HSDYKVKGLCDSNLISMDITFFSE DGELSSLGKEGTGFRSNYFAYETGG KACKMQYCKHWGVR LPSGVWFEMAD KDLFAAARFPECPEGSSISAPSQTS VDVSLIQDVERILDYSLCQETWSKIR AGLPISPVDLSYLAPKNPGTGPAFTI INGTLKYFETRYIRVDIAAPILSRM VGMISGTTEAELWDDWAPYEDVEIG PNGLVLRSSGYKFPLYMIGHGMLD SDLHLSKAQVFEHPHIQDAASQLP DDESLFF

GGACGAGTACACCGGAGAATGGG TCGATTCCCAGTTCATCAACGGCA AGTGCTCCAACTACATTTGCCCAA CCGTGCACAACAGCACTACTTGGC ACAGCGACTACAAAGTGAAGGGTC TGTGTGATTCCAACCTGATCTCCAT GGATATCACTTTCTTCTCGGAAGA CGGCGAACTGTCTCACTGGGCAA AGAAGGAACTGGGTTCGCTCAA TTACTTCGCCTACGAACTGGAGG AAAAGCCTGCAAGATGCAGTACTG CAAGCACTGGGGCGTGAGACTACC CAGCGGTGTCTGGTTCGAGATGGC CGATAAGGACCTGTTTGCAGCAGC GAGATTCCCGGAATGCCCTGAGGG ATCGAGCATCTCCGCTCCAAGCCA AACTTCAGTGGACGTGAGCCTGAT CCAGGACGTGGAACGGATTCTCGA CTACTCGCTGTGCCAGGAGACCTG GTCGAAGATCAGAGCGGGACTGCC CATCTACCGGTGGACCTGTCTTA CCTGGCGCCAAAGAATCCGGGCAC TGGACCGGCGTTCACCATCATCAA CGGCACCCTCAAATACTTCGAGAC GCGGTACATCCGGGTGGACATCGC AGCTCCGATCCTCTCCCGGATGGT GGGAATGATCTCGGGGACTACTAC CGAAGCCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGAT CGGACCTAACGGAGTGCTCCGGAC CTCCTCCGGGTACAAGTTCCCTCTG TACATGATCGGCCATGGCATGCTG GACTCGGATCTGCATCTGTCGTCC AAAGCACAGGTGTTTGAACACCCA CACATTCAAGACGCCGCCAGCCAG CTGCCGGACGATGAGTCGCTGTTC TTCGGAGACACGGGCTTGTCAAAG AATCCCATCGAGCTGGTGGAAGGA TGGTTTTTCATCCTGGAAAAGCAGC ATCGCTTCATTCTTCTTCATCATTG GCCTGATCATCGGCCTATTTCTAGT CCTGCGGGTGGGAATTCATCTGTG CATCAAGCTCAAGCACACTAAGAA GCGGCAAATCTACACTGATATCGA GATGAATCGCCTGGGCAAG (SEQ ID NO: 73)	GDTGLSKNPIELVEGWFSSWKSSIAS FFFIIGLIIGLFLVLRVGIHLCKLKHT KKRQIYTDIEMNRLGK (SEQ ID NO: 74)
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In another embodiment, the tandem expression cassette of the VSV-G env plasmid encodes a CD80 targeting protein, an anti-CD3 targeting protein, and a mutated VSV-G envelope protein. For example, in a specific embodiment, the tandem expression cassette may comprise a CD80 targeting polynucleotide according to SEQ ID NO: 1, which encodes an amino acid sequence of SEQ ID NO: 2. In a specific embodiment, the tandem expression cassette may comprise an anti-CD3 targeting polynucleotide according to SEQ ID NO: 9, which encodes an amino acid sequence of SEQ ID NO: 10. Such an embodiment is described in more detail in Table 3. As illustrated in Table 3, the CD80 targeting protein is separated from the anti-CD3 targeting protein via a P2A self-cleaving peptide encoded by SEQ ID NO: 29 and having an amino acid sequence according to SEQ ID NO: 30.

In some embodiments, where the tandem expression cassette of the VSV-G env plasmid encodes a CD80 targeting protein, anti-CD3 targeting protein, and a mutated VSV-G envelope protein, the tandem expression cassette may include a polynucleotide encoding the CD80 targeting protein and anti-CD3 targeting protein 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: 21, which encodes an amino acid sequence 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: 22. In certain embodiments, the CD80 targeting protein is separated from the anti-CD3 targeting protein via a P2A self-cleaving peptide encoded a nucleic acid sequence 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: 29, and the P2A self-cleaving peptide includes an amino acid 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: 30.

In some embodiments, wherein the tandem expression cassette of the VSV-G env plasmid encodes a CD80 targeting protein, anti-CD3 targeting protein, and a mutated VSV-G envelope protein, the CD80 targeting protein and anti-CD3 targeting protein are separated from the mutated VSV-G envelope protein via a 2A self-cleaving peptide.

In some embodiments of a four plasmid LVV system, the GagPol plasmid includes a tandem expression cassette that encodes the Gag polyprotein precursor and

Pol polyprotein precursor and a targeting protein as disclosed herein. In specific embodiments, the tandem expression cassette included in the GagPol plasmid includes a polynucleotide that encodes a first signal peptide, a polynucleotide that encodes a targeting protein, a polynucleotide that encodes one of an internal ribosomal entry site (IRES), a furin cleavage site, or a viral 2A peptide, a polynucleotide that encodes a second signal peptide, and a polynucleotide that encodes Gag polyprotein precursor and Pol polyprotein precursor. In certain embodiments, the polynucleotide encoding the Gag polyprotein precursor and Pol polyprotein precursor is positioned 5' to the polynucleotide encoding the targeting protein. In other embodiments, the polynucleotide encoding the Gag polyprotein precursor and Pol polyprotein precursor is positioned 3' to the polynucleotide encoding the targeting protein. The polynucleotide that encodes the targeting protein and the polynucleotide that encodes the Gag polyprotein precursor and Pol polyprotein precursor are separated in the tandem cassette by the polynucleotide that encodes the IRES, furin cleavage site, or viral 2A peptide, which allows for co-expression of Gag polyprotein precursor and Pol polyprotein precursor and the targeting protein from a single mRNA. In certain embodiments, a viral 2A peptide is a porcine teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or a variant thereof. Specific examples of amino acid and nucleotide sequences for the viral 2A peptides are provided in Table 7.

In some embodiments, the GagPol plasmid containing the tandem expression cassette encoding Gag polyprotein precursor and Pol polyprotein precursor and the targeting protein is transfected into producer cells at a higher molar concentration than the VSV-G env plasmid. In some embodiments, the GagPol plasmid containing the tandem expression cassette encoding Gag polyprotein precursor and Pol polyprotein precursor and the targeting protein is transfected into producer cells at a lower molar concentration than the VSV-G env plasmid.

In one embodiment, the tandem expression cassette of the GagPol plasmid encodes an anti-CD3 targeting protein and Gag polyprotein precursor and Pol polyprotein precursor. In some embodiments, the anti-CD3 targeting protein is separated from the Gag polyprotein precursor and Pol polyprotein precursor via a P2A self-cleaving peptide.

In another embodiment, the tandem expression cassette of the GagPol plasmid encodes a CD80 targeting protein and Gag polyprotein precursor and Pol polyprotein precursor. In some embodiments, the CD80 targeting protein is separated from Gag polyprotein precursor and Pol polyprotein precursor via a P2A self-cleaving peptide.

5 In some embodiments of a four plasmid LVV system, the Rev plasmid includes a tandem expression cassette that encodes the Rev protein and a targeting protein as disclosed herein. In specific embodiments, the tandem expression cassette included in the Rev plasmid includes a polynucleotide that encodes a first signal peptide, a polynucleotide that encodes a targeting protein, a polynucleotide that encodes one of an
10 internal ribosomal entry site (IRES), a furin cleavage site, or a viral 2A peptide, a polynucleotide that encodes a second signal peptide, and a polynucleotide that encodes a Rev protein. In certain embodiments, the polynucleotide encoding the Rev protein is positioned 5' to the polynucleotide encoding the targeting protein. In other embodiments, the polynucleotide encoding the Rev protein is positioned 3' to the
15 polynucleotide encoding the targeting protein. The polynucleotide that encodes the targeting protein and the polynucleotide that encodes the Rev protein are separated in the tandem cassette by the polynucleotide that encodes the IRES, furin cleavage site, or viral 2A peptide, which allows for co-expression of the Rev protein and the targeting protein from a single mRNA. In certain embodiments, a viral 2A peptide is a porcine
20 teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or a variant thereof. Specific examples of amino acid and nucleotide sequences for the viral 2A peptides are provided in Table 7.

In some embodiments, the Rev plasmid containing the tandem expression cassette encoding the Rev protein and the targeting protein is transfected into producer
25 cells at a higher molar concentration than the VSV-G env plasmid. In some embodiments, the Rev plasmid containing the tandem expression cassette encoding the Rev protein and the targeting protein is transfected into producer cells at a lower molar concentration than the VSV-G env plasmid.

In one embodiment, the tandem expression cassette of the Rev plasmid encodes
30 an anti-CD3 targeting protein and a Rev protein. In some embodiments, the anti-CD3 targeting protein is separated from the Rev protein via a P2A self-cleaving peptide.

In another embodiment, the tandem expression cassette of the GagPol plasmid encodes a CD80 targeting protein and a Rev protein. In some embodiments, the CD80 targeting protein is separated from the Rev protein via a P2A self-cleaving peptide.

5 Examples of mutated VSV-G sequences that may be used in the four plasmid, 3rd generation lentiviral vector system of the present disclosure are provided in Table 10.

Table 10: WT VSV-G reference and mutant sequences

WT VSV-G	
Nucleotide	Amino acid (signal sequence <u>underlined</u>)
ATGAAGTGTCTGCTGTACCTGGCGT TCCTGTTTATCGGGGTGAACTGCAA GTTCACTATCGTGTTTCCGCACAAC CAAAAGGGCAACTGGAAAAACGTG CCTTCAAATTACCATTATTGCCCA GCAGCTCGGACCTGAACTGGCACA ATGACCTCATTGGAACCGCGCTGCA GGTGAAGATGCCAAAGAGCCACAA GGCTATCCAGGCTGACGGATGGAT GTGCCACGCGTCAAAATGGGTGAC TACCTGCGATTTCGCTGGTACGGA CCAAAATACATCACGCACAGCATC AGATCATTCACCCCGTCAGTGGAAC AATGCAAAGAATCCATCGAACAGA CTAAGCAGGGAACCTGGCTGAACC CTGGATTTCCGCCGAGTCGTGTGG GTACGCAACCGTGACCGATGCAGA GGCCGTGATCGTGCAAGTCACGCC GCATCACGTGCTTGTGGACGAGTAC ACCGGAGAATGGGTGCGATTCCCAG TTCATCAACGGCAAGTGCTCCAAC ACATTTGCCCAACCGTGCACAACAG CACTACTTGGCACAGCGACTACAA AGTGAAGGGTCTGTGTGATTCCAAC CTGATCTCCATGGATATCACTTTCT TCTCGGAAGACGGCGAACTGTCCTC ACTGGGCAAAGAAGGAACTGGGTT TCGCTCAAATTACTTCGCCTACGAA ACTGGAGGAAAAGCCTGCAAGATG CAGTACTGCAAGCACTGGGGCGTG AGACTACCCAGCGGTGTCTGGTTTCG AGATGGCCGATAAGGACCTGTTTGC	<u>MKCLLYLAFLFIGVNCKFTIVFPHN</u> QKGNWKNVPSNYHYCPSSSDLNW HNDLIGTALQVKMPKSHKAIQADG WMCHASKWVTTCDFRWYGPKYIT HSIRSFTPSVEQCKESIEQTKQGTWL NPGFPPQSCGYATVTDAAEAVIVQVT PHHVLVDEYTGEWVDSQFINGKCS NYICPTVHNSTTWHSYKVKGLCD SNLISMDITFFSEDGELSSLGKEGTG FRSNYFAYETGGKACKMQYCKHW GVRLPSGVWFEMADKDLFAAARFP ECPEGSSISAPSQTSVDVSLIQDVERI LDYSLCQETWSKIRAGLPISPVDLS YLAPKNPGTGPAFTIINGTLKYFETR YIRVDIAAPILSRMVGMISGTTTTERE LWDDWAPYEDVEIGPNGVLRITSSG YKFPLYMIGHGMLDSDLHLSSKAQ VFEHPHIQDAASQLPDDESFFGDT GLSKNPIELVEGWFWSSWKSSIASFFF IIGLIIGLFLVLRVGIHLCKLKHTKK RQIYTDIEMNRLGK (SEQ ID NO: 78)

AGCAGCGAGATTCCCGGAATGCCC TGAGGGGATCGAGCATCTCCGCTCCA AGCCAAACTTCAGTGGACGTGAGC CTGATCCAGGACGTGGAACGGATT CTCGACTACTCGCTGTGCCAGGAGA CCTGGTCGAAGATCAGAGCGGGAC TGCCCATCTCACCGGTGGACCTGTC CTACCTGGCGCCAAAGAATCCGGG CACTGGACCGGCGTTCACCATCATC AACGGCACCTCAAATACTTCGAG ACGCGGTACATCCGGGTGGACATC GCAGCTCCGATCCTCTCCCGGATGG TGGGAATGATCTCGGGGACTACTAC CGAACGCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGAT CGGACCTAACGGAGTGCTCCGGAC CTCCTCCGGGTACAAGTTCCCTCTG TACATGATCGGCCATGGCATGCTGG ACTCGGATCTGCATCTGTCGTCCAA AGCACAGGTGTTTGAACACCCACA CATTCAAGACGCCGCCAGCCAGCT GCCGGACGATGAGTCGCTGTTCTTC GGAGACACGGGCTTGTCAAAGAAT CCCATCGAGCTGGTGGAAGGATGG TTTTCATCCTGGAAAAGCAGCATCG CTTCATTCTTCTTCATCATTGGCCTG ATCATCGGCCTATTTCTAGTCCTGC GGGTGGGAATTCATCTGTGCATCAA GCTCAAGCACACTAAGAAGCGGCA AATCTACACTGATATCGAGATGAAT CGCCTGGGCAAG (SEQ ID NO: 77)	
WT VSV-G Signal Sequence	
Nucleotide ATGAAGTGTCTGCTGTACCTGGCGT TCCTGTTTATCGGGGTGAACTGC (SEQ ID NO: 87)	Amino acid MKCLLYLAFLFIGVNC (SEQ ID NO: 88)
WT VSV-G without signal sequence	
Nucleotide AAGTTCACTATCGTGTTTCCGCACA ACCAAAAGGGCAACTGGAAAAACG TGCCTTCAAATTACCATTATTGCCC CAGCAGCTCGGACCTGAACTGGCA CAATGACCTCATTGGAACCGCGCTG CAGGTGAAGATGCCAAAGAGCCAC AAGGCTATCCAGGCTGACGGATGG ATGTGCCACGCGTCAAATGGGTG	Amino acid KFTIVFPHNQKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPKS HKAIQADGWMCHASKWVTTCDFR WYGPKYITHSIRSFTPSVEQCKESIE QTKQGTWLNPGFPPQSCGYATVTD AEAVIVQVTPHHVLVDEYTG EWVD SQFINGKCSNYICPTVHNSTTWHS DKYVKGLCDSNLSMDITFFSEDGEL

ACTACCTGCGATTTCCGCTGGTACG
 GACCAAAATACATCACGCACAGCA
 TCAGATCATTACCCCGTCAGTGGA
 ACAATGCAAAGAATCCATCGAACA
 GACTAAGCAGGGAACCTGGCTGAA
 CCCTGGATTTCCGCCGCAGTCGTGT
 GGGTACGCAACCGTGACCGATGCA
 GAGGCCGTGATCGTGCAAGTCACG
 CCGCATCACGTGCTTGTGGACGAGT
 ACACCGGAGAATGGGTCGATTCCC
 AGTTCATCAACGGCAAGTGCTCCAA
 CTACATTTGCCCAACCGTGCAACA
 AGCACTACTTGGCACAGCGACTAC
 AAAGTGAAGGGTCTGTGTGATTCCA
 ACCTGATCTCCATGGATATCACTTT
 CTTCTCGGAAGACGGCGAACTGTCC
 TCACTGGGCAAAGAAGGAACTGGG
 TTTCGCTCAAATTACTTCGCCTACG
 AAAGTGGAGGAAAAGCCTGCAAGA
 TGCAGTACTGCAAGCACTGGGGCG
 TGAGACTACCCAGCGGTGTCTGGTT
 CGAGATGGCCGATAAGGACCTGTTT
 GCAGCAGCGAGATTCCCGGAATGC
 CCTGAGGGATCGAGCATCTCCGCTC
 CAAGCCAAACTTCAGTGGACGTGA
 GCCTGATCCAGGACGTGGAACGGA
 TTCTCGACTACTCGCTGTGCCAGGA
 GACCTGGTCGAAGATCAGAGCGGG
 ACTGCCCATCTCACCGGTGGACCTG
 TCCTACCTGGCGCCAAAGAATCCGG
 GCACTGGACCGGCGTTCACCATCAT
 CAACGGCACCCCTCAAATACTTCGAG
 ACGCGGTACATCCGGGTGGACATC
 GCAGCTCCGATCCTCTCCCGGATGG
 TGGGAATGATCTCGGGGACTACTAC
 CGAACGCGAGCTCTGGGACGACTG
 GGCACCTTACGAGGATGTCGAGAT
 CGGACCTAACGGAGTGCTCCGGAC
 CTCCTCCGGGTACAAGTTCCCTCTG
 TACATGATCGGCCATGGCATGCTGG
 ACTCGGATCTGCATCTGTCGTCCAA
 AGCACAGGTGTTTGAACACCCACA
 CATTCAAGACGCCGCCAGCCAGCT
 GCCGGACGATGAGTCGCTGTTCTTC
 GGAGACACGGGCTTGTCAAAGAAT
 CCCATCGAGCTGGTGGAAGGATGG
 TTTTCATCCTGGAAAAGCAGCATCG
 CTTTATTCTTCTTCATCATTGGCCTG
 ATCATCGGCCTATTTCTAGTCCTGC

SSLGKEGTGFRSNYFAYETGGKAC
 KMQYCKHWGVRLPSGVWFEMAD
 KDLFAAARFPECPEGSSISAPSQTSV
 DVSLIQDVERILDYSLCQETWSKIR
 AGLPISPVDLSYLAPKNPGTGPAFTI
 INGTLYFETRYIRVDIAAPILSRMV
 GMISGTTTERELWDDWAPYEDVEI
 GPNGLVLTSSGYKFPLYMIGHGML
 DSDLHLSSKAQVFEHPHIQDAASQL
 PDDESLFFGDTGLSKNPIELVEGWF
 SSWKSSIASFFFIIIGLIIGLFLVLRVGI
 HLCIKLKHTKKRQIYTDIEMNRLGK
 (SEQ ID NO: 90)

GGGTGGGAATTCATCTGTGCATCAA GCTCAAGCACACTAAGAAGCGGCA AATCTACACTGATATCGAGATGAAT CGCCTGGGCAAG (SEQ ID NO: 89)	
Trop-002 Mutated VSV-G	
Nucleotide	Amino Acid
AAGTTCATCTCGTGTTTCCGCACA ACCAAAAGGGCAACTGGAAAAACG TGCCTTCAAATTACCATTATTGCCC CAGCAGCTCGGACCTGAACTGGCA CAATGACCTCATTGGAACCGCGCTG CAGGTGAAGATGCCACAGAGCCAC AAGGCTATCCAGGCTGACGGATGG ATGTGCCACGCGTCAAAATGGGTG ACTACCTGCGATTTCCGCTGGTACG GACCAAAATACATCACGCACAGCA TCAGATCATTACCCCGTCAGTGGA ACAATGCAAAGAATCCATCGAACA GACTAAGCAGGGAACCTGGCTGAA CCCTGGATTTCCGCCGCAGTCGTGT GGGTACGCAACCGTGACCGATGCA GAGGCCGTGATCGTGCAAGTCACG CCGCATCACGTGCTTGTGGACGAGT ACACCGGAGAATGGGTGCGATTCCC AGTTCATCAACGGCAAGTGCTCCAA CTACATTTGCCCAACCGTGCAACAC AGCACTACTTGGCACAGCGACTAC AAAGTGAAGGGTCTGTGTGATTCCA ACCTGATCTCCATGGATATCACTTT CTTCTCGGAAGACGGCGAACTGTCC TCACTGGGCAAAGAAGGAACTGGG TTTCGCTCAAATTACTTCGCCTACG AACTGGAGGAAAAGCCTGCAAGA TGCAGTACTGCAAGCACTGGGGCG TGAGACTACCCAGCGGTGTCTGGTT CGAGATGGCCGATAAGGACCTGTTT GCAGCAGCGAGATTCCCGGAATGC CCTGAGGGATCGAGCATCTCCGCTC CAAGCCAACTTCAGTGGACGTGA GCCTGATCCAGGACGTGGAACGGA TTCTCGACTACTCGCTGTGCCAGGA GACCTGGTCGAAGATCAGAGCGGG ACTGCCCATCTCACCGGTGGACCTG TCCTACCTGGCGCCAAAGAATCCGG GCACTGGACCGGCGTTCACCATCAT CAACGGCACCCCTCAAATACTTCGAG ACGCGGTACATCCGGGTGGACATC	KFTIVFPHNQKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPQS HKAIQADGWMCHASKWVTTCDFR WYGPKYITHSIRSFTPSVEQCKESIE QTKQGTWLNPGFPPQSCGYATVTD AEAVIVQVTPHHVLVDEYTG EWVD SQFINGKCSNYICPTVHNSTTWHSD YKVKGLCDSNLISMDITFFSEDGEL SSLGKEGTGFRSNYFAYETGGKAC KMQYCKHWGVRLPSGVWFEMAD KDLFAAARFPECPEGSSISAPSQTSV DVS LIQDVERILDYSLCQETWSKIR AGLPISPVDLSYLAPKNPGTGPAFTI INGTLKYFETRYIRVDIAAPILSRMV GMISGTTTEAELWDDWAPYEDVEI GPNGVLRTSSGYKFPLYMIGHGML DSDLHLSSKAQVFEHPHIQDAASQL PDDESLFFGDTGLSKNPIELVEGWF SSWKSSIASFFFIIIGLIIGLFLVLRVGI HLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO: 74)

GCAGCTCCGATCCTCTCCCGGATGG TGGGAATGATCTCGGGGACTACTAC CGAAGCCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGAT CGGACCTAACGGAGTGCTCCGGAC CTCCTCCGGGTACAAGTTCCCTCTG TACATGATCGGCCATGGCATGCTGG ACTCGGATCTGCATCTGTCTGTCCTAA AGCACAGGTGTTTGAACACCCACA CATTCAAGACGCCGCCAGCCAGCT GCCGGACGATGAGTCGCTGTTCTTC GGAGACACGGGCTTGTCAAAGAAT CCCATCGAGCTGGTGGAAAGGATGG TTTTTCATCCTGGAAAAGCAGCATCG CTTCATTCTTCTTCATCATTGGCCTG ATCATCGGCCTATTTCTAGTCCTGC GGGTGGGAATTCATCTGTGCATCAA GCTCAAGCACACTAAGAAGCGGCA AATCTACACTGATATCGAGATGAAT CGCCTGGGCAAG (SEQ ID NO: 73)	
Trop-002 Mutated VSV-G	
Nucleotide	Amino acid
AAGTTCACATATCGTGTTTCCGCACA ACCAAAAGGGCAACTGGAAAAACG TGCCTTCAAATTACCATTATTGCCC CAGCAGCTCGGACCTGAACTGGCA CAATGACCTCATTGGAACCGCGCTG CAGGTGAAGATGCCACAGAGCCAC AAGGCTATCCAGGCTGACGGATGG ATGTGCCACGCGTCAAAATGGGTG ACTACCTGCGATTTCCGCTGGTACG GACCAAAATACATCACGCACAGCA TCAGATCATTACCCCGTCAGTGGA ACAATGCAAAGAATCCATCGAACA GACTAAGCAGGGAACCTGGCTGAA CCCTGGATTTCCGCCGCAGTCGTGT GGGTACGCAACCGTGACCGATGCA GAGGCCGTGATCGTGCAAGTCACG CCGCATCACGTGCTTGTGGACGAGT ACACCGGAGAATGGGTCGATTCCC AGTTCATCAACGGCAAGTGCTCCAA CTACATTTGCCCAACCGTGCAACAC AGCACTACTTGGCATAGCGACTACA AAGTGAAGGGTCTGTGTGATTCCAA CCTGATCTCCATGGATATCACTTTC TTCTCGGAAGACGGCGAACTGTCCT CACTGGGCAAAGAAGGAAGTGGGT TTCGCTCAAATTACTTCGCCTACGA	KFTIVFPHNQKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPQS HKAIQADGWMCHASKWVTTCDFR WYGPKYITHSIRSFTPSVEQCKESIE QTKQGTWLNPGFPPQSCGYATVTD AEAVIVQVTPHHVLVDEYTGWVVD SQFINGKCSNYICPTVHNSTTWHSD YKVKGLCDSNLISMDITFFSEDGEL SSLGKEGTGFRSNYFAYETGGKAC KMQYCKHWGVRLPSGVWFEMAD KDLFAAARFPECPEGSSISAPSQTSV DVS LIQDVERILDYSLCQETWSKIR AGLPISPVDLSYLAPKNPGTGPAFTI INGTLKYFETRYIRVDIAAPILSRMV GMISGTTTEAELWDDWAPYEDVEI GPNGVLR TSSGYKFPLYMIGHGML DSDLHLSSKAQVFEHPHIQDAASQL PDDESLFFGDTGLSKNPIELVEGWF SSWKSSIASFFFIIGLIIGLFLVLRVGI HLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO: 74)

A A C T G G A G G A A A A G C C T G C A A G A T G C A G T A C T G C A A G C A C T G G G G C G T G A G A C T A C C C A G C G G T G T C T G G T T C G A G A T G G C C G A T A A G G A C C T G T T T G C A G C A G C G A G A T T C C C G G A A T G C C C T G A G G G A T C G A G C A T C T C C G C T C C A A G C C A A A C T T C A G T G G A C G T G A G C C T G A T C C A G G A C G T G G A A C G G A T T C T C G A C T A C T C G C T G T G C C A G G A G A C C T G G T C G A A G A T C A G A G C G G G A C T G C C C A T C T C A C C G G T G G A C C T G T C C T A C C T G G C G C C A A A G A A T C C G G G C A C T G G A C C G G C G T T C A C C A T C A T C A A C G G C A C C C T C A A A T A C T T C G A G A C G C G G T A C A T C C G G G T G G A C A T C G C A G C T C C G A T C C T C T C C C G G A T G G T G G G A A T G A T C T C G G G G A C T A C T A C C G A A G C C G A G C T C T G G G A C G A C T G G G C A C C T T A C G A G G A T G T C G A G A T C G G A C C T A A C G G A G T G C T C C G G A C C T C C T C C G G G T A C A A G T T C C C T C T G T A C A T G A T C G G C C A T G G C A T G C T G G A C T C G G A T C T G C A T C T G T C G T C C A A A G C A C A G G T G T T T G A A C A C C C A C A C A T T C A A G A C G C C G C C A G C C A G C T G C C G G A C G A T G A G T C G C T G T T C T T C G G A G A C A C G G G C T T G T C A A A G A A T C C C A T C G A G C T G G T G G A A G G A T G G T T T T C A T C C T G G A A A A G C A G C A T C G C T T C A T T C T T C T T C A T C A T T G G C C T G A T C A T C G G C C T A T T T C T A G T C C T G C G G G T G G G A A T T C A T C T G T G C A T C A A G C T C A A G C A C A C T A A G A A G C G G C A A A T C T A C A C T G A T A T C G A G A T G A A T C G C C T G G G C A A G T A G (SEQ ID NO: 91)	
Trop-051 Mutated VSV-G	
Nucleotide	Amino acid
A A G T T C A C T A T C G T G T T T C C G C A C A A C C A A A A G G G C A A C T G G A A A A A C G T G C C T T C A A A T T A C C A T T A T T G C C C C A G C A G C T C G G A C C T G A A C T G G C A C A A T G A C C T C A T T G G A A C C G C G C T G C A G G T G A A G A T G C C A A A G A G C C A C A A G G C T A T C C A G G C T G A C G G A T G G A T G T G C C A C G C G T C A A A A T G G G T G A C T A C C T G C G A T T T C C G C T G G T A C G	K F T I V F P H N Q K G N W K N V P S N Y H Y C P S S S D L N W H N D L I G T A L Q V K M P K S H K A I Q A D G W M C H A S K W V T T C D F R W Y G P K Y I T H S I R S F T P S V E Q C K E S I E Q T K Q G T W L N P G F P P Q S C G Y A T V T D A E A V I V Q V T P H H V L V D E Y T G E W V D S Q F I N G K C S N Y I C P T V H N S T T W H S D Y K V K G L C D S A L A S M D I T F F S E D G E L S S L G K E G T G F R S N Y F A Y E T G G K A C

GACCAAAATACATCACGCACAGCA
 TCAGATCATTACCCCCGTCAGTGGA
 ACAATGCAAAGAATCCATCGAACA
 GACTAAGCAGGGAACCTGGCTGAA
 CCCTGGATTTCCGCCGCAGTCGTGT
 GGGTACGCAACCGTGACCGATGCA
 GAGGCCGTGATCGTGCAAGTCACG
 CCGCATCACGTGCTTGTGGACGAGT
 ACACCGGAGAATGGGTCGATTCCC
 AGTTCATCAACGGCAAGTGCTCCAA
 CTACATTTGCCCAACCGTGCAACA
 AGCACTACTTGGCACAGCGACTAC
 AAAGTGAAGGGTCTGTGTGATTCCG
 CCCTGGCCTCCATGGATATCACTTT
 CTTCTCGGAAGACGGCGAACTGTCC
 TCACTGGGCAAAGAAGGAACTGGG
 TTTCGCTCAAATTACTTCGCCTACG
 AAAGTGGAGGAAAAGCCTGCAAGA
 TGCAGTACTGCAAGCACTGGGGCG
 TGAGACTACCCAGCGGTGTCTGGTT
 CGAGATGGCCGATAAGGACCTGTTT
 GCAGCAGCGAGATTCCCGGAATGC
 CCTGAGGGATCGAGCATCTCCGCTC
 CAAGCCAACTTCAGTGGACGTGA
 GCCTGATCCAGGACGTGGAACGGA
 TTCTCGACTACTCGCTGTGCCAGGA
 GACCTGGTCGAAGATCAGAGCGGG
 ACTGCCCATCTCACCGGTGGACCTG
 TCCTACCTGGCGCCAAAGAATCCGG
 GCACTGGACCGGCGTTCACCATCAT
 CAACGGCACCCCTCAAATACTTCGAG
 ACGCGGTACATCCGGGTGGACATC
 GCAGCTCCGATCCTCTCCCGGATGG
 TGGGAATGATCTCGGGGACTACTGC
 CGCCCGCGAGCTCTGGGACGACTG
 GGCACCTTACGAGGATGTCGAGAT
 CGGACCTAACGGAGTGCTCCGGAC
 CTCCTCCGGGTACAAGTTCCCTCTG
 TACATGATCGGCCATGGCATGCTGG
 ACTCGGATCTGCATCTGTCGTCCAA
 AGCACAGGTGTTTGAACACCCACA
 CATTCAAGACGCCGCCAGCCAGCT
 GCCGGACGATGAGTCGCTGTTCTTC
 GGAGACACGGGCTTGTCAAAGAAT
 CCCATCGAGCTGGTGGAAGGATGG
 TTTTCATCCTGGAAAAGCAGCATCG
 CTTTATTCTTCTTCATCATTGGCCTG
 ATCATCGGCCTATTTCTAGTCCTGC
 GGGTGGGAATTCATCTGTGCATCAA

KMQYCKHWGVRLPSGVWFEMAD
 KDLFAAARFPECPEGSSISAPSQTSV
 DVSLIQDVERILDYSLCQETWSKIR
 AGLPISPVDLSYLAPKNPGTGPAFTI
 INGTLYKFETRYIRVDIAAPILSRMV
 GMISGTTAARELWDDWAPYEDVEI
 GPNGVLRTSSGYKFPLYMIGHGML
 DSDLHLSSKAQVFEHPHIQDAASQL
 PDDESLFFGDTGLSKNPIELVEGWF
 SSWKSSIASFFFIIIGLIIGLFLVLRVGI
 HLCIKLKHTKKRQIYTDIEMNRLGK
 (SEQ ID NO: 93)

GCTCAAGCACACTAAGAAGCGGCA AATCTACACTGATATCGAGATGAAT CGCCTGGGCAAGTAG (SEQ ID NO: 92)	
Trop-052 Mutated VSV-G	
Nucleotide	Amino acid
AAGTTCACACTATCGTGTTTCCGCACA ACCAAAAGGGCAACTGGAAAAACG TGCCTTCAAATTACCATTATTGCCC CAGCAGCTCGGACCTGAACTGGCA CAATGACCTCATTGGAACCGCGCTG CAGGTGAAGATGCCAAAGAGCCAC AAGGCTATCCAGGCTGACGGATGG ATGTGCCACGCGTCAAAATGGGTG ACTACCTGCGATTTCCGCTGGTACG GACCAAAATACATCACGCACAGCA TCAGATCATTACCCCGTCAGTGGA ACAATGCAAAGAATCCATCGAACA GACTAAGCAGGGAACCTGGCTGAA CCCTGGATTTCCGCCGCAGTCGTGT GGGTACGCAACCGTGACCGATGCA GAGGCCGTGATCGTGCAAGTCACG CCGCATCACGTGCTTGTGGACGAGT ACACCGGAGAATGGGTGCGATTCCC AGTTCATCAACGGCAAGTGCTCCAA CTACATTTGCCCAACCGTGCAACA AGCACTACTTGGCACAGCGACTAC AAAGTGAAGGGTCTGTGTGATTCCA ACCTGATCTCCATGGATATCACTTT CTTCTCGGAAGACGGCGAACTGTCC TCACTGGGCAAAGAAGGAAGTGGG TTTCGCTCAAATTACTTCGCCTACG AACTGGAGGAAAAGCCTGCAAGA TGCACTACTGCAAGCACTGGGGCG TGAGACTACCCAGCGGTGTCTGGTT CGAGATGGCCGATAAGGACCTGTTT GCAGCAGCGAGATTCCCGGAATGC CCTGAGGGATCGAGCATCTCCGCTC CAAGCCAACTTCAGTGGACGTGA GCCTGATCCAGGACGTGGAACGGA TTCTCGACTACTCGCTGTGCCAGGA GACCTGGTCGAAGATCAGAGCGGG ACTGCCCATCTCACCGGTGGACCTG TCCTACCTGGCGCCAAAGAATCCGG GCACTGGACCGGCGTTACCATCAT CAACGGCACCCCTCAAATACTTCGAG ACGCGGTACATCCGGGTGGACATC	KFTIVFPHNQKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPKS HKAIQADGWMCHASKWVTTCDFR WYGPKYITHSIRSFTPSVEQCKESIE QTKQGTWLNPGFPPQSCGYATVTD AEAVIVQVTPHHVLVDEYTG EWVD SQFINGKCSNYICPTVHNSTTWHS YKVKGLCDSNLISMDITFFSEDGEL SSLGKEGTGFRSNYFAYETGGKAC KMQYCKHWGVRLPSGVWFEMAD KDLFAAARFPECPEGSSISAPSQTSV DVS LIQDVERILDYSLCQETWSKIR AGLPISPVDLSYLAPKNPGTGPAFTI INGTLKYFETRYIRVDIAAPILSRMV GMISGTTWARELWDDWAPYEDVEI GPNGVLRTSSGYKFPLYMIGHGML DSDLHLSSKAQVFEHPHIQDAASQL PDDESLFFGDTGLSKNPIELVEGWF SSWKSSIASFFFIIGLIIGLFLVLRVGI HLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO: 95)

GCAGCTCCGATCCTCTCCCGGATGG TGGGAATGATCTCGGGGACTACTTG GGCCCGCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGAT CGGACCTAACGGAGTGCTCCGGAC CTCCTCCGGGTACAAGTTCCCTCTG TACATGATCGGCCATGGCATGCTGG ACTCGGATCTGCATCTGTCTGTCCTAA AGCACAGGTGTTTGAACACCCACA CATTCAAGACGCCGCCAGCCAGCT GCCGGACGATGAGTCGCTGTTCTTC GGAGACACGGGCTTGTCAAAGAAT CCCATCGAGCTGGTGGGAAGGATGG TTTTTCATCCTGGAAGCAGCATCG CTTCATTCTTCTTCATCATTGGCCTG ATCATCGGCCTATTTCTAGTCCTGC GGGTGGGAATTCATCTGTGCATCAA GCTCAAGCACACTAAGAAGCGGCA AATCTACACTGATATCGAGATGAAT CGCCTGGGCAAGTAG (SEQ ID NO: 94)	
Trop-055 Mutated VSV-G	
Nucleotide	Amino acid
AAGTTCACATATCGTGTTTCCGCACG CCGCCAAGGGCAACTGGAAAAACG TGCCTTCAAATTACCATTATTGCCC CAGCAGCTCGGACCTGAACTGGCA CAATGACCTCATTGGAACCGCGCTG CAGGTGAAGATGCCAAAGAGCCAC AAGGCTATCCAGGCTGACGGATGG ATGTGCCACGCGTCAAAATGGGTG ACTACCTGCGATTTCGCTGGTACG GACCAAAATACATCACGCACAGCA TCAGATCATTACCCCGTCAGTGGA ACAATGCAAAGAATCCATCGAACA GACTAAGCAGGGAACCTGGCTGAA CCCTGGATTTCCGCCGCAGTCGTGT GGGTACGCAACCGTGACCGATGCA GAGGCCGTGATCGTGCAAGTCACG CCGCATCACGTGCTTGTGGACGAGT ACACCGGAGAATGGGTCGATTCCC AGTTCATCAACGGCAAGTGCTCCAA CTACATTTGCCCAACCGTGCAACA AGCACTACTTGGCACAGCGACTAC AAAGTGAAGGGTCTGTGTGATTCCG CCCTGATCTCCATGGATATCACTTT	KFTIVFPHAAKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPKS HKAIQADGWMCHASKWVTTCDFR WYGPKYITHSIRSFTPSVEQCKESIE QTKQGTWLNPGFPQSCGYATVTD AEAVIVQVTPHHVLVDEYTGEWVD SQFINGKCSNYICPTVHNSTTWHS YKVKGLCDSALISMDITFFSEDGEL SSLGKEGTGFRSNYFAYETGGKAC KMQYCKHWGVRLPSGVWFEMAD KDLFAAARFPECPEGSSISAPSQTSV DVS LIQDVERILDYSLCQETWSKIR AGLPISPVDLSYLAPKNPGTGPAFTI INGTLKYFETRYIRVDIAAPILSRMV GMISGTTTERELWDDWAPYEDVEI GPNGVLR TSSGYKFPLYMIGHGML DSDLHLSSKAQVFEHPHIQDAASQL PDDES LFFGDTGLSKNPIELVEGWF SSWKSSIASFFFIIIGLIIGLFLVLRVGI HLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO: 97)

CTTCTCGGAAGACGGCGAACTGTCC TCACTGGGCAAAGAAGGAACTGGG TTTCGCTCAAATTACTTCGCCTACG AAACTGGAGGAAAAGCCTGCAAGA TGCAGTACTGCAAGCACTGGGGCG TGAGACTACCCAGCGGTGTCTGGTT CGAGATGGCCGATAAAGGACCTGTTT GCAGCAGCGAGATTCCCGGAATGC CCTGAGGGATCGAGCATCTCCGCTC CAAGCCAACTTCAGTGGACGTGA GCCTGATCCAGGACGTGGAACGGA TTCTCGACTACTCGCTGTGCCAGGA GACCTGGTCGAAGATCAGAGCGGG ACTGCCCATCTCACCGGTGGACCTG TCCTACCTGGCGCCAAAGAATCCGG GCACTGGACCGGCGTTCACCATCAT CAACGGCACCCCTCAAATACTTCGAG ACGCGGTACATCCGGGTGGACATC GCAGCTCCGATCCTCTCCCGGATGG TGGGAATGATCTCGGGGACTACTAC CGAACGCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGAT CGGACCTAACGGAGTGCTCCGGAC CTCCTCCGGGTACAAGTTCCCTCTG TACATGATCGGCCATGGCATGCTGG ACTCGGATCTGCATCTGTCTGTCCTAA AGCACAGGTGTTTGAACACCCACA CATTCAAGACGCCGCCAGCCAGCT GCCGGACGATGAGTCGCTGTTCTTC GGAGACACGGGCTTGTCAAAGAAT CCCATCGAGCTGGTGGAAAGGATGG TTTTTCATCCTGGAAAAGCAGCATCG CTTCATTCTTCTTCATCATTGGCCTG ATCATCGGCCTATTTCTAGTCCTGC GGGTGGGAATTCATCTGTGCATCAA GCTCAAGCACACTAAGAAGCGGCA AATCTACACTGATATCGAGATGAAT CGCCTGGGCAAGTAG (SEQ ID NO: 96)	
Trop-056 Mutated VSV-G	
Nucleotide	Amino acid
AAGTTCACTATCGTGTTTCCGCACA ACACCACACAAAAGGGCAACTGGA AAAACGTGCCTTCAAATTACCATTA TTGCCCCAGCAGCTCGGACCTGAAC TGGCACAATGACCTCATTGGAACCG CGCTGCAGGTGAAGATGCCAAAGA	KFTIVFPHTTQKGNWKNVPSNYH YCPSSDLNWHNDLIGTALQVKMP KSHKAIQADGWMCHASKWVTTC FRWYGPKYITHSIRSFTPSVEQCKES IEQTKQGTWLNPGFPPQSCGYATVT DAEAVIVQVTPHHVLVDEYTG EWV

GCCACAAGGCTATCCAGGCTGACG GATGGATGTGCCACGCGTCAAAAT GGGTGACTACCTGCGATTTCCGCTG GTACGGACCAAAATACATCACGCA CAGCATCAGATCATTCACCCCGTCA GTGGAACAATGCAAAGAATCCATC GAACAGACTAAGCAGGGAACCTGG CTGAACCCTGGATTTCCGCCGCGAGT CGTGTGGGTACGCAACCGTGACCG ATGCAGAGGCCGTGATCGTGCAAG TCACGCCGCATCACGTGCTTGTGGA CGAGTACACCGGAGAATGGGTGCA TTCCCAGTTCATCAACGGCAAGTGC TCCAACACTATTTGCCCAACCGTGC ACAACAGCACTACTTGGCACAGCG ACTACAAAGTGAAGGGTCTGTGTG ATTCCAACCTGATCTCCATGGATAT CACTTTCTTCTCGGAAGACGGCGAA CTGTCTCACTGGGCAAAGAAGGA ACTGGGTTTCGCTCAAATTAATTCG CCTACGAAACTGGAGGAAAAGCCT GCAAGATGCAGTACTGCAAGCACT GGGGCGTGAGACTACCCAGCGGTG TCTGGTTCGAGATGGCCGATAAGG ACCTGTTTGCAGCAGCGAGATTCCC GGAATGCCCTGAGGGATCGAGCAT CTCCGCTCCAAGCCAACTTCAGTG GACGTGAGCCTGATCCAGGACGTG GAACGGATTCTCGACTACTCGCTGT GCCAGGAGACCTGGTCTGAAGATCA GAGCGGGACTGCCCATCTCACCGGT GGACCTGTCCTACCTGGCGCCAAAG AATCCGGGCACTGGACCGGCGTTC ACCATCATCAACGGCACCCCTCAAAT ACTTCGAGACGCGGTACATCCGGGT GGACATCGCAGCTCCGATCCTCTCC CGGATGGTGGGAATGATCTCGGGG ACTACTACCGAACGCGAGCTCTGG GACGACTGGGCACCTTACGAGGAT GTCGAGATCGGACCTAACGGAGTG CTCCGGACCTCCTCCGGGTACAAGT TCCCTCTGTACATGATCGGCCATGG CATGCTGGACTCGGATCTGCATCTG TCGTCCAAAGCACAGGTGTTTGAAC ACCCACACATTCAAGACGCCGCCA GCCAGCTGCCGGACGATGAGTCGC TGTTCTTCGGAGACACGGGCTTGTC AAAGAATCCCATCGAGCTGGTGGA AGGATGGTTTTCATCCTGGAAAAGC	DSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCDSNLISMDITFFSEGE LSSLGKEGTGFRSNYFAYETGGKA CKMQYCKHWGVRLPSGVWFEMA DKDLFAAARFPECPEGSSISAPSQTS VDVSLIQDVERILDYSLCQETWSKI RAGLPISPVDLSYLAPKNPGTGPAF TIINGTLKYFETRYIRVDIAAPILSR MVGMIISGTTTERELWDDWAPYED VEIGPNGVLRTSSGYKFPLYMIGHG MLDSDLHLSSKAQVFEHPHIQDAA SQLPDDESLFFGDTGLSKNPIELVEG WFSSWKSSIASFFFIIGLIIGLFLVLR VGIHLCKLKHHTKKRQIYTDIEMNR LGK (SEQ ID NO: 99)
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AGCATCGCTTCATTCTTCTTCATCAT TGGCCTGATCATCGGCCTATTTCTA GTCCTGCGGGTGGGAATTCATCTGT GCATCAAGCTCAAGCACACTAAGA AGCGGCAAATCTACACTGATATCG AGATGAATCGCCTGGGCAAGTAG (SEQ ID NO: 98)	
Trop-058 Mutated VSV-G	
Nucleotide	Amino acid
AAGTTCACTATCGTGTTTCCGCACA ACCAAAAGGGCAACTGGAAAAACG TGCCTTCAAATTACCATTATTGCCC CAGCAGCTCGGACCTGAACTGGCA CAATGACCTCATTGGAACCGCGCTG CAGGTGAAGATGCCAGGCGGCAGC AAGAGCCACAAGGCTATCCAGGCT GACGGATGGATGTGCCACGCGTCA AAATGGGTGACTACCTGCGATTTCC GCTGGTACGGACCAAAATACATCA CGCACAGCATCAGATCATTACCCCC GTCAGTGGAACAATGCAAAGAATC CATCGAACAGACTAAGCAGGGAAC CTGGCTGAACCCTGGATTTCGCCCG CAGTCGTGTGGGTACGCAACCGTG ACCGATGCAGAGGCCGTGATCGTG CAAGTCACGCCGCATCACGTGCTTG TGGACGAGTACACCGGAGAATGGG TCGATTCCCAGTTCATCAACGGCAA GTGCTCCAACACTATTTGCCCAACC GTGCACAACAGCACTACTTGGCAC AGCGACTACAAAGTGAAGGGTCTG TGTGATTCCAACCTGATCTCCATGG ATATCACTTTCTTCTCGGAAGACGG CGAACTGTCCTCACTGGGCAAAGA AGGAACTGGGTTTCGCTCAAATTAC TTCGCCTACGAAACTGGAGGAAAA GCCTGCAAGATGCAGTACTGCAAG CACTGGGGCGTGAGACTACCCAGC GGTGTCTGGTTCGAGATGGCCGATA AGGACCTGTTTGCAGCAGCGAGATT CCCGGAATGCCCTGAGGGATCGAG CATCTCCGCTCCAAGCCAAACTTCA GTGGACGTGAGCCTGATCCAGGAC GTGGAACGGATTCTCGACTACTCGC TGTGCCAGGAGACCTGGTCGAAGA TCAGAGCGGGACTGCCCATCTCACC GGTGGACCTGTCCTACCTGGCGCCA	KFTIVFPHNQKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPGG SKSHKAIQADGWMCHASKWV TTC DFRWYGP KYITHSIRSFTPSVEQCK ESIEQTKQGTWLNPGFPPQSCGYAT VTDAEAVIVQVTPHHVLVDEYTGE WVDSQFINGKCSNYICPTVHNSTT WHS DYKVKGLCDSNLISMDITFFSE DGELSSLGKEGTGFRSNYFAYETG GKACKMQYCKHWGVRLPSGVWFE MADKDLFAAARFPECPEGSSISAPS QTSVDVSLIQDVERILDYSLCQETW SKIRAGLPISPVDLSYLA PKNPGTGP AFTIINGTLKYFETRYIRVDIAAPILS RMVGMISGTTTERELWDDWAPYE DVEIGPNGVLRTSSGYKFPLYMIGH GMLDSDLHLSSKAQVFEHPHIQDA ASQLPDDES LFFGDTGLSKNP IELVE GWFSSWKSSIASFFFIIIGLIIGLFLVL RVGIHLCKIKLKHTKKRQIYTDIEMN RLGK (SEQ ID NO: 101)

AAGAATCCGGGCACTGGACCGGCG TTCACCATCATCAACGGCACCTCA AATACTTCGAGACGCGGTACATCCG GGTGGACATCGCAGCTCCGATCCTC TCCCGGATGGTGGGAATGATCTCGG GGACTACTACCGAACGCGAGCTCT GGGACGACTGGGCACCTTACGAGG ATGTCGAGATCGGACCTAACGGAG TGCTCCGGACCTCCTCCGGGTACAA GTTCCCTCTGTACATGATCGGCCAT GGCATGCTGGACTCGGATCTGCATC TGTCGTCCAAAGCACAGGTGTTTGA ACACCCACACATTCAAGACGCCGC CAGCCAGCTGCCGGACGATGAGTC GCTGTTCTTCGGAGACACGGGCTTG TCAAAGAATCCCATCGAGCTGGTG GAAGGATGGTTTTTCATCCTGGAAAA GCAGCATCGCTTCATTCTTCTTCAT CATTGGCCTGATCATCGGCCTATTT CTAGTCCTGCGGGTGGGAATTCATC TGTGCATCAAGCTCAAGCACACTAA GAAGCGGCAAATCTACACTGATAT CGAGATGAATCGCCTGGGCAAGTA G (SEQ ID NO: 100)	
Trop-061 Mutated VSV-G	
Nucleotide	Amino acid
AAGTTCACATATCGTGTTTCCGCAC GGCGGAAGCAACGGCGGGAGCCA AAAGGGCAACTGGAAAAACGTGC CTTCAAATTACCATTATTGCCCA GCAGCTCGGACCTGAACTGGCAC AATGACCTCATTGGAACCGCGCT GCAGGTGAAGATGCCAAAGAGCC ACAAGGCTATCCAGGCTGACGGA TGGATGTGCCACGCGTCAAAATG GGTGACTACCTGCGATTTCCGCTG GTACGGACCAAAATACATCACGC ACAGCATCAGATCATTACCCCGT CAGTGGAACAATGCAAAGAATCC ATCGAACAGACTAAGCAGGGAAC CTGGCTGAACCCTGGATTTCCGCC GCAGTCGTGTGGGTACGCAACCG TGACCGATGCAGAGGCCGTGATC GTGCAAGTCACGCCGCATCACGT GCTTGTGGACGAGTACACCGGAG AATGGGTCGATTCCCAGTTCATCA ACGGCAAGTGCTCCAACACTACATT GCCCAACCGTGCACAACAGCACT	KFTIVFPHGGSNGGSQKGNWKNVPS NYHYCPSSSDLNWHNDLIGTALQVK MPKSHKAIQADGWMCHASKWVTTC DFRWYGPKYITHSIRSFTPSVEQCKES IEQTKQGTWLNPGFPPQSCGYATVTD AEAVIVQVTPHHVLVDEYTGEWVDS QFINGKCSNYICPTVHNSTTWHSDYK VKGLCDSNLISMDITFFSEDGELSSLG KEGTGFRSNYFAYETGGKACKMQYC KHWGVRLPSGVWFEMADKDLFAAA RFPECPEGSSISAPSQTSVDVSLIQDVE RILDYSLCQETWSKIRAGLPISPVDLS YLAPKNPGTGPAFTIINGTLKYFETRY IRVDIAAPILSRMVGMISGTTTERELW DDWAPYEDVEIGPNGVLRRTSSGYKFP LYMIGHGMLDSDLHLSSKAQVFEHP HIQDAASQLPDDESFFGDTGLSKNPI ELVEGWFSWKSSIASFFFFIIGLIIGLFL VLRVGIHLCKLKHKKRQIYTDIEM NRLGK (SEQ ID NO: 103)

ACTTGGCACAGCGACTACAAAGT GAAGGGTCTGTGTGATTCCAACCT GATCTCCATGGATATCACTTTCTT CTCGGAAGACGGCGAACTGTCCT CACTGGGCAAAGAAGGAACTGGG TTTCGCTCAAATTACTTCGCCTAC GAAACTGGAGGAAAAGCCTGCAA GATGCAGTACTGCAAGCACTGGG GCGTGAGACTACCCAGCGGTGTC TGGTTCGAGATGGCCGATAAGGA CCTGTTTGCAGCAGCGAGATTCCC GGAATGCCCTGAGGGATCGAGCA TCTCCGCTCCAAGCCAACTTCAG TGGACGTGAGCCTGATCCAGGAC GTGGAACGGATTCTCGACTACTCG CTGTGCCAGGAGACCTGGTCGAA GATCAGAGCGGGACTGCCCATCT CACCGGTGGACCTGTCCTACCTGG CGCCAAAGAATCCGGGCACTGGA CCGGCGTTCACCATCATCAACGGC ACCCTCAAATACTTCGAGACGCG GTACATCCGGGTGGACATCGCAG CTCCGATCCTCTCCCGGATGGTGG GAATGATCTCGGGGACTACTACC GAACGCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGA TCGGACCTAACGGAGTGCTCCGG ACCTCCTCCGGGTACAAGTTCCCT CTGTACATGATCGGCCATGGCATG CTGGACTCGGATCTGCATCTGTCTG TCCAAAGCACAGGTGTTTGAACA CCCACACATTCAAGACGCCGCCA GCCAGCTGCCGGACGATGAGTCG CTGTTCTTCGGAGACACGGGCTTG TCAAAGAATCCCATCGAGCTGGT GGAAGGATGGTTTTTCATCCTGGA AAAGCAGCATCGCTTCATTCTTCT TCATCATTGGCCTGATCATCGGCC TATTTCTAGTCCTGCGGGTGGGAA TTCATCTGTGCATCAAGCTCAAGC AACTAAGAAGCGGCAAATCTAC ACTGATATCGAGATGAATCGCCT GGGCAAGTAG (SEQ ID NO: 102)	
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In other embodiments, the methods for producing LVV according to the present disclosure may utilize a five plasmid system. Where a five plasmid system is used, the LVV production system utilizes four plasmids from a third generation vector system

(i.e., a transgene plasmid, a GagPol plasmid, an Env plasmid, and a VSV-G env plasmid). In addition, a five plasmid system as described herein includes a fifth plasmid that encodes a targeting protein (the “targeting protein plasmid”). The GagPol and Env packaging plasmids included in a five plasmid system may be standard
 5 packaging plasmids as described in *Production of Lentiviral Vectors*, Merten et al., *Molecular Therapy – Methods & Clinical Development* (2016), 3, 16017. The transgene plasmid includes an expression cassette that encodes one or more CAR transgenes according to the present disclosure, and the VSV-G env plasmid includes an expression cassette that encodes a mutant VSV-G as described herein.

10 In some embodiment, the fifth plasmid comprises an expression cassette encoding a CD80 targeting protein. In some embodiments, the fifth plasmid comprises an expression cassette encoding an anti-CD3 targeting protein. In some embodiments, the fifth plasmid comprises an expression cassette encoding a CD80 targeting protein and an anti-CD3 targeting protein. Examples of CD80 targeting protein and anti-CD3
 15 targeting proteins that may be used in the fifth plasmid are provided in Tables 1-3.

In certain embodiments, the present disclosure provides a method for producing a lymphocyte targeted lentiviral vector according to any one of the preceding claims, the method comprising: transfecting a producer cell with a GagPol plasmid, a Rev plasmid, a transgene plasmid, a VSV-G env plasmid, and a lymphocyte targeting
 20 protein plasmid; 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 protein and a lentiviral pol protein within the producer cell; the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral rev protein within the producer cell; the transgene plasmid comprises an
 25 expression cassette comprising a polynucleotide encoding a CAR; and the VSV-G env plasmid comprises a polynucleotide encoding a mutated VSV-G envelope protein as described herein and is capable of expressing the mutated VSV-G envelope protein within the producer cell; and the lymphocyte targeting protein plasmid comprises a polynucleotide encoding a lymphocyte targeting protein as described herein and is
 30 capable of expressing the lymphocyte targeting protein within the producer cell; culturing the producer cell in a culture medium; and harvesting lentiviral vector from the culture medium.

In some embodiments, the producer cells are transfected with a defined ratio of the transgene plasmid, GagPol plasmid, VSV-G env plasmid, Rev plasmid, and lymphocyte targeting plasmid. The lymphocyte targeting plasmid may be at a ratio of about 0.25 to about 5 relative to the VSV-G env plasmid (by mass). For example, using the defined ratios for the four packaging plasmid system (transgene plasmid, GagPol plasmid, VSV-G env plasmid, Rev plasmid) described herein, the ratio of the lymphocyte targeting protein plasmid can be adjusted relative to the VSV-G env plasmid. For example, the lymphocyte targeting plasmid may be at a ratio of about 0.25:1, 0.5:1, 1:1, 1.25:1, 1.5:1, 1.75:1, 2:1, 2.25:1, 2.5:1, 2.75:1, 3:1, 3.25:1, 3.5:1, 3.75:1, 4:1, 4.25:1, 4.5:1, 4.75:1 or 5:1 relative to the VSV-G env plasmid.

In embodiments of LVV production methods that utilize a combination of five plasmids, the fifth plasmid is a targeting protein plasmid and includes an expression cassette that encodes one or more targeting proteins. For example the targeting protein plasmid may include an expression cassette that includes a polynucleotide that encodes a CD80 targeting protein, an anti-CD3 targeting protein, or both a CD80 targeting protein and an anti-CD3 targeting protein. In certain embodiments, a targeting protein vector plasmid an expression cassette comprising a polynucleotide according to SEQ ID NO: 1, which encodes a CD80 targeting protein according to SEQ ID NO: 2. In other embodiments, a targeting protein plasmid includes an expression cassette comprising a polynucleotide according to SEQ ID NO: 9, which encodes an anti-CD3 targeting protein according to SEQ ID NO: 10. In other embodiments, a targeting protein plasmid includes an expression cassette comprising a polynucleotide according to SEQ ID NO: 116, which encodes an anti-CD3 targeting protein according to SEQ ID NO: 117. In other embodiments, a targeting protein plasmid includes a tandem expression cassette that comprises a polynucleotide according to SEQ ID NO: 21, which encodes a polypeptide according to SEQ ID NO: 22. Examples of mutated VSV-G sequences that may be used in the five plasmid, 3rd generation lentiviral vector system of the present disclosure are provided in Table 10.

In the four plasmid packaging system, the concentration of the lymphocyte targeting protein on the surface of the transduced cells may be associated with the

concentration of the plasmid containing the targeting protein. For example if the lymphocyte targeting protein plasmid is contained in the VSV-G env plasmid, the concentration of the lymphocyte targeting protein on the surface of the transduced cells may be associated with the concentration of the VSV-G env plasmid. In another example, if the lymphocyte targeting protein plasmid is contained in the
5 GagPol plasmid, the concentration of the lymphocyte targeting protein on the surface of the transduced cells may be associated with the concentration of the GagPol plasmid. In another example, if the lymphocyte targeting protein plasmid is contained in the Rev plasmid, the concentration of the lymphocyte targeting protein on the surface of
10 the transduced cells may be associated with the concentration of the Rev plasmid. In the five plasmid packaging system, the concentration of the lymphocyte targeting protein on the surface of the transduced cells may be associated with the concentration of the plasmid containing the targeting protein.

In the four plasmid packaging system, the transduction efficiency may be
15 associated with the concentration of the plasmid containing the targeting protein. For example if the lymphocyte targeting protein plasmid is contained in the VSV-G env plasmid, the transduction efficiency may be associated with the concentration of the VSV-G env plasmid. In another example, if the lymphocyte targeting protein plasmid is contained in the GagPol plasmid, the transduction efficiency may be
20 associated with the concentration of the GagPol plasmid. In another example, if the lymphocyte targeting protein plasmid is contained in the Rev plasmid, the transduction efficiency may be associated with the concentration of the Rev plasmid. In the five plasmid packaging system, the transduction efficiency of may be associated with the concentration of the lymphocyte targeting protein plasmid.

25 Following transfection of producer cells, the lentiviral particles product may be harvested from the cell supernatant or culture media. Downstream processes of LVV to maximize LVV recovery while minimizing components which may negatively impact efficacy or safety are known in the art. A typical process, involves sequential purification steps comprising removing cells and their debris followed by enrichment of
30 LVV and the removal of host cell or serum proteins, nucleic acids and lipids. The LVV product may be further concentrated prior to exchanging into a suitable formulation buffer for stability and then finally undergoing sterile filtration prior to storage or

application. A clarification step may be initially performed on the harvested LVV supernatants to remove large impurities such as aggregates and cell debris. In some embodiments, the clarification step comprises centrifugation and/or conventional flow filtration. In some embodiments, the clarification step includes a nuclease digestion step. In some embodiments, the LVV product undergoes further purification steps, including for example, ion exchange chromatography (e.g., anion exchange chromatography). The LVV may also be concentrated, e.g., by tangential flow filtration or ultrafiltration/diafiltration. In some embodiments, harvesting LVV from culture medium comprises centrifugation. In some embodiments, harvesting LVV from culture medium comprises anion exchange chromatography. In some embodiments, harvesting LVV from culture medium comprises anion exchange chromatography and tangential flow filtration.

In some embodiments, the lentiviral vectors described herein, which incorporate a mutated VSV-G env and one or more lymphocyte targeting proteins, are capable of producing a high titer LVV product, as compared to standard LVV incorporating another fusogenic env protein (e.g., cocl env, paramyxovirus env, truncated VSV-G env). As used herein viral titer refers to infectious virus particle titer as measured by transducing units (TU) per mL. In some embodiments, the titer of LVV described herein is at least $1e7$ TU/mL, at least $1e8$ TU/mL, at least $1e9$ TU/mL, at least $1e10$ TU/mL, at least $1e11$ TU/mL, or at least $1e12$ TU/mL as measured in concentrated LVV product. In some embodiments, the titer of LVV described herein is about $1e7$ TU/mL to about $1e12$ TU/mL in concentrated LVV product.

Engineered Lymphocytes

The LVV described herein can be used to modify targeted lymphocytes (e.g., T cells, B cells, or NK cells) to express a CAR encoded by a transgene carried by the LVV. In certain embodiments, the engineered lymphocytes have been transduced by an LVV according to the present disclosure by contacting the lymphocytes with the LVV of the present disclosure. In such embodiments, the engineered lymphocytes (e.g., T cells, B cells, or NK cells) express a CAR encoded by the transgene carried by the LVV. These lymphocytes are also referred to herein as “CAR modified lymphocytes.” In specific embodiments, the engineered T cells are referred to herein as “CAR

modified T cells.” In other specific embodiments, the engineered NK cells are referred to herein as “CAR modified NK cells.” In specific embodiments, the engineered T cells are referred to herein as “CAR modified B cells.” In specific embodiments the CAR modified T cell expresses a CAR encoded by a transgene carried by an LVV described
5 herein and is selected from naïve T cells (CD45RA⁺, CCR7⁺, CD62L⁺, CD27⁺, CD45RO⁻), central memory T cells (CD45RA⁻, CD45RO⁺, CD62L⁺, CCR7⁺, CD27⁺), effector memory T cells (CD45RA⁻, CD45RO⁺, CCR7⁻, CD62L⁻, CD27⁻), $\gamma\delta$ T cells, mucosal-associated invariant T (MAIT) cells, Tregs, natural killer T cells, and tissue resident T cells.

10 Transduction of targeted lymphocytes (e.g., T cells, B cells, or NK cells) by LVV described herein may be performed *ex vivo* or *in vivo*.

In certain embodiments, the lymphocytes (e.g., T cells, B cells, or NK cells) can be primary cells or cell lines derived from human, mouse, rat, or other mammals. If obtained from a mammal, a lymphocyte can be obtained from numerous sources,
15 including blood, bone marrow, lymph node, thymus, or other tissues or fluids. A lymphocyte composition (e.g., T cell composition, B cell composition, or NK cell composition) may be enriched or purified. T cell lines are well known in the art, some of which are described in Sandberg *et al.*, *Leukemia* 21:230, 2000. In certain embodiments, the T cells lack endogenous expression of a TCR α gene, TCR β gene, or
20 both. Such T cells may naturally lack endogenous expression of TCR α and β chains, or may have been modified to block expression (e.g., T cells from a transgenic mouse that does not express TCR α and β chains or cells that have been manipulated to inhibit expression of TCR α and β chains) or to knockout a TCR α chain, a TCR β chain, or both genes.

25 In some embodiments, prior to genetic modification of the lymphocytes (e.g., T cells, B cells, or NK cells) with a polynucleotide encoding a CAR molecule, a source of lymphocytes (e.g., T cells or NK cells) may be obtained from a subject (e.g., whole blood, peripheral blood mononuclear cells (PBMCs), bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion,
30 spleen tissue). The lymphocytes, T cells, B cells, or NK cells can be enriched or isolated from a sample taken from the subject using methods known in the art. For example, T cells can be enriched in a whole blood sample taken from a subject using

known methods for hypotonic lysis of red blood cells or T cells can be isolated from a sample taken from a subject using known gradient sedimentation, e.g., Ficoll® reduction) techniques. Alternatively, T cells can be isolated from whole blood using magnetic antibody-labeled beads followed by column separation. Specific host cell
5 subsets can be collected in accordance with known techniques and enriched or depleted by known techniques, such as affinity binding to antibodies, flow cytometry and/or immunomagnetic selection. In some embodiments, after enrichment and/or depletion steps, the T cells are placed in contact with LVV as disclosed such that T cells obtained from the subject are transduced by the LVV and express the CAR encoded by the
10 transgene carried by the LVV. In some embodiments, after enrichment and/or depletion steps, the NK cells are placed in contact with LVV as disclosed such that NK cells obtained from the subject are transduced by the LVV and express the CAR encoded by the transgene carried by the LVV. In some embodiments, after enrichment and/or depletion steps, the B cells are placed in contact with LVV as disclosed such that B
15 cells obtained from the subject are transduced by the LVV and express the CAR encoded by the transgene carried by the LVV.

Generally, resting T cells, such as resting CD4 and CD8 lymphocytes, resting B cells, and resting NK cells are refractory to genetic transduction by lentiviral vectors. Resting T cells, also known as quiescent T cells or naïve T cells, refer to T cells that are
20 not mitotically active or have not been exposed to a cognate antigen presented on an antigen presenting cell, such as a macrophage or dendritic cell. An example of a marker for resting T cells is CD28. Alternatively, markers that are expressed on activated T cells but not resting T cells include, for example, 4-1BB, PD-1, and HLA-DR. Similarly, resting B cells and resting NK cells, refer to B cells and NK cells that
25 are not mitotically active or have not been exposed to a cognate antigen, respectively. Examples of markers for resting B cells include CD21 and CD23, and the absence of CD80, CD86, CD95, or CD25. CD137 and GITR, which are expressed on activated NK cells, are absent on resting NK cells. As a result, in order to facilitate transduction of T cells, B cells, or NK cells using a lentiviral vector, the T cells, B cells, or NK cells are
30 typically activated in vitro using stimulation reagents before genetic modification via a lentiviral vector can occur. Following stimulation and transduction, the genetically modified cells are then typically expanded in vitro and subsequently reintroduced into a

patient. However, LVV described herein are capable of transducing resting T cells, B cells, and/or resting NK cells. In certain embodiments, methods of transducing T cells with an LVV according to the present description include, placing the LVV in contact with a population of T cells, wherein in the T cells are not activated and the T cells are not exposed to an exogenous stimulating agent during the transduction process. In some such embodiments, the methods of transducing T cells wherein the T cells are not activated and the T cells are not exposed to an exogenous stimulating agent during the transduction process comprises transducing T cells with a LVV comprising a CD80 targeting protein, an anti-CD3 targeting protein, or both a CD80 targeting protein and an anti-CD3 targeting protein. Examples of exogenous T cell stimulating agents include an anti-CD3 antibody or antigen binding fragment thereof (i.e., anti-CD3 antibody or antigen binding fragment thereof that is not part of the LVV), an anti-CD28 antibody or antigen binding fragment thereof (i.e., anti-CD28 antibody or antigen binding fragment thereof that is not part of the LVV), anti-CD2 antibody or antigen binding fragment thereof, IL-2, IL-7, IL-15, PHA, or any combination thereof. Exogenous stimulating agents may be contacted to the T cell in soluble form or immobilized on a solid substrate, such as a bead or cell culture plate. In some embodiments, an anti-CD3 antibody or antigen binding fragment thereof, an anti-CD28 antibody or antigen binding fragment thereof, or both the anti-CD3 antibody and anti-CD28 antibody or antigen binding fragments thereof are contacted to the T cells in soluble form or immobilized on a solid substrate. Examples of anti-CD3 antibodies include OKT3, UCTH1, and BW264/56. An example of an anti-CD2 antibody is LT2. An example of an anti-CD28 antibody is 15E8. In certain embodiments, methods of transducing NK cells with an LVV according to the present description include, placing the LVV in contact with a population of NK cells, wherein in the NK cells are not activated and the NK cells are not exposed to an exogenous stimulating agent during the transduction process. Examples of exogenous NK cell stimulating agents include anti-CD2 antibody or antigen binding fragment thereof, anti-CD335 antibody or antigen binding fragment thereof, IL-2, IL-15, IL-12, IL-18, IL-21, or any combination thereof. In certain embodiments, methods of transducing B cells with an LVV according to the present description include, placing the LVV in contact with a population of B cells, wherein in the B cells are not activated and the B cells are not exposed to an exogenous

stimulating agent during the transduction process. Examples of exogenous B cell stimulating agents include CD154 and mixed Ig F(ab)₂.

In some embodiments, a lentiviral vector having a CD80 targeting protein and an anti-CD3 targeting protein is capable of efficiently transducing both CD4 and CD8 T cells
5 as compared to standard LVV.

In some embodiments, a lentiviral vector having a CD80 targeting protein and an anti-CD3 targeting protein is capable of activating T cells without exogenous stimulation (e.g., T cell stimulating agent) to a comparable level as T cells transduced with standard LVV that are treated with exogenous stimulation. Examples of exogenous T cell
10 stimulating agents include an anti-CD3 antibody or antigen binding fragment thereof (i.e., anti-CD3 antibody or antigen binding fragment thereof that is not part of the LVV), an anti-CD28 antibody or antigen binding fragment thereof (i.e., anti-CD28 antibody or antigen binding fragment thereof that is not part of the LVV), anti-CD2
15 antibody or antigen binding fragment thereof, IL-2, IL-7, IL-15, PHA, or any combination thereof..

In certain embodiments, methods of transducing T cells with an LVV according to the present description exhibit enhanced transduction of CD4 T cells compared to standard LVV. In some such embodiments, the methods of enhancing transduction of CD4 T cells in a mixed population of T cells (e.g., composed of CD4 and CD8 T cells)
20 comprises transducing the mixed population of T cells with a LVV according to the present description comprising a CD80 targeting protein.

In certain embodiments, methods of transducing T cells with an LVV according to the present description efficiently transduce both CD4 and CD8 T cells. In some such embodiments, the methods of transducing T cells wherein both CD4 and CD8 T
25 cells are efficiently transduced comprises transducing T cells with a LVV comprising a CD80 targeting protein and an anti-CD3 targeting protein.

In certain embodiments, an LVV according to the present description is capable of transducing T cells under “stressed” conditions, e.g., at low multiplicity of infection (MOI) or without treatment with exogenous IL-2 during transduction. In some
30 embodiments, the LVV comprises a CD80 targeting protein and an anti-CD3 targeting protein. In some embodiments, the LVV according to the present description is capable

of transducing T cells at a LVV concentration about 5 to about 25X lower than a non-lymphocyte targeting LVV.

The expression of a CAR molecule on host cells may be assessed by methods known in the art, such as quantitative PCR or flow cytometry following staining with a
5 fluorescently labeled antigen for the CAR binding domain.

The expression of a CAR molecule on host cells may be functionally characterized according to any of a large number of art-accepted methodologies for assaying host T cell activity, including determination of T cell binding, activation or induction and also including determination of T cell responses that are antigen-specific.
10 Examples include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, CTL activity (*e.g.*, by detecting ^{51}Cr or Europium release from pre-loaded target cells, induction of caspase activity in target cells, extracellular release of lactate dehydrogenase by target cells), changes in T cell phenotypic marker expression, and other measures of T cell functions. Assaying host NK cell activity may
15 also be assayed using similar methodologies. Procedures for performing these and similar assays are may be found, for example, in Lefkovits (*Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*, 1998). *See, also, Current Protocols in Immunology*; Weir, *Handbook of Experimental Immunology*, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) *Selected Methods in Cellular*
20 *Immunology*, Freeman Publishing, San Francisco, CA (1979); Green and Reed, *Science* 281:1309 (1998) and references cited therein. Cytokine levels may be determined according to methods known in the art, including for example, ELISA, ELISPOT, intracellular cytokine staining, flow cytometry, and any combination thereof (*e.g.*, intracellular cytokine staining and flow cytometry). Immune cell proliferation and
25 clonal expansion resulting from an antigen-specific elicitation or stimulation of an immune response may be determined by isolating lymphocytes, such as circulating lymphocytes in samples of peripheral blood cells or cells from lymph nodes, stimulating the cells with antigen, and measuring cytokine production, cell proliferation and/or cell viability, such as by incorporation of tritiated thymidine or non-radioactive
30 assays, such as MTT assays and the like.

Methods of & Compositions for Treatment

In one aspect, the present disclosure provides methods of treating a disease in a subject comprising administering to the subject an effective amount of an LVV as described herein, a CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) as described herein, or pharmaceutical compositions thereof. In another aspect, the methods of treating a disease in a subject according to the present description include administering to the subject an effective amount of an LVV as described herein, a CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) as described herein, or pharmaceutical compositions thereof, in combination with one or more additional therapeutic agents.

Diseases that may be treated with the LVV or CAR modified lymphocytes (e.g., T cells, B cells, or NK cells) according to the present description include cancers. Adoptive immune and gene therapies are promising treatments for various types of cancer (Morgan *et al.*, *Science* 314:126, 2006; Schmitt *et al.*, *Hum. Gene Ther.* 20:1240, 2009; June, *J. Clin. Invest.* 117:1466, 2007) and infectious disease (Kitchen *et al.*, *PLoS One* 4:38208, 2009; Rossi *et al.*, *Nat. Biotechnol.* 25:1444, 2007; Zhang *et al.*, *PLoS Pathog.* 6:e1001018, 2010; Luo *et al.*, *J. Mol. Med.* 89:903, 2011).

A wide variety of cancers, including solid tumors and leukemias are amenable to the compositions and methods disclosed herein. Exemplary cancers that may be treated using the receptors, modified host cells, and composition described herein include adenocarcinoma of the breast, prostate, and colon; all forms of bronchogenic carcinoma of the lung; myeloid leukemia; melanoma; hepatoma; neuroblastoma; papilloma; apudoma; choristoma; branchioma; malignant carcinoid syndrome; carcinoid heart disease; and carcinoma (e.g., Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell). Additional types of cancers that may be treated using the receptors, modified host cells, and composition described herein include histiocytic disorders; malignant histiocytosis; leukemia; Hodgkin's disease; immunoproliferative small; non-Hodgkin's lymphoma; plasmacytoma; multiple myeloma; chronic myeloid leukemia (CML); acute myeloid leukemia (AML); plasmacytoma; reticuloendotheliosis; melanoma; chondroblastoma; chondroma; chondrosarcoma; fibroma; fibrosarcoma; giant cell

tumors; histiocytoma; lipoma; liposarcoma; mesothelioma; myxoma; myxosarcoma; osteoma; osteosarcoma; chordoma; craniopharyngioma; dysgerminoma; hamartoma; mesenchymoma; mesonephroma; myosarcoma; ameloblastoma; cementoma; odontoma; teratoma; thymoma; trophoblastic tumor. Further, the following types of cancers are

5 also contemplated as amenable to treatment using the receptors, modified host cells, and composition described herein: adenoma; cholangioma; cholesteatoma; cyclindroma; cystadenocarcinoma; cystadenoma; granulosa cell tumor; gynandroblastoma; hepatoma; hidradenoma; islet cell tumor; Leydig cell tumor; papilloma; sertoli cell tumor; theca cell tumor; leiomyoma; leiomyosarcoma; myoblastoma; myomma; myosarcoma;

10 rhabdomyoma; rhabdomyosarcoma; ependymoma; ganglioneuroma; glioma; medulloblastoma; meningioma; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neuroma; paraganglioma; paraganglioma nonchromaffin. The types of cancers that may be treated also include angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angioma sclerosing; angiomatosis; glomangioma;

15 hemangioendothelioma; hemangioma; hemangiopericytoma; hemangiosarcoma; lymphangioma; lymphangiomyoma; lymphangiosarcoma; pinealoma; carcinosarcoma; chondrosarcoma; cystosarcoma phyllodes; fibrosarcoma; hemangiosarcoma; leiomyosarcoma; leukosarcoma; liposarcoma; lymphangiosarcoma; myosarcoma; myxosarcoma; ovarian carcinoma; rhabdomyosarcoma; sarcoma; neoplasms;

20 neurofibromatosis; cervical dysplasia, and peritoneal cancer.

Examples of hyperproliferative disorders amenable to therapy using the receptors, modified host cells, and composition described herein include B-cell cancers (B-cell malignancies), including B-cell lymphomas (such as various forms of Hodgkin's disease, non-Hodgkin's lymphoma (NHL) or central nervous system lymphomas),

25 leukemias (such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia, B cell blast transformation of chronic myeloid leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia, and myelomas (such as multiple myeloma). Additional B cell cancers that may be treated using the receptors, modified host cells, and composition described herein include small lymphocytic

30 lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-

associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, Burkitt's lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

The CAR modified lymphocytes included in the compositions disclosed herein and administered to a subject may include, CAR modified T cells, *e.g.*, CD4⁺ T cells, CD8⁺ T cells, Natural Killer T cells, gamma delta T cells, or MAIT cells; CAR modified B cells, or CAR modified NK cells. In certain embodiments, methods of treating a subject comprise administering an effective amount of an LVV as described herein or CAR modified lymphocytes (*i.e.*, recombinant cells that express one or more CARs) according to the present disclosure. In some embodiments, CAR modified T cells are administered to a subject. In some embodiments, CAR modified NK cells are administered to a subject. In some embodiments, CAR modified B cells are administered to a subject. The CAR modified lymphocytes (*e.g.*, T cells, B cells, or NK cells) may be xenogeneic, syngeneic, allogeneic, or autologous to the subject.

Pharmaceutical compositions including the LVV or CAR engineered lymphocytes may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose, suitable duration, and frequency of administration of the compositions will be determined by such factors as the condition of the patient, size, weight, body surface area, age, sex, type and severity of the disease, particular therapy to be administered, particular form of the active ingredient, time and the method of administration, and other drugs being administered concurrently. The present disclosure provides pharmaceutical compositions comprising LVV or CAR modified lymphocytes and a pharmaceutically acceptable carrier, diluent, or excipient. Suitable excipients include water, saline, dextrose, glycerol, or the like and combinations thereof. Other suitable infusion medium can be any isotonic medium formulation, including saline, Normosol R (Abbott), Plasma-Lyte A (Baxter), 5% dextrose in water, or Ringer's lactate.

A treatment effective amount of engineered lymphocytes (e.g., T cells, B cells, or NK cells) in a pharmaceutical composition is at least one cell (for example, one CAR modified T cell) and is more typically greater than 10^2 cells, for example, up to 10^6 , up to 10^7 , up to 10^8 cells, up to 10^9 cells, up to 10^{10} cells, or up to 10^{11} cells or more. In certain embodiments, the cells are administered in a range from about 10^6 to about 10^{10} cells/m², preferably in a range of about 10^7 to about 10^9 cells/m². The number of cells will depend upon the ultimate use for which the composition is intended as well the type of cells included therein. For example, a composition comprising T cells modified to contain a CAR will comprise a T cell population containing from about 5% to about 95% or more of such cells. In certain embodiments, a composition comprising CAR modified T cells comprises a cell population comprising at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. In certain embodiments, a composition comprising CAR modified NK cells comprises a cell population comprising at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. In certain embodiments, a composition comprising CAR modified B cells comprises a cell population comprising at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. For uses provided herein, the lymphocytes (e.g., T cells, B cells, or NK cells) are generally in a volume of a liter or less, 500 mls or less, 250 mls or less, or 100 mls or less. Hence the density of the desired cells is typically greater than 10^4 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The lymphocytes (e.g., T cells, B cells, or NK cells) may be administered as a single infusion or in multiple infusions over a range of time. Repeated infusions of CAR modified lymphocytes (e.g., T cells, B cells, or NK cells) may be separated by days, weeks, months, or even years if relapses of disease or disease activity are present. A clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , or 10^{11} cells. A preferred dose for administration of a host cell comprising a recombinant expression vector as described herein is about 10^7 cells/m², about 5×10^7 cells/m², about 10^8 cells/m², about 5×10^8 cells/m², about 10^9 cells/m², about 5×10^9 cells/m², about 10^{10} cells/m², about 5×10^{10} cells/m², or about 10^{11} cells/m².

The LVV and/or CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) compositions as described herein may be administered to a subject intravenously, intraperitoneally, intratumorally, into the bone marrow (e.g., intraosseous administration), into the lymph node (intranodally), and /or into cerebrospinal fluid.

5 The LVV vectors and/or CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) compositions may be administered to a subject in combination with one or more additional therapeutic agents. Examples of therapeutic agents that may be administered in combination with the LVV vectors or CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) according to the present description include radiation therapy, adoptive cellular immunotherapy agent (e.g., recombinant TCR, enhanced affinity TCR, 10 CAR, TCR-CAR, scTCR fusion protein, dendritic cell vaccine), antibody therapy, immune checkpoint molecule inhibitor therapy, UV light therapy, electric pulse therapy, high intensity focused ultrasound therapy, oncolytic virus therapy, or a pharmaceutical therapy, such as a chemotherapeutic agent, a therapeutic peptide, a hormone, an aptamer, antibiotic, anti-viral agent, anti-fungal agent, anti-inflammatory agent, a small molecule therapy, or any combination thereof. 15

Radiation therapy includes external beam radiation therapy (e.g., conventional external beam radiation therapy, stereotactic radiation, 3-dimensional conformal radiation therapy, intensity-modulated radiation therapy, volumetric modulated arc 20 therapy, particle therapy, proton therapy, and auger therapy), brachytherapy, systemic radioisotope therapy, intraoperative radiotherapy, or any combination thereof.

Exemplary antibodies that may be used in conjunction with the LVV or CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) compositions described herein include rituximab, pertuzumab, trastuzumab, alemtuzumab, Ibritumomab tiuxetan, 25 Brentuximab vedotin, cetuximab, bevacizumab, abciximab, adalimumab, alefacept, basilizimab, belimumab, bezlotoxumab, canakinumab, certolizumab pegol, daclizumab, denosumab, efalizumab, golimumab, olaratumab, palivizumab, panitumumab, and tocilizumab.

Exemplary inhibitors of immune checkpoint molecules that that may be used in 30 conjunction with the LVV or CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) compositions described herein include checkpoint inhibitors targeting PD-L1, PD-L2, CD80, CD86, B7-H3, B7-H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1,

CEACAM-3, CEACAM-5, PVRL2, PD-1, CTLA-4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1, PVRIG/CD112R, or any combination thereof. In certain embodiments, an immune checkpoint inhibitor may be an antibody, a peptide, an RNAi agent, or a small molecule. An antibody specific for CTLA-4 may be
 5 ipilimumab or tremelimumab. An antibody specific for PD-1 may be pidilizumab, nivolumab, or pembrolizumab. An antibody specific for PD-L1 may be durvalumab, atezolizumab, or avelumab.

Exemplary chemotherapeutics that may be used in conjunction with the LVV or CAR modified lymphocyte (e.g., T cell, B cell, or NK cell) compositions described
 10 herein may include an alkylating agent, a platinum based agent, a cytotoxic agent, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), and a DNA repair inhibitor.

As referred to herein, a chemotherapeutic includes non-specific cytotoxic agents that inhibit mitosis or cell division, as well as molecularly targeted therapy that blocks the growth and spread of cancer cells by targeting specific molecules that are involved in tumor growth, progression, and metastasis (e.g., oncogenes). Exemplary non-specific chemotherapeutics for use in conjunction with the expression cassette compositions
 20 described herein may include an alkylating agent, a platinum based agent, a cytotoxic agent, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), hypomethylating
 25 agent, and a DNA repair inhibitor.

Examples of chemotherapeutic agents considered for use in combination therapies contemplated herein include vemurafenib, dabrafenib, trametinib, cobimetinib, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine
 30 (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytosan® or Neosar®), cytarabine, cytosine

arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegen), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Acrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycytidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), fdabra tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), ibrutinib, venetoclax, crizotinib, alectinib, brigatinib, ceritinib, and vinorelbine (Navelbine®).

Exemplary alkylating agents for use in combination therapies contemplated herein include nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen Mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytosan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents for use in combination therapies contemplated herein include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan

(Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytosan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine
 5 (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednimustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESP and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytosan®, Neosar®, Procytox®,
 10 Revimmune®); and Bendamustine HCl (Treanda®).

Exemplary platinum based agents for use in combination therapies contemplated herein include carboplatin, cisplatin, oxaliplatin, nedaplatin, picoplatin, satraplatin, phenanthriplatin, and triplatin tetranitrate.

Exemplary hypomethylating agents for use in combination therapies include
 15 azacitidine and decitabine.

Exemplary molecularly targeted inhibitors for use in combination therapies contemplated herein include small molecules that target molecules involved in cancer cell growth and survival, including for example, receptor tyrosine kinase inhibitors, RAF inhibitors, BCL-2 inhibitors, ABL inhibitors, TRK inhibitors, c-KIT inhibitors, c-
 20 MET inhibitors, CDK4/6 inhibitors, FAK inhibitors, FGFR inhibitors, FLT3 inhibitors, IDH1 inhibitors, IDH2 inhibitors, PDGFRA inhibitors, and RET inhibitors

Exemplary molecularly targeted therapy includes hormone antagonists, signal transduction inhibitors, gene expression inhibitors (*e.g.*, translation inhibitors), apoptosis inducers, angiogenesis inhibitors (*e.g.*, a VEGF pathway inhibitor), tyrosine
 25 kinase inhibitors (*e.g.*, an EGF/EGFR pathway inhibitor), growth factor inhibitors, GTPase inhibitors, serine/threonine kinase inhibitors, transcription factor inhibitors, inhibitors of driver mutations associated with cancer, B-Raf inhibitors, RAF inhibitors, a MEK inhibitors, mTOR inhibitors, adenosine pathway inhibitors, EGFR inhibitors, PI3K inhibitors, BCL2 inhibitors, VEGFR inhibitors, MET inhibitors, MYC inhibitors,
 30 BCR-ABL inhibitors, ABL inhibitors, HER2 inhibitors, H-RAS inhibitors, K-RAS inhibitors, PDGFR inhibitors, ALK inhibitors, ROS1 inhibitors, BTK inhibitors, TRK inhibitors, c-KIT inhibitors, c-MET inhibitors, CDK4/6 inhibitors, FAK inhibitors,

FGFR inhibitors, FLT3 inhibitors, IDH1 inhibitors, IDH2 inhibitors, PARP inhibitors, PARP inhibitors, PDGFRA inhibitors, and RET inhibitors.

Exemplary angiogenesis inhibitors include, without limitation A6 (Angstrom Pharmaceuticals), ABT-510 (Abbott Laboratories), ABT-627 (Atrasentan) (Abbott Laboratories/Xinlay), ABT-869 (Abbott Laboratories), Actimid (CC4047, Pomalidomide) (Celgene Corporation), AdGVPEDF.11D (GenVec), ADH-1 (Exherin) (Adherex Technologies), AEE788 (Novartis), AG-013736 (Axitinib) (Pfizer), AG3340 (Prinomastat) (Agouron Pharmaceuticals), AGX1053 (AngioGenex), AGX51 (AngioGenex), ALN-VSP (ALN-VSP O2) (Alnylam Pharmaceuticals), AMG 386 (Amgen), AMG706 (Amgen), Apatinib (YN968D1) (Jiangsu Hengrui Medicine), AP23573 (Ridaforolimus/MK8669) (Ariad Pharmaceuticals), AQ4N (Novavea), ARQ 197 (ArQule), ASA404 (Novartis/Antisoma), Atiprimod (Callisto Pharmaceuticals), ATN-161 (Attenuon), AV-412 (Aveo Pharmaceuticals), AV-951 (Aveo Pharmaceuticals), Avastin (Bevacizumab) (Genentech), AZD2171 (Cediranib/Recentin) (AstraZeneca), BAY 57-9352 (Telatinib) (Bayer), BEZ235 (Novartis), BIBF1120 (Boehringer Ingelheim Pharmaceuticals), BIBW 2992 (Boehringer Ingelheim Pharmaceuticals), BMS-275291 (Bristol-Myers Squibb), BMS-582664 (Brivanib) (Bristol-Myers Squibb), BMS-690514 (Bristol-Myers Squibb), Calcitriol, CCI-779 (Torisel) (Wyeth), CDP-791 (ImClone Systems), Ceflatonin (Homoharringtonine/HHT) (ChemGenex Therapeutics), Celebrex (Celecoxib) (Pfizer), CEP-7055 (Cephalon/Sanofi), CHIR-265 (Chiron Corporation), NGR-TNF, COL-3 (Metastat) (Collagenex Pharmaceuticals), Combretastatin (Oxigene), CP-751,871 (Figitumumab) (Pfizer), CP-547,632 (Pfizer), CS-7017 (Daiichi Sankyo Pharma), CT-322 (Angiocept) (Adnexus), Curcumin, Dalteparin (Fragmin) (Pfizer), Disulfiram (Antabuse), E7820 (Eisai Limited), E7080 (Eisai Limited), EMD 121974 (Cilengitide) (EMD Pharmaceuticals), ENMD-1198 (EntreMed), ENMD-2076 (EntreMed), Endostar (Simcere), Erbitux (ImClone/Bristol-Myers Squibb), EZN-2208 (Enzon Pharmaceuticals), EZN-2968 (Enzon Pharmaceuticals), GC1008 (Genzyme), Genistein, GSK1363089 (Foretinib) (GlaxoSmithKline), GW786034 (Pazopanib) (GlaxoSmithKline), GT-111 (Vascular Biogenics Ltd.), IMC-1121B (Ramucirumab) (ImClone Systems), IMC-18F1 (ImClone Systems), IMC-3G3 (ImClone LLC), INCB007839 (Incyte Corporation), INGN 241 (Introgen Therapeutics), Iressa

(ZD1839/Gefitinib), LBH589 (Faridak/Panobinostat) (Novartis), Lucentis (Ranibizumab) (Genentech/Novartis), LY317615 (Enzastaurin) (Eli Lilly and Company), Macugen (Pegaptanib) (Pfizer), MEDI522 (Abegrin) (MedImmune), MLN518 (Tandutinib) (Millennium), Neovastat (AE941/Benefin) (Aeterna Zentaris),
 5 Nexavar (Bayer/Onyx), NM-3 (Genzyme Corporation), Noscaphine (Cougar Biotechnology), NPI-2358 (Nereus Pharmaceuticals), OSI-930 (OSI), Palomid 529 (Paloma Pharmaceuticals, Inc.), Panzem Capsules (2ME2) (EntreMed), Panzem NCD (2ME2) (EntreMed), PF-02341066 (Pfizer), PF-04554878 (Pfizer), PI-88 (Progen Industries/Medigen Biotechnology), PKC412 (Novartis), Polyphenon E (Green Tea
 10 Extract) (Polypheno E International, Inc.), PPI-2458 (Praecis Pharmaceuticals), PTC299 (PTC Therapeutics), PTK787 (Vatalanib) (Novartis), PXD101 (Belinostat) (CuraGen Corporation), RAD001 (Everolimus) (Novartis), RAF265 (Novartis), Regorafenib (BAY73-4506) (Bayer), Revlimid (Celgene), Retaane (Alcon Research), SN38 (Liposomal) (Neopharm), SNS-032 (BMS-387032) (Sunesis), SOM230
 15 (Pasireotide) (Novartis), Squalamine (Genaera), Suramin, Sutent (Pfizer), Tarceva (Genentech), TB-403 (Thrombogenics), Tempostat (Collard Biopharmaceuticals), Tetrathiomolybdate (Sigma-Aldrich), TG100801 (TargeGen), Thalidomide (Celgene Corporation), Tinzaparin Sodium, TKI258 (Novartis), TRC093 (Tacon Pharmaceuticals Inc.), VEGF Trap (Aflibercept) (Regeneron Pharmaceuticals), VEGF
 20 Trap-Eye (Regeneron Pharmaceuticals), Veglin (VasGene Therapeutics), Bortezomib (Millennium), XL184 (Exelixis), XL647 (Exelixis), XL784 (Exelixis), XL820 (Exelixis), XL999 (Exelixis), ZD6474 (AstraZeneca), Vorinostat (Merck), and ZSTK474.

Exemplary B-Raf inhibitors include vemurafenib, dabrafenib, and encorafenib.

25 Exemplary MEK inhibitors include binimetinib, cobimetinib, refametinib, selumetinib, and trametinib.

Exemplary BTK inhibitors include ibrutinib, Loxo-305, tirabrutinib, GDC-0853, acalabrutinib, ONO-4059, spebrutinib, BGB-3111, HM71224, and M7583.

30 Exemplary TRK inhibitors include entrectinib, larotrectinib, CH7057288, ONO-7579, LOXO-101, lestaurtinib, and LOXO-195.

Exemplary c-KIT inhibitors include imatinib, sunitinib, and ponatinib.

Exemplary c-MET inhibitors include capmatinib, crizotinib, tivantinib, onartuzumab, INCB28060, AMG-458, savolitinib, and tepotinib.

Exemplary CDK4/6 inhibitors include palbociclib, ribociclib, abemaciclib, and trilaciclib.

5 Exemplary FAK inhibitors include defactinib, GSK2256098, BI853520, and PF-00562271.

Exemplary FGFR inhibitors include erdafitinib, pemigatinib, infigratinib, rogaratinib, AZD4547, BGJ398, FP-1039, and ARQ 087.

10 Exemplary FLT-3 inhibitors include quizartinib, crenolanib, gilteritinib, midostaurin, and lestaurtinib.

Exemplary IDH1 inhibitors include ivosidenib, BAY-1436032, and AGI-5198.

An exemplary IDH2 inhibitor includes enasidenib.

15 Exemplary PARP inhibitors include talazoparib, niraparib, rucaparib, olaparib, veliparib, CEP 9722, E7016, AG014699, MK4827, BMN-673, and Pamiparib (BGB-290).

Exemplary PDGFRA inhibitors include imatinib, regorafenib, crenolanib, and olaratumab.

Exemplary pan-RAF inhibitors include belvarafenib, LXH254, LY3009120, INU-152, and HM95573.

20 Exemplary RET inhibitors include lenvatinib, alectinib, vandetanib, cabozantinib, BLU-667, and LOXO-292.

Exemplary ROS1 inhibitors include ceritinib, lorlatinib, entrectinib, crizotinib, TPX-0005, and DS-6051b.

25 Exemplary Vascular Endothelial Growth Factor (VEGF) receptor inhibitors include, but are not limited to, Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (S)—((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib

(GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[(3 α ,5 β ,6 α)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[1-methyl-6-(3-pyridinyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino]-N-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); and Aflibercept (Eylea®).

Exemplary EGF pathway inhibitors include, without limitation tyrphostin 46, EKB-569, erlotinib (Tarceva®), gefitinib (Iressa®), erbitux, nimotuzumab, lapatinib (Tykerb®), cetuximab (anti-EGFR mAb), ¹⁸⁸Re-labeled nimotuzumab (anti-EGFR mAb), and those compounds that are generically and specifically disclosed in WO 97/02266, EP 0 564 409, WO 99/03854, EP 0 520 722, EP 0 566 226, EP 0 787 722, EP 0 837 063, U.S. Pat. No. 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and WO 96/33980. Exemplary EGFR antibodies include, but are not limited to, Cetuximab (Erbitux®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Trastuzumab (Herceptin®); Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1). Exemplary Epidermal growth factor receptor (EGFR) inhibitors include, but not limited to, Erlotinib hydrochloride (Tarceva®); ceritinib; brigatinib; osimertinib; icotinib; Gefitinib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[(3''S'')-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide, Tovok®; Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-((3-

methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperazinyl)methyl]phenyl]-N-[(1R)-1-phenylethyl]-7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569);
 5 Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[[(3 α ,5 β ,6 α)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-[4-[(1R)-1-Phenylethyl]amino]-7H-
 10 pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PKI166, CAS 187724-61-4); rocletinib.

Exemplary mTOR inhibitors include, without limitation, rapamycin (Rapamune®), and analogs and derivatives thereof; SDZ-RAD; Temsirolimus (Torisel®; also known as CCI-779); Ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-
 15 2[(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-penta-oxo-11,36-dioxo-4-azatricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or
 20 RAD001); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-51-3); (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-d]pyrimidin-7(8H)-one (PF04691502, CAS 1013101-36-4); and N²-[1,4-dioxo-[[4-(4-oxo-8-phenyl-4H-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-
 25 arginylglycyl-L- α -aspartyl-L-serine-, inner salt (SF1126, CAS 936487-67-1).

Exemplary Phosphoinositide 3-kinase (PI3K) inhibitors include, but are not limited to, duvelisib, idelalisib, 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as GDC 0941 and
 30 described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in

PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

Exemplary Protein Kinase B (PKB) or AKT inhibitors include, but are not limited to. 8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-f][1,6]naphthyridin-3(2H)-one (MK-2206, CAS 1032349-93-1); Perifosine (KRX0401); 4-Dodecyl-N-1,3,4-thiadiazol-2-yl -benzenesulfonamide (PHT-427, CAS 1191951-57-1); 4-[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-[(3S)-3-piperidinylmethoxy]-1H-imidazo[4,5-c]pyridin-4-yl]-2-methyl-3-butyn-2-ol (GSK690693, CAS 937174-76-0); 8-(1-Hydroxyethyl)-2-methoxy-3-[(4-methoxyphenyl)methoxy]-6H-dibenzo[b,d]pyran-6-one (palomid 529, P529, or SG-00529); Tricirbine (6-Amino-4-methyl-8-(β-D-ribofuranosyl)-4H,8H-pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine); (αS)-α-[[[5-(3-Methyl-1H-indazol-5-yl)-3-pyridinyl]oxy]methyl]-benzeneethanamine (A674563, CAS 552325-73-2); 4-[(4-Chlorophenyl)methyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-4-piperidinamine (CCT128930, CAS 885499-61-6); 4-(4-Chlorophenyl)-4-[4-(1H-pyrazol-4-yl)phenyl]-piperidine (AT7867, CAS 857531-00-1); and Archexin (RX-0201, CAS 663232-27-7).

In certain embodiments, a tyrosine kinase inhibitor used in combination with the LVV or CAR modified T cells is an anaplastic lymphoma kinase (ALK) inhibitor. Exemplary ALK inhibitors include crizotinib, ceritinib, alectinib, brigatinib, dalantercept, entrectinib, and lorlatinib.

In certain embodiments where the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered in combination with one or more additional therapies, the LVV, CAR modified lymphocytes (e.g., T cells or NK cells), or one or more additional therapies may be administered at a dose that might otherwise be considered sub-therapeutic if administered as a monotherapy. Combination therapy

includes administration of an LVV or CAR modified lymphocyte (e.g., T cell or NK cell) composition as described herein before an additional therapy (e.g., 1 day to 30 days or more before the additional therapy), concurrently with an additional therapy (on the same day), or after an additional therapy (e.g., 1 day – 30 days or more after the additional therapy). In certain embodiments, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered concurrently with the one or more additional therapies. In further embodiments, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days before or after administration of the one or more additional therapies. In still further embodiments, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered within 4 weeks, within 3 weeks, within 2 weeks, or within 1 week before or after administration of the one or more additional therapies. Where the one or more additional therapies involves multiple doses, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) may be administered before or after the initial dose of the one or more additional therapies, after the final dose of the one or more additional therapies, or in between multiple doses of the one or more additional therapies.

Subjects that can be treated by the compositions and methods of the present disclosure include animals, such as humans, primates, cows, horses, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, or pigs. The subject may be male or female, and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects.

EXAMPLES

Example 1: Methods for generating lentiviral vectors with defined tropism

Self-inactivating lentiviral vectors (LVV) as described herein were produced using a third generation production system. An expression plasmid that contains the gene sequences desired for delivery by the LVV was combined at defined ratios with three packaging plasmids, VSV-G, GagPol, and Rev, and used to transfect HEK293 producer cells. Productive viral particles were harvested from the HEK293 culture media 2-3 days later.

The tropism of the resulting LVV was tested to determine whether it can be redirected using a non-viral targeting protein as described herein expressed from one of

the three packaging plasmids or by addition of a fifth plasmid to the transfection mix.

The LVV vector includes a mutated VSV-G envelope protein, where the mutated VSV-G includes mutations that abolish binding of the mutated VSV-G to its native receptor such that viral entry occurs via the non-viral, tropism defining targeting protein.

5 Mutations in the LDL-receptor binding site of VSV-G were found to abolish binding to its natural receptor. VSV-G bearing a variety of mutations (Trop-002 (SEQ ID NO: 74), Trop-051 (SEQ ID NO: 93), Trop-052 (SEQ ID NO: 95), Trop-055 (SEQ ID NO: 97), or Trop-061 (SEQ ID NO: 103)) was expressed together with CD80 (SEQ ID NO: 2) on the LVV. CD80 is the natural ligand to T cell surface receptors, CD28 and
10 CTLA4, and provides tropism to T cells and not B cells. Binding to the natural receptor for VSV-G is assessed by transduction of Raji B cells. Several mutations in VSV-G prohibit transduction of Raji B cells yet transduction of Jurkat T cells is maintained (**FIGS. 2A-2B**).

In a first method, a tropism-defining targeting protein derived from CD80 (SEQ
15 ID NO: 76) was expressed from the VSV-G packaging plasmid. CD80 is the natural ligand to T cell surface receptors, CD28 and CTLA4, and provides tropism to T cells. A codon optimized CD80 molecule (encoding SEQ ID NO: 2) was cloned downstream of the mutated VSV-G (Trop-002, encoding SEQ ID NO: 74), which contains mutations to abolish LDL receptor binding and was used to package a green fluorescent protein
20 (GFP)-expressing LVV. CD80 and VSV-G were expressed at relatively equivalent levels on the surface of HEK293 producer cells, suggesting comparable decoration of the LVV particles (**FIG. 3A**). LVV generated with this approach were capable of transducing T cells as assessed by GFP expression (**FIG. 3B**).

In a second method, the tropism-defining CD80 targeting protein (encoding
25 SEQ ID NO: 2) was cloned into the Rev packaging plasmid and transfected at a lower molar concentration to the VSV-G env (encoding Trop-002 (SEQ ID NO: 74)) plasmid. The modified plasmids was used to package a GFP-expressing LVV. Assessment of VSV-G and CD80 expression on the HEK-293 producer cells showed relative expression levels trended with the molar concentrations of the packaging plasmids.
30 LVV generated with these packaging approaches were capable of transducing T cells as assessed by GFP expression (**FIG. 4**). Reduced expression of CD80 was observed in

LVVs packaged with CD80 targeting protein contained in the Rev packaging plasmid due to the lower ratio of the Rev plasmid.

In a third method, the tropism defining CD80 targeting protein (encoding SEQ ID NO: 2) was added as an additional plasmid during transfection of HEK293 cells intended to produce LVV (Trop-002 VSV-G env). The amount of the CD80 plasmid used during LVV production was associated with the expression on HEK293 producer cells and is likely representative of decoration of the LVV particles. LVV generated with this packaging approach were capable of transducing T cells as assessed by GFP expression and transduction efficiency was associated with CD80 levels on the LVV particles (**FIG. 5**).

Regardless of the method selected, high titer, tropism-redirectioned LVV can be generated with adherent or suspension HEK293 producer cells and concentrated by centrifugation or anion exchange chromatography (AEX) followed by tangential flow filtration (TFF) (**FIGS. 6A-6C**). Concentration by AEX/TFF resulted in LVV preparations with a high level of purity and recovery (**FIG. 6D**).

Example 2: CD4 T Cells Enriched by CD80 LVV

The tropism-defining targeting molecule precisely defines the specific cell type transduced with this LVV. The ligand for CD80, CD28, is naturally expressed on T cells. However, a higher frequency of CD4 T cells express CD28 in human peripheral blood compared to CD8 T cells (80% vs 50%). When a LVV was packaged to decorate the Trop-002 VSV-G envelope with a CD80 targeting protein (SEQ ID NO: 76) was used to transduce human PBMC, a higher composition of transduced CD4 T cells was observed compared to transduced CD8 T cells and was maintained for up to a week in culture (**FIG. 13**).

Example 3: Methods for ex vitro generation of tumor-reactive T cells engineered to express a chimeric antigen receptor (CAR) using a lentiviral vector containing T cell-specific tropism

Compared to standard lentiviral vectors (LVV) that use common third
5 generation packaging systems, LVV packaged to redirect tropism as provided herein
may have improved transduction efficiencies and specificity. The levels of available
viral particles are likely increased when transducing a specific cell type as part of a
heterogeneous mixture, as the LVV particles are not taken up by other, non-desired cell
types. Whether improved transduction of T cells with a CAR molecule is possible in a
10 heterogenous peripheral blood mononuclear cell (PBMC) mixture was evaluated. A
representative anti-BCMA CAR (SEQ ID NO: 58) used to treat Multiple Myeloma or a
representative anti-CD19 CAR (SEQ ID NO: 42) used to treat non-Hodgkin Lymphoma
was used. GFP reporter was used as control. The LVV was packaged to decorate the
envelope with anti-CD3 targeting protein and CD80 targeting protein and redirect
15 tropism toward the T cell surface molecules, CD3, CD28 and CTLA4. The CD80
targeting protein was cloned into the Trop-002 VSV-G env packaging plasmid (SEQ ID
NO: 76) and the anti-CD3 targeting protein (encoding SEQ ID NO: 10) was expressed
from a fifth packaging plasmid.

The efficiency of T cell transduction within PBMC obtained from healthy
20 donors leukapheresis after informed consent was tested. The PBMC were enriched
using red cell lysis and matched donors were transduced with standard LVV or LVV
with T cell redirected tropism (anti-CD3 and CD80) at LVV concentrations 5 to 25
times lower than typically used for CAR T cell transduction. Expression of an anti-
BCMA CAR was assessed by flow cytometry after staining with a fluorescently labeled
25 BCMA molecule to specifically detect anti-BCMA CAR T cells. Even with extremely
low viral levels, T cells transduced using the T cell tropic LVV showed higher levels of
transduction compared to standard LVV (**FIG. 7**).

In the same study whether anti-BCMA CAR T cells can be generated without
antibodies to activate T cells was evaluated. T cell activating antibodies (e.g., anti-
30 CD28 and anti-CD3) are typically required for transduction using standard LVV during
CAR T cell generation. It was confirmed that standard LVV indeed requires activation
using antibodies to transduce normal human primary T cells (**FIG. 7**). By contrast, a T

cell redirected LVV (anti-CD3 and CD80) can cause transduction even in the absence of prior T cell activation (without the presence of IL-2 and exogenous activating antibodies anti-CD3 and anti-CD28) (**FIG. 7**). A consequence of T cell activation is the subsequent cellular expansion when cultured in media containing IL-2. PBMCs

5 transduced in the presence or absence of exogenous activating anti-CD3 and anti-CD28 antibodies after standard LVV or T cell redirected LVV (anti-CD3 and CD80) showed that T cell redirected LVV was uniquely able to expand T cells even in the absence of antibody activation (**FIG. 8**). In another example, transduction of an anti-CD19 CAR also was achieved without prior T cell activation when using the T cell redirected LVV

10 (anti-CD3 and CD80) (**FIG. 9**).

T cell activation and transduction efficiency can be dictated by the selection of anti-CD3 antibodies used to generate the T cell redirected LVV. Examples of envelope-bound anti-CD3 binding proteins having 12F6 binding domains in VH (SEQ ID NO:113)-VL (SEQ ID NO:115) orientation and VL (SEQ ID NO:115)-VH (SEQ ID

15 NO:113) orientation are shown to cause a variable amount of T cell transduction as well as T cell activation (**FIG. 10**).

Example 4: T Cell Specificity of T cell targeted lentiviral vector

The T cell specificity of an LVV packaged to decorate the envelope (Trop-002 VSV-G

20 env) with an anti-CD3 targeting protein (SEQ ID NO: 10) and CD80 targeting protein (SEQ ID NO: 2) was confirmed by transduction of a T cell line and a panel of B cell lines (Raji, Ramos, Jeko-1, NALM-6). Transduction of these cell lines assessed by flow cytometry for a GFP transgene was compared to standard LVV. Both standard LVV and a T cell-targeted LVV effectively transduced the T cell line (Jurkat). The standard

25 LVV effectively transduced all the B cell lines, confirming these lines are sensitive to LVV transduction, but no substantial transduction by the T cell-targeting LVV was detected in any of the B cell lines (**FIG. 14**).

Example 5: Tumor-specific T cells generated using a T cell targeted lentiviral vector

30 Using methods described herein, T cells expressing an anti-BCMA CAR (SEQ ID NO: 58) were generated by transducing PBMC from healthy donors with LVVs having

Trop-002 VSV-G env and CD80 targeting protein and anti-CD3 targeting protein. The CD80 targeting protein was cloned into the Trop-002 VSV-G env packaging plasmid (SEQ ID NO: 76) and the anti-CD3 targeting protein (encoding SEQ ID NO: 10) was expressed from a fifth packaging plasmid to produce the T cell targeting LVVs. The T cell transductions were performed in the absence of any T cell stimulation typically required for efficient LVV-mediated gene transfer (exogenous anti-CD3 and anti-CD28).

First, the activity of anti-BCMA CAR T cells generated by T cell-redirectioned LVV was examined compared to standard LVV. Anti-tumor activity was assessed by intracellular cytokine staining for interferon-gamma and tumor necrosis factor-alpha after co-culture with a BCMA-negative cell line (Nalm-6) or BCMA-positive cell line (RPMI-8826). Anti-BCMA CAR T cells exhibited increased expression of T cell effector cytokines after culture with BCMA-positive cell lines that is not observed with BCMA-negative cell lines whether generated by standard LVV or T cell-redirectioned LVV (**FIG. 11**).

Example 6: Methods of T cell-specific in vivo delivery of genetic material using a T cell tropic lentiviral vector

The efficient and specific transduction of T cells with a T cell targeted LVV could permit safe and effective delivery of genetic material to T cells in vivo. A humanized mouse model infused intravenously with a GFP-expressing, T cell targeted LVV was used to test this hypothesis. LVVs having Trop-002 mutated VSV-G and coated with anti-CD3 targeting protein and CD80 targeting protein were produced. The CD80 targeting protein was cloned into the Trop-002 VSV-G env packaging plasmid (SEQ ID NO: 76) and the anti-CD3 targeting protein (encoding SEQ ID NO: 10) was expressed from a fifth packaging plasmid to produce the T cell targeting LVVs.

Immunocompromised NCG mice were humanized via intravenous injection of human PBMC and supported by daily intraperitoneal administration of recombinant IL-2 for 4 days. One day after PBMC administration, the T cell targeted lentiviral vector is administered intravenously to the mice. Peripheral blood and splenic cells were harvested on day 7, and T cell specificity was assessed by flow cytometry. GFP positive cells were only detected in human T cells (CD3+) and not human B cells

(CD20+), confirming T cell specificity of LVV transduction (**FIG. 12A**). GFP was detected in both CD8+ and CD8- (CD4) T cells, indicating that the T cell specific LVV transduces both CD4 and CD8 T cells in vivo (**FIG. 12B**).

Specific T cell transduction has a key safety advantages to other in vivo CAR delivery approaches by minimizing risk of transducing tumor cells. Transduction of T cells without transduction of tumor cells is evaluated using PBMCs from chronic lymphocytic leukemia patients with detectable CD19 tumor cells. The PBMC are transduced with T cell targeted LVV and transduced cells are assessed using flow cytometry.

10 The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including but not limited to U.S. Provisional Patent Application No. 63/154,639, filed on
15 Feburary 26, 2021, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

 These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should
20 not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

25

CLAIMS

1. A lentiviral vector, comprising:
 - (a) a viral envelope comprising:
 - a heterologous lymphocyte targeting protein, the lymphocyte targeting protein
 - 5 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
 - 10 (c) an expression cassette that comprises a heterologous transgene.
2. The lentiviral vector according to claim 1, wherein the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein comprising an extracellular domain comprising a T cell targeting domain.
3. The lentiviral vector according to claim 1, wherein the heterologous lymphocyte
 - 15 targeting protein is a heterologous natural killer (NK) cell targeting protein comprising an extracellular domain comprising a NK cell targeting domain.
- 4. The lentiviral vector according to claim 1, wherein the heterologous lymphocyte targeting protein is a heterologous B cell targeting protein comprising an extracellular domain comprising a B cell targeting domain.
- 20 5. A lentiviral vector according to claim 1 or 2, wherein the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein that specifically binds to:
 - (a) 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;
 - 25 (b) a $\gamma\delta$ T cell marker selected from $\gamma\delta$ TCR, Vdelta1, Vdelta2, and NKG2D (KLRK1, CD314);

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

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

5

6. A lentiviral vector according to claim 1, 2, or 5, wherein 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.

10

7. A lentiviral vector according to claim 1 or 3, wherein 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.

15

8. A lentiviral vector according to claim 1 or 4, wherein 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.

20

9. A lentiviral vector according to any one of claims 1, 2, 5, and 6, wherein 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.

25

10. A lentiviral vector according to any one of claims 1, 2, 5, 6, and 9, wherein 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

11. A lentiviral vector according to claim 1, 2, 5, 6, 9, or 10, wherein 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.
12. A lentiviral vector according to any one of claims 1, 2, 5, and 6, wherein the
5 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.
- 10 13. A lentiviral vector according to claim 12 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.
14. A lentiviral vector according to any preceding claim, wherein the targeting protein
15 as expressed in a host cell comprises a signal sequence.
15. A lentiviral vector according to any preceding claim, wherein the mature targeting protein lacks a signal sequence.
16. A lentiviral vector according to any of claims 1-14, wherein the mature targeting protein includes at least one amino acid from a signal sequence.
- 20 17. A lentiviral vector according to any preceding claim, wherein the mutated VSV-G envelope protein comprises a mutation selected from one 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
25 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.

18. A lentiviral vector according to any preceding claim, wherein the mutated VSV-G envelope protein comprises a mutation selected from 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;

5 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.

10

19. A lentiviral vector according to any preceding claim, wherein 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.

20. A lentiviral vector according to any preceding claim, wherein the mutated VSV-G envelope protein comprises a mutation selected from one or more of H8A, K47A, K47Q, Y209A, R354A, and R354Q.

21. A lentiviral vector according to any of the preceding claims, wherein the mutated VSV-G envelope protein comprises:

- (a) a K47 mutation and a R354 mutation;
- 20 (b) a N180 mutation, a I182 mutation, a T352 mutation, and a E353 mutation;
- (c) a T352 mutation and E353 mutation;
- (d) a N9 mutation, a Q10 mutation, and a N180 mutation; or
- (e) an insertion of GGS between H8 and N9 and an insertion of GGS between N9 and Q10;
- 25 (f) an insertion of TT between N9 and Q10; or
- (g) an insertion of GGS between P46 and K47.

22. The lentiviral vector according to claim 21, 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.

23. The lentiviral vector according to any one of the preceding claims, wherein the
5 mutated VSV-G envelope protein comprises the amino acid sequence of any one of
SEQ ID NOS: 74, 93, 95, 97, 99 and 103.

24. A lentiviral vector according to any preceding claim, 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
10 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.

25. A lentiviral vector according to claim 24, wherein the CAR encoded by the
15 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,
20 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,
25 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),
30 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, SIRP α , pT α , TCR α , TCR β , TIM3, TRIM, LPA5, and Zap70 transmembrane domain;

(c) an intracellular signaling domain selected from a CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ ,
5 CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcR γ , 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
10 (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:

15 (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
20 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.

26. A lentiviral vector according to either of claims 24 or 25, wherein the CAR encoded by the transgene is an anti-CD19 CAR comprising:

25 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;

30 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 CD3 ζ 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.

5

27. A lentiviral vector according to claim 26, 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.

10 28. A lentiviral vector according to either of claim 24 or 25, wherein 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

15 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;

20 a CD3 ζ 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.

25 29. A lentiviral vector according to claim 26, 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.

30. 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
30 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;
- 5 (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,
- 10 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;
- 15 (b) culturing the producer cell in a culture medium; and
- (c) harvesting lentiviral vector from the culture medium.

31. A method for producing a lymphocyte targeted lentiviral vector according to any one of claims 1-29, the method comprising:
- 20 (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
- 25 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
- 30 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.

32. A method for producing a lymphocyte targeted lentiviral vector according to any
5 one of claims 1-29, 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
10 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
15 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
20 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.

25 33. A method for producing a lymphocyte targeted lentiviral vector according to any
one of claims 1-29, 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
30 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;

5 (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

10 (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.

15

34. The method according to any one of claims 30-33, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a T cell targeting protein.

20 35. The method according to any one of claims 30-33, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a NK cell targeting protein.

36. The method according to any one of claims 30-33, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a B cell
25 targeting protein.

37. A method according to any one of claims 30-33 and 34, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a T cell targeting protein that specifically binds to:

(a) a T cell marker selected from CD3, CD28, CD80, 4-1BB, AhR, CD3, CD2,
30 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;

(b) a $\gamma\delta$ T cell marker selected from $\gamma\delta$ TCR, Vdelta1, Vdelta2, and NKG2D
5 (KLRK1, CD314, or any combination thereof);

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

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

10 38. A method according to claim 37, wherein 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.

15 39. A method according to either of claims 37 and 38, wherein 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

40. The method according to any one of claim 37, 38, and 39, wherein the targeting protein comprises a CD80 protein comprising SEQ ID NO: 2 or SEQ ID NO: 2 absent
20 the signal peptide of SEQ ID NO: 4.

41. A method according any of claims 30-34 and 37, wherein 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
25 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.

42. A method according to any one of claims 30-34, 37, and 41, wherein the targeting
 5 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.

43. The method according to any one of claims 30-33 and 35, wherein the lymphocyte
 10 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.

44. The method according to any one of claims 30-33 and 36, wherein the lymphocyte
 15 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.
 20

45. A method according to any of claims 30-44, wherein the mutated VSV-G envelope protein encoded by the VSV-G env plasmid comprises a mutation selected from one or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184,
 25 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
 30 a deletion of residues 1-8.

46. A method according to any of claims 30-45, wherein the mutated VSV-G envelope protein encoded by the VSV-G env plasmid comprises a mutation selected from two or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209,
5 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

10 a deletion of residues 1-8.

47. A method according to any of claims 30-46, wherein 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.

15 48. A method according to any of claims 30-47, wherein 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.

20 49. A method according to any of claims 30-48, wherein the mutated VSV-G envelope protein comprises:

- (a) a K47 mutation and a R354 mutation;
- (b) a N180 mutation, a I182 mutation, a T352 mutation, and a E353 mutation;
- (c) a T352 mutation and E353 mutation;
- (d) a N9 mutation, a Q10 mutation, and a N180 mutation; or
- 25 (e) an insertion of GGS between H8 and N9 and an insertion of GGS between N9 and Q10;
- (f) an insertion of TT between N9 and Q10; or
- (g) an insertion of GGS between P46 and K47.

50. The method according to claim 49, 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;
- 5 (c) a T352W mutation and a E353A mutation; or
- (d) a N9A mutation, a Q10A mutation, and a N180A mutation.

51. The method according to any one claims 30-50, wherein 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.

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52. A method according to any of claims 30-51, wherein 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
 15 motif (ITAM) and optionally a costimulatory signaling domain; and a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

53. A method according to any of claims 30-52, wherein the CAR encoded by the transgene plasmid comprises:

- 20 (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,
 25 HLA-A2 + NY-ESO-1, HLA-A3 + NY-ESO-1, HLADR, IL-11Ralpha, IL-13Ralpha2, 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, CD3 ϵ , CD3 δ , CD3 ζ , 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),
 5 CD270 (HVEM), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), CD279 (PD-1), CD300, CD357 (GITR), A2aR, DAP10, FcR α , FcR β , FcR γ , Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRP α , pT α , TCR α , TCR β , TIM3, TRIM, LPA5, and Zap70 transmembrane domain;
- 10 (c) an intracellular signaling domain selected from a CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcR γ , 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),
 15 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
 20 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;
- 25 (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.

54. A method according to any of claims 30-53, wherein the CAR encoded by the
 30 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 CD3 ζ 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.

55. The method according to claim 54, 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.

56. A method according to any of claims 30-53, wherein 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 CD3 ζ 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.

57. The method of claim 56, 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.

58. The method of any one of claims 30-57, 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, 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.

59. The method of claim 58, 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.

60. The method of claim 58, 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 3.125:3.125:2.5:1.25.

61. The method of any one of claims 30-60, wherein harvesting lentiviral vector from the culture medium comprises centrifugation, anion exchange, chromatography, or any combination thereof.

62. The method of any one of claims 30-61, wherein 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.

63. A method for producing a T cell expressing a heterologous transgene, the method comprising:

transducing the T cell with one or more LVVs according to any one of claims 1, 2, 5, 6, and 9-29, wherein transduction of the T cell results in expression of the heterologous transgene by the T cell.

64. The method according to claim 63, wherein the T cell is a resting T cell.

65. The method of according to claim 63 or 64, wherein transducing the T cell is performed in vivo or ex vivo.

66. The method according to any one of claims 63-65, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorally, intraosseously, or intranodally.
67. The method according to any one of claims 63-66, wherein transducing the T cell
5 comprises contacting T cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject with the one or more LVVs.
68. A method according to any one of claims 63-67, wherein transduction is accomplished without use of an exogenous T cell stimulating agent.
69. The method according to claim 68, wherein the exogenous T cell stimulating agent
10 comprises an anti-CD3 antibody or antigen binding fragment thereof, an anti-CD28 antibody or antigen binding fragment thereof, or both.
70. A method for producing a T cell expressing a CAR, the method comprising:
transducing the T cell with one or more LVVs according to any one of claims
24-29 and transduction of the T cell results in expression of the CAR transgene by the T
15 cell.
71. The method according to claim 70, wherein the T cell is a resting T cell.
72. The method of according to claim 70 or 71, wherein transducing the T cell is performed in vivo or ex vivo.
73. The method according to any one of claims 70-72, wherein the one or more LVVs is
20 administered to a subject intravenously, intraperitoneally, intratumorally, intraosseously, or intranodally.
74. The method according to any one of claims 70-72, wherein transducing the T cell comprises contacting T cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject with the one or more LVVs.
- 25 75. A method according to any one of claims 70-74, wherein transduction is accomplished without use of an exogenous T cell stimulating agent.

76. The method of claim 75, 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.

77. A method for producing a NK cell expressing a heterologous transgene, the method
5 comprising:

transducing the NK cell with one or more LVVs according to any one of claims 1, 3, 7, and 14-29, wherein transduction of the NK cell results in expression of the heterologous transgene by the NK cell.

78. The method according to claim 77, wherein the NK cell is a resting NK cell.

10 79. The method of according to claim 77 or 78, wherein transducing the NK cell is performed in vivo or ex vivo.

80. The method according to any one of claims 77-79, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorally, intraosseously, or intranodally.

15 81. The method according to any one of claims 77-79, wherein transducing the NK cell comprises contacting NK cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject.

82. A method according to any one of claims 77-81, wherein transduction is accomplished without use of an exogenous NK cell stimulating agent.

20 83. A method for producing a NK cell expressing a CAR, the method comprising:
transducing the NK cell with one or more LVVs according to any one of claims 24-29 and transduction of the NK cell results in expression of the CAR transgene by the NK cell.

84. The method according to claim 83, wherein the NK cell is a resting NK cell.

25 85. The method of according to claim 83 or 84, wherein transducing the NK cell is performed in vivo or ex vivo.

86. The method according to any one of claims 83-85, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorally, intraosseously, or intranodally.
87. The method according to any one of claims 83-85, wherein transducing the NK cell
5 comprises contacting NK cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject with the one or more LVVs.
88. A method according to any one of claims 83-87, wherein transduction is accomplished without use of an exogenous NK cell stimulating agent.
89. A method for producing a B cell expressing a heterologous transgene, the method
10 comprising:
transducing the B cell with one or more LVVs according to any one of claims 1, 4, 8 and 14-29, wherein transduction of the B cell results in expression of the heterologous transgene by the B cell.
90. The method according to claim 89, wherein the B cell is a resting B cell.
- 15 91. The method of according to claim 89 or 90, wherein transducing the B cell is performed in vivo or ex vivo.
92. The method according to any one of claims 89-91, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorally, intraosseously, or intranodally.
- 20 93. The method according to any one of claims 89-91, wherein transducing the B cell comprises contacting B cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject.
94. A method according to any one of claims 89-93, wherein transduction is accomplished without use of B cell stimulating agents.
- 25 95. A method for producing a B cell expressing a CAR, the method comprising:

transducing the B cell with one or more LVVs according to any one of claims 24-29 and transduction of the NK cell results in expression of the CAR transgene by the B cell.

96. The method according to claim 95, wherein the B cell is a resting B cell.

- 5 97. The method of according to claim 95 or 96, wherein transducing the B cell is performed in vivo or ex vivo.

98. The method according to any one of claims 95-97, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorally, intraosseously, or intranodally.

- 10 99. The method according to any one of claims 95-97, wherein transducing the B cell comprises contacting B cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject with the one or more LVVs.

100. A method according to any one of claims 95-99, wherein transduction is accomplished without use of an exogenous B cell stimulating agent.

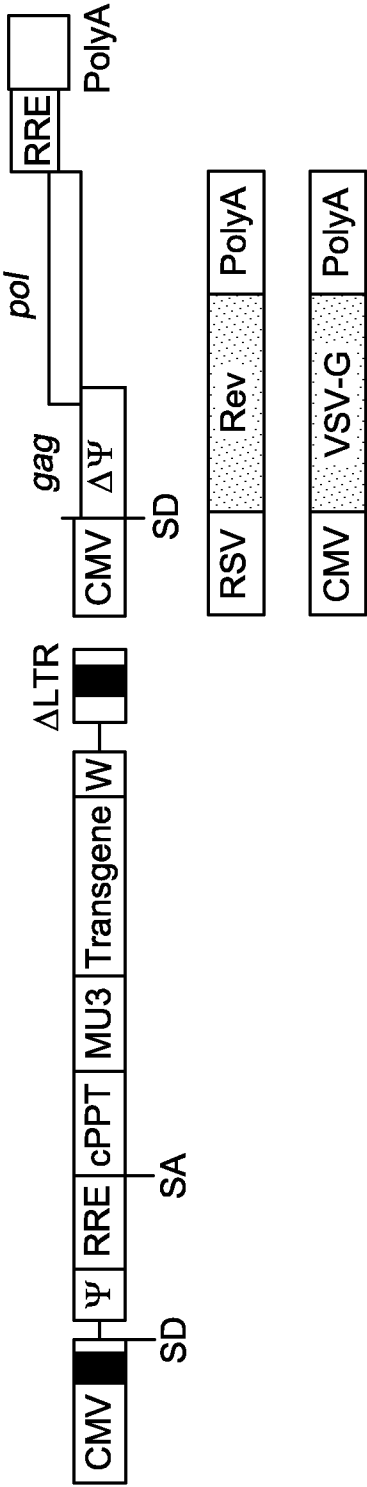


FIG. 1

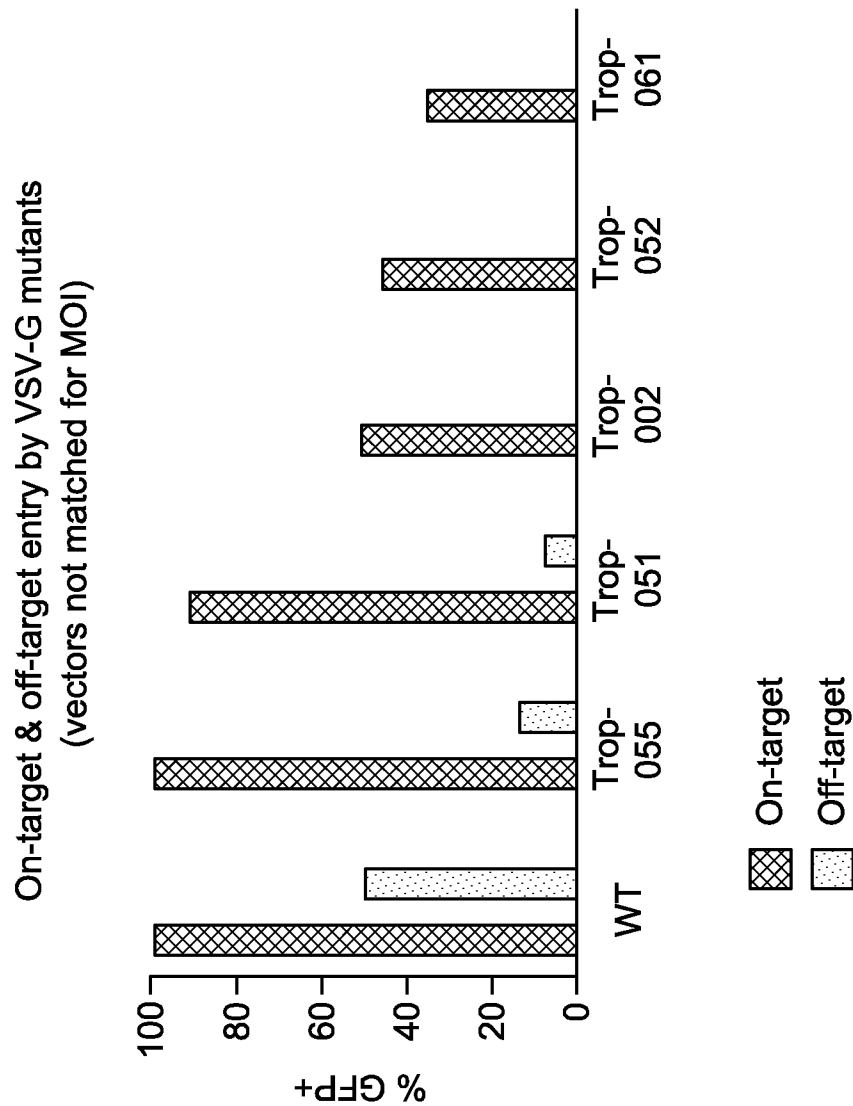
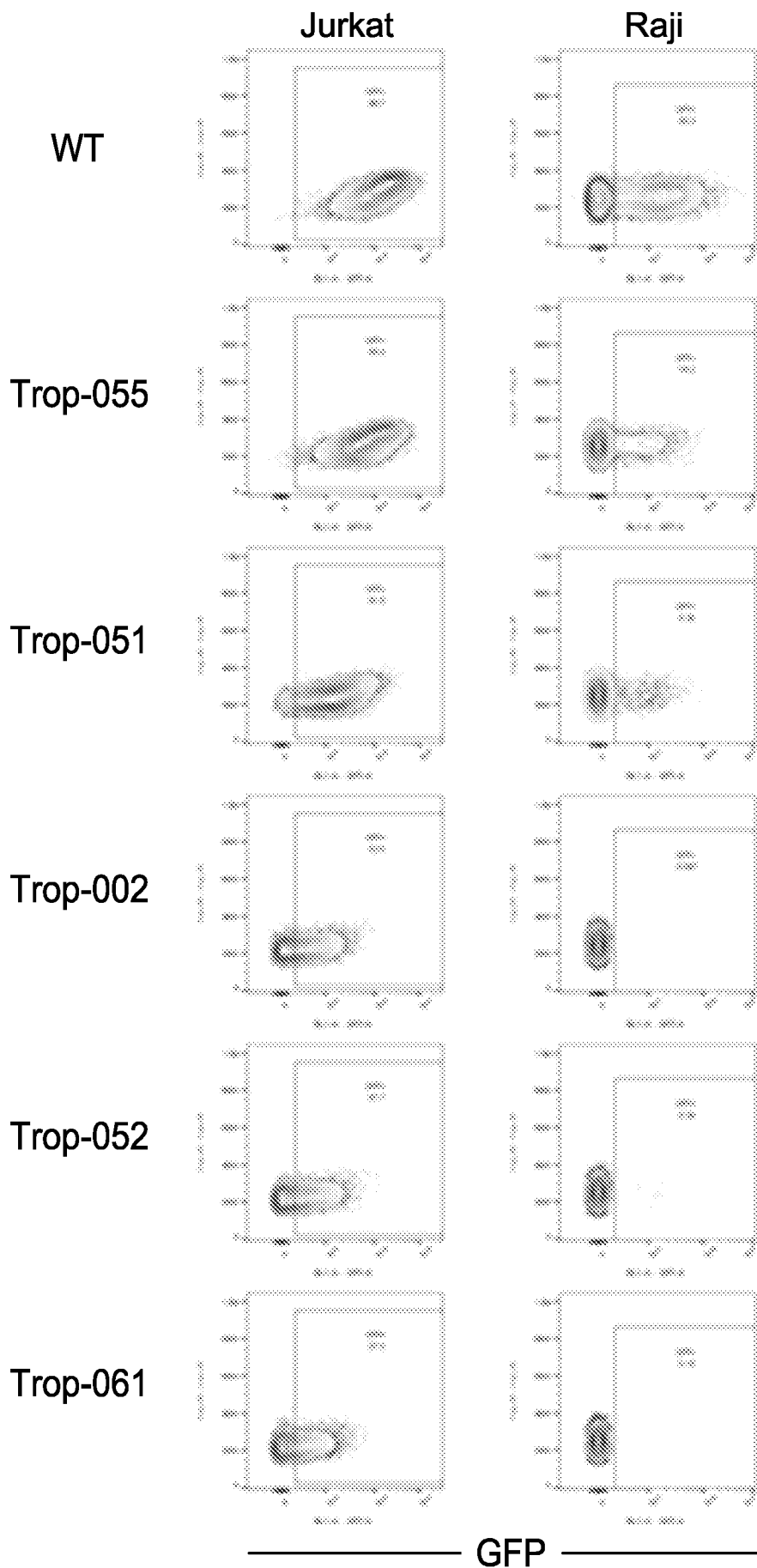


FIG. 2A

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**FIG. 2B**

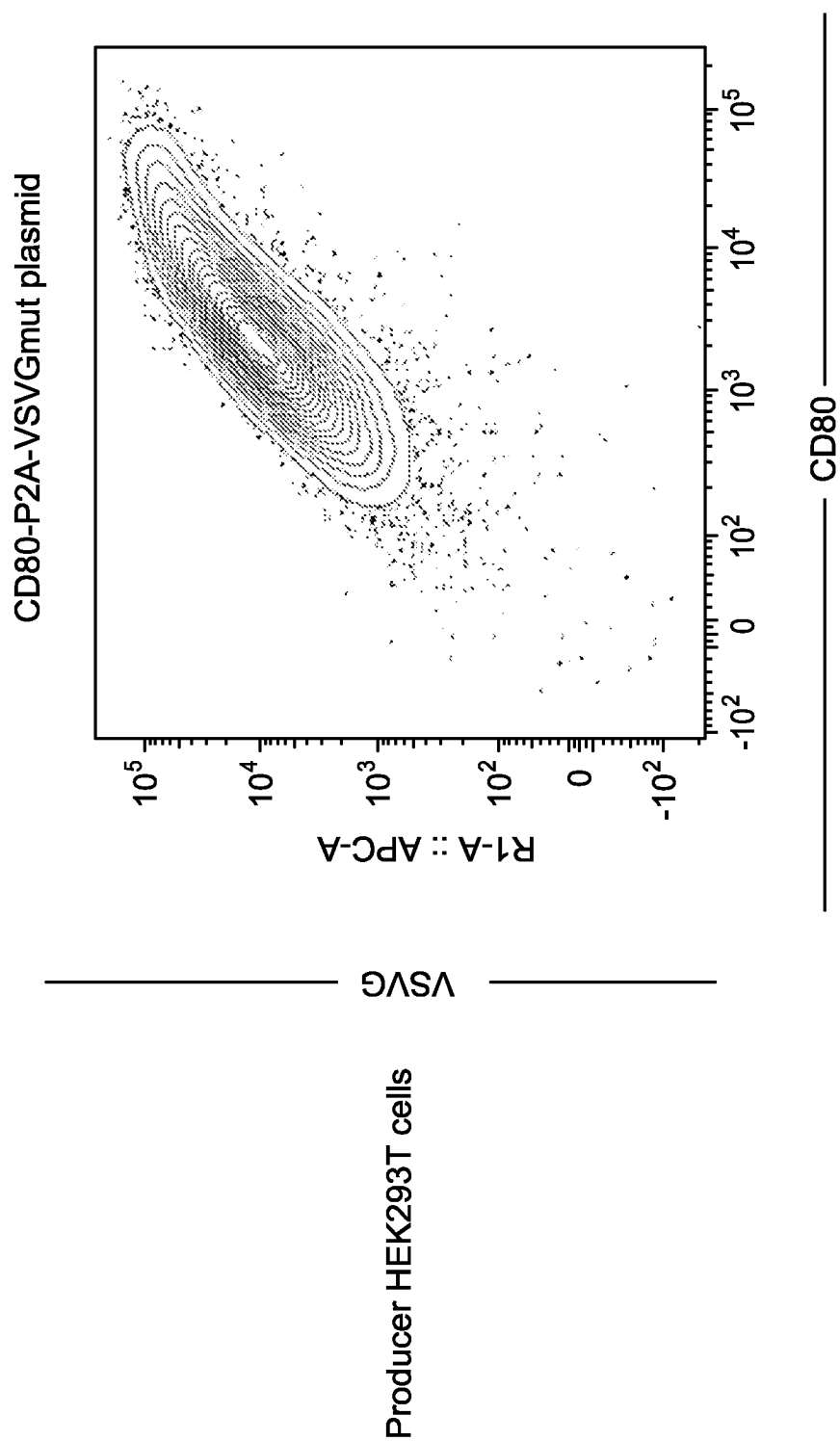
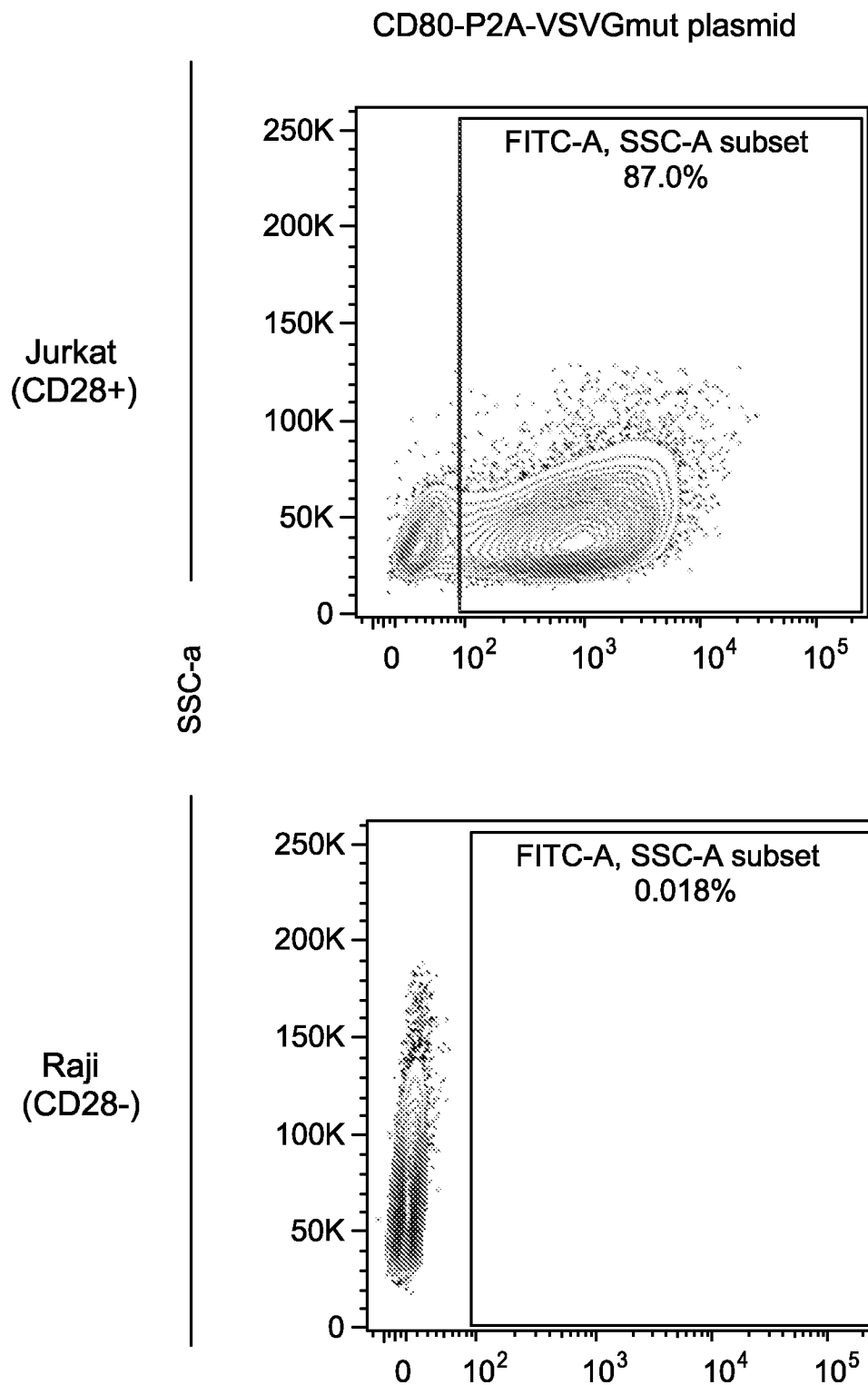


FIG. 3A

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**FIG. 3B**

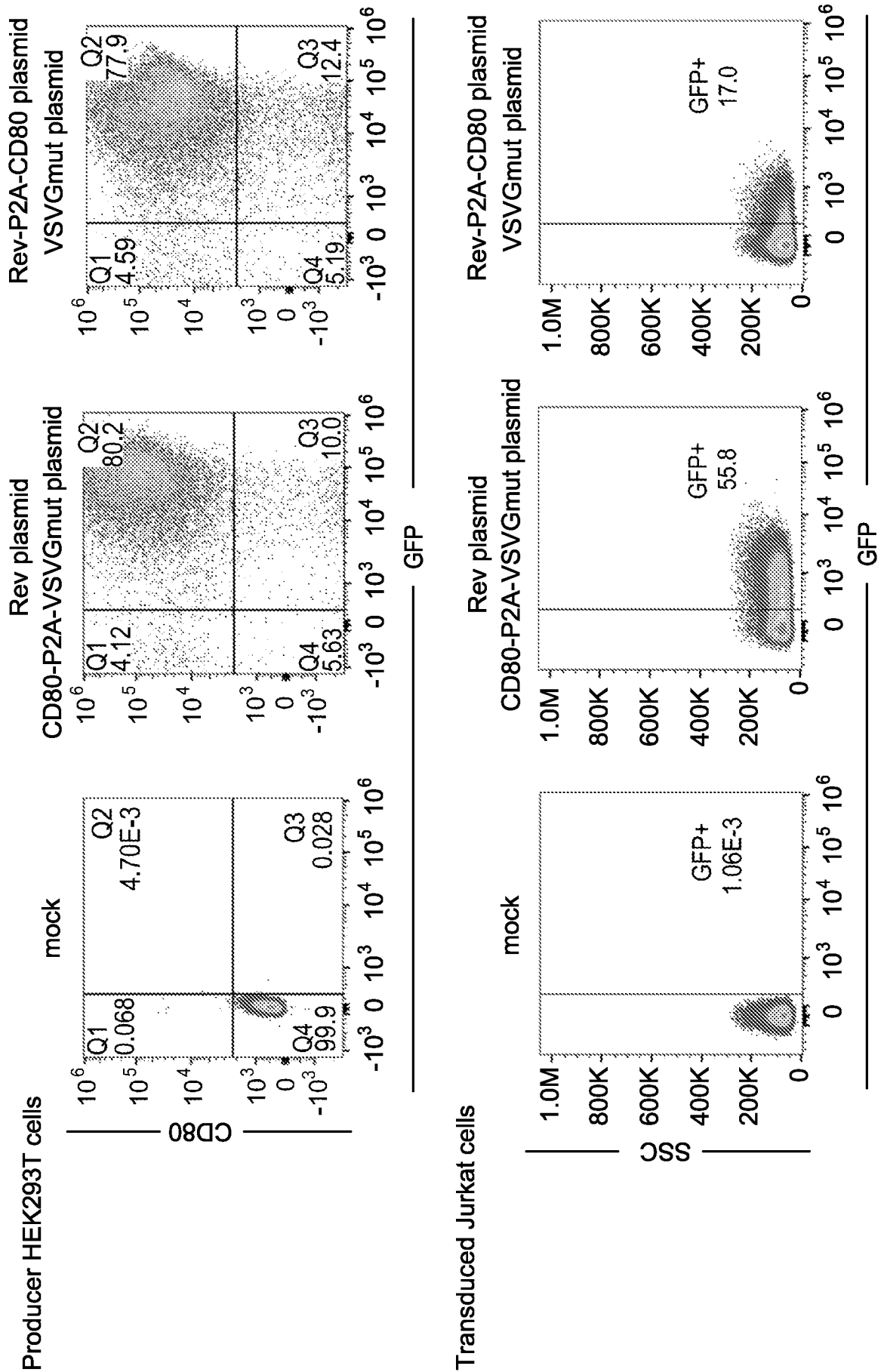


FIG. 4

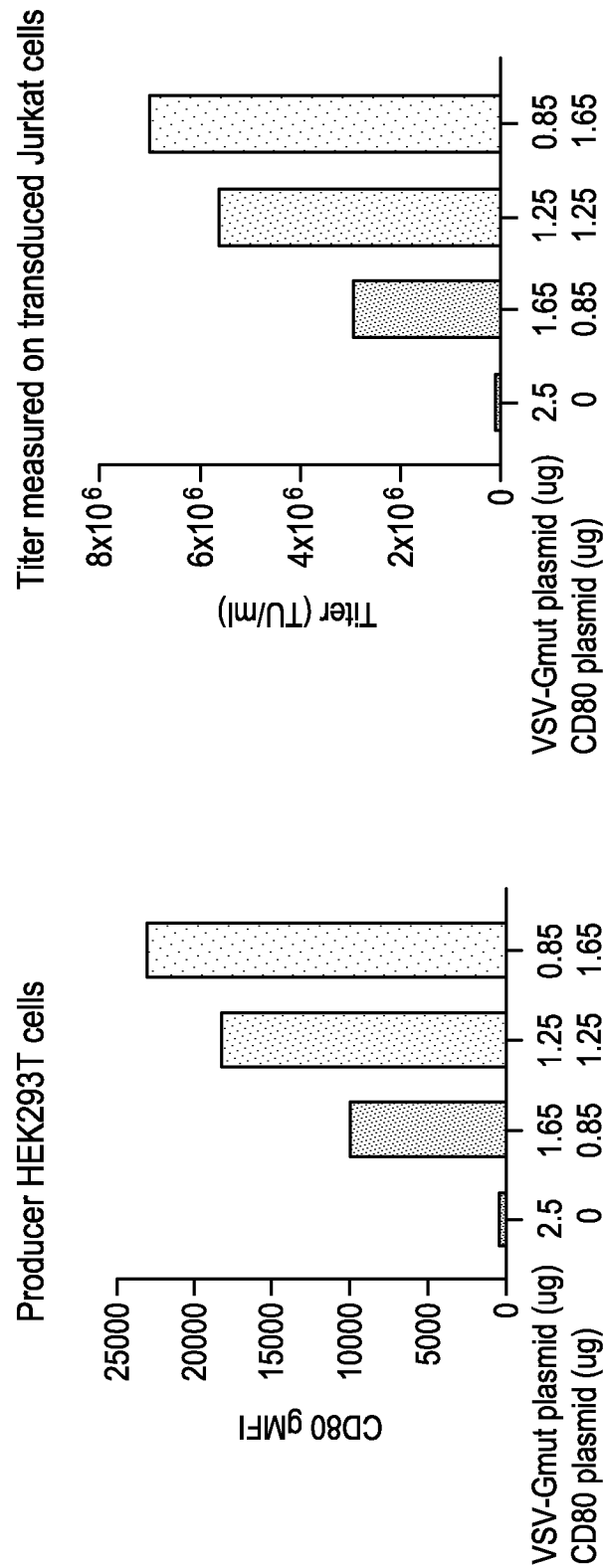


FIG. 5

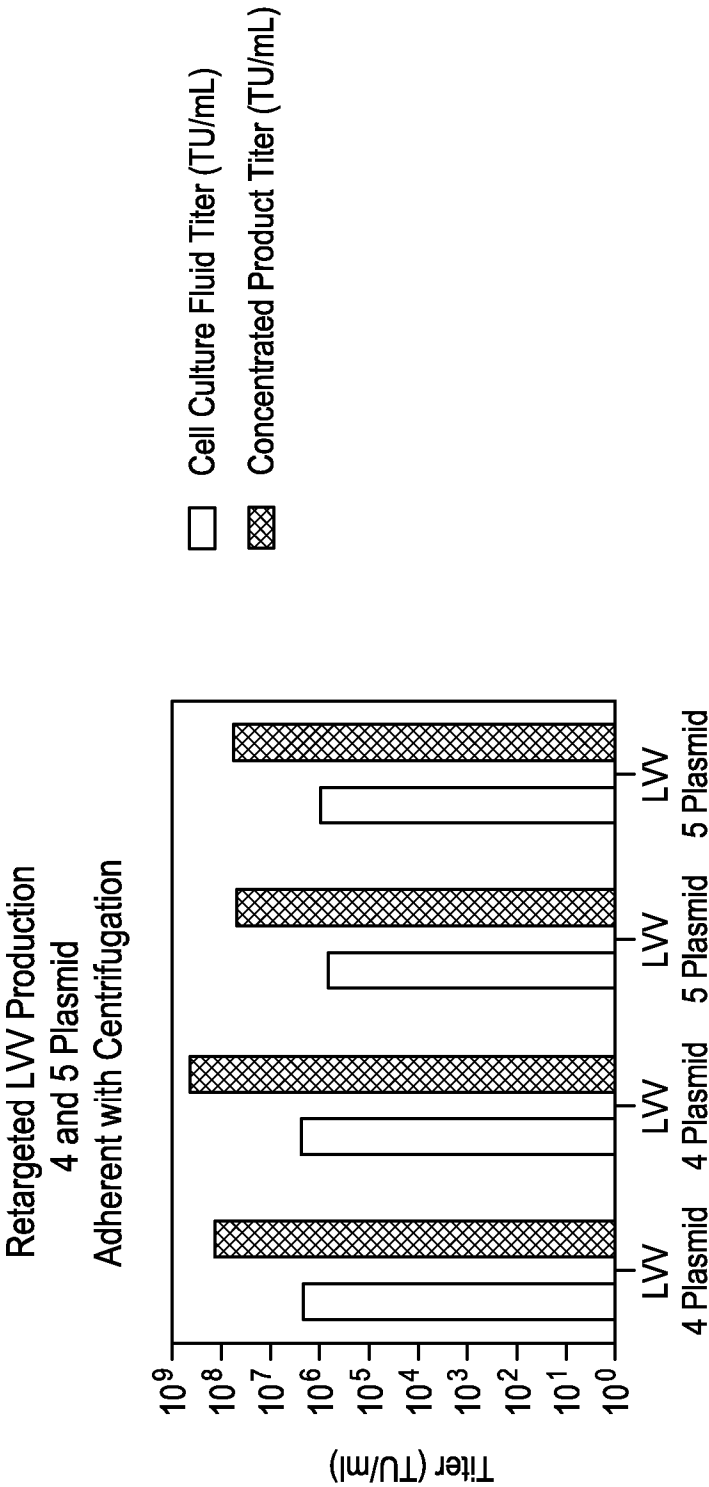


FIG. 6A

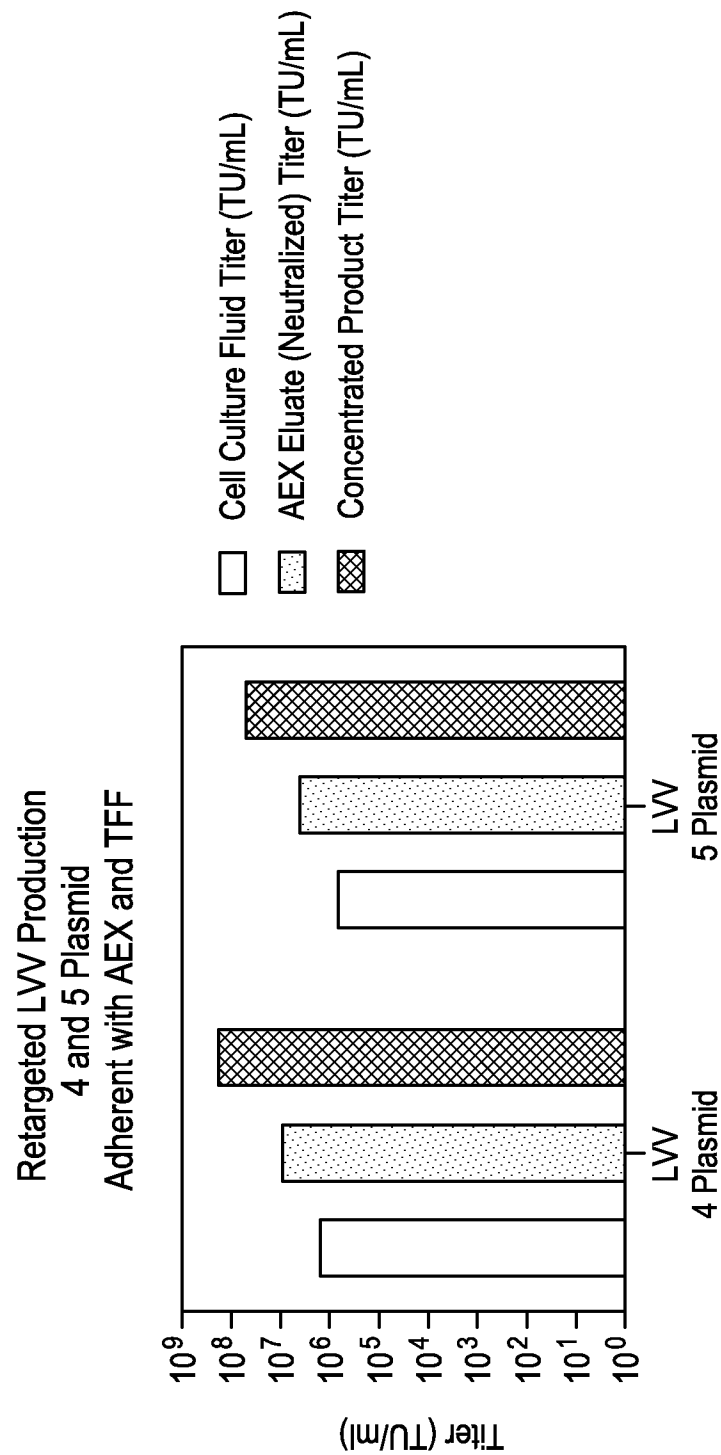


FIG. 6B

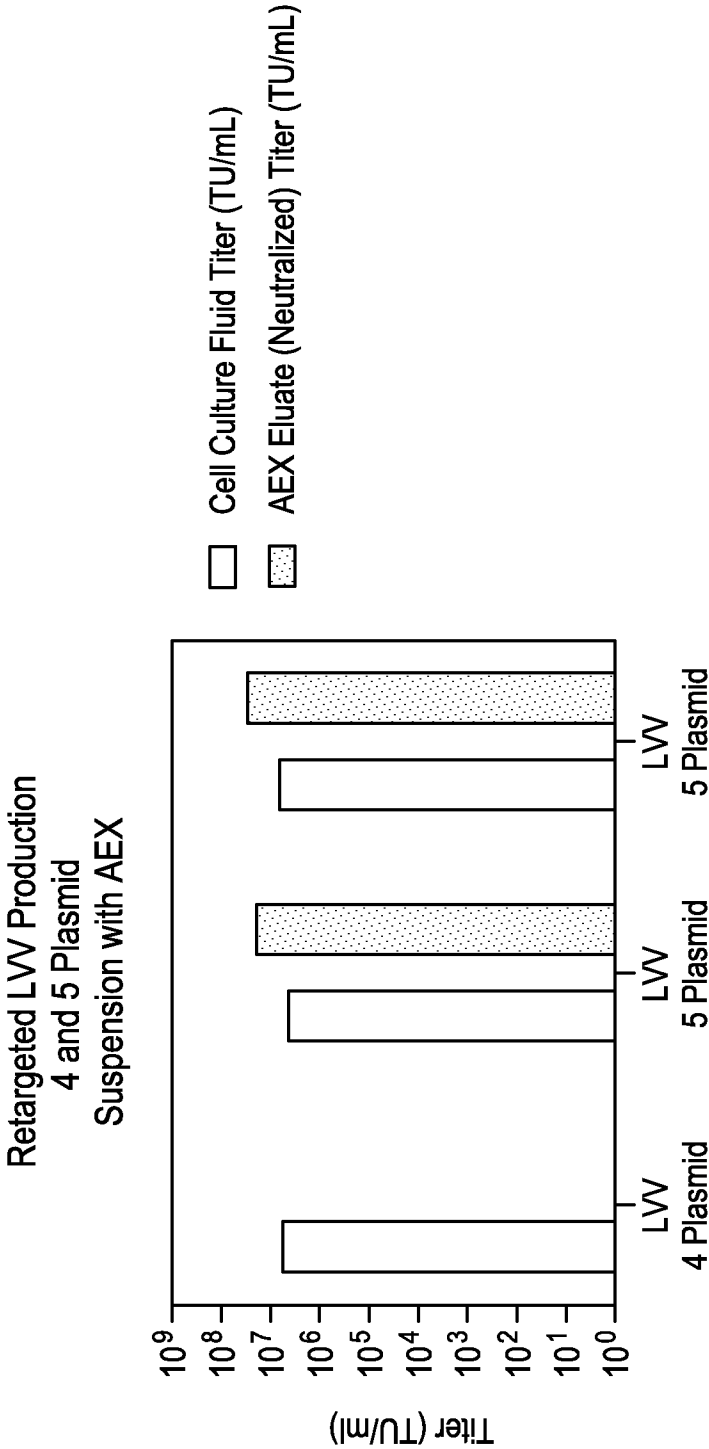


FIG. 6C

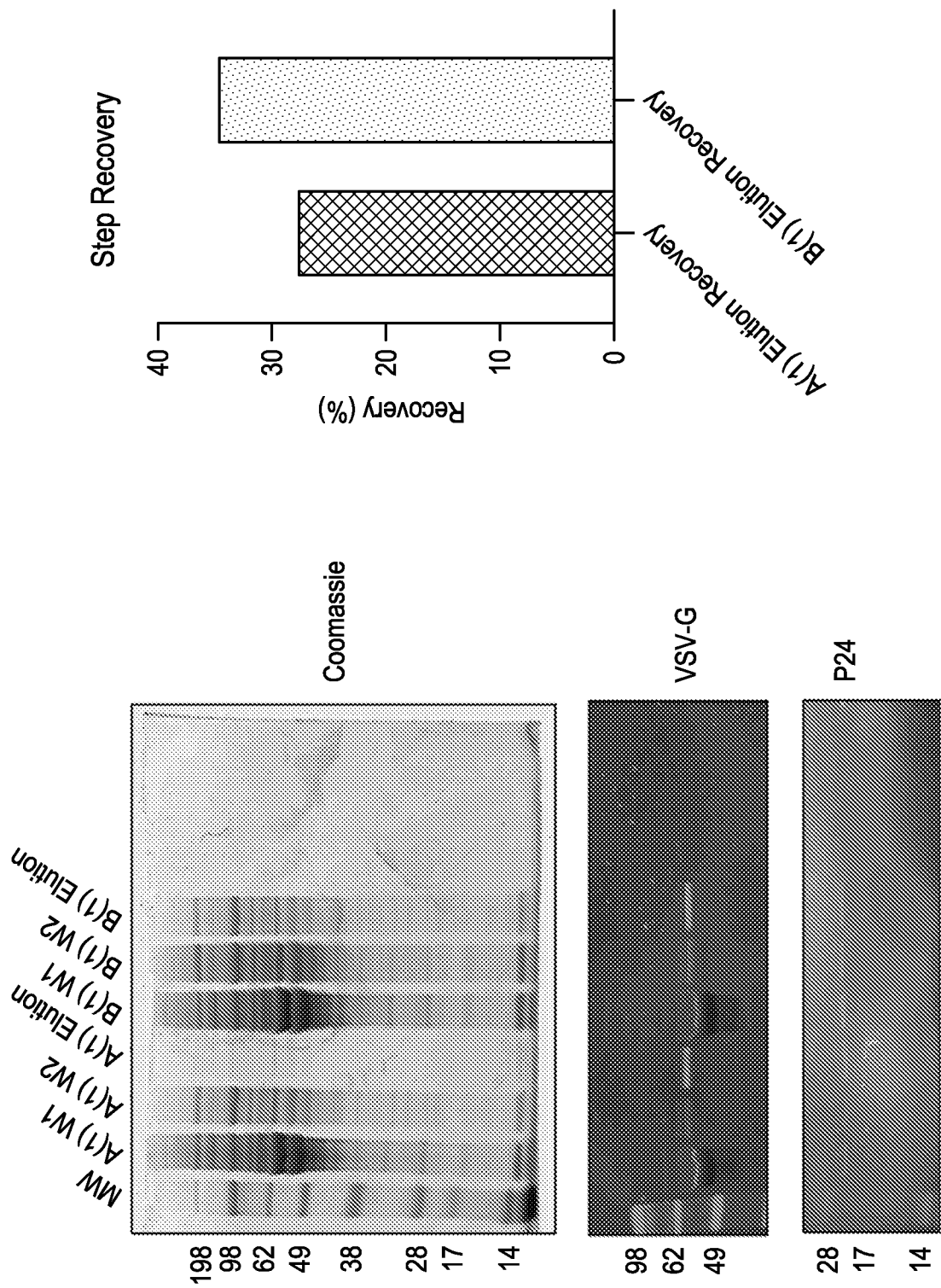
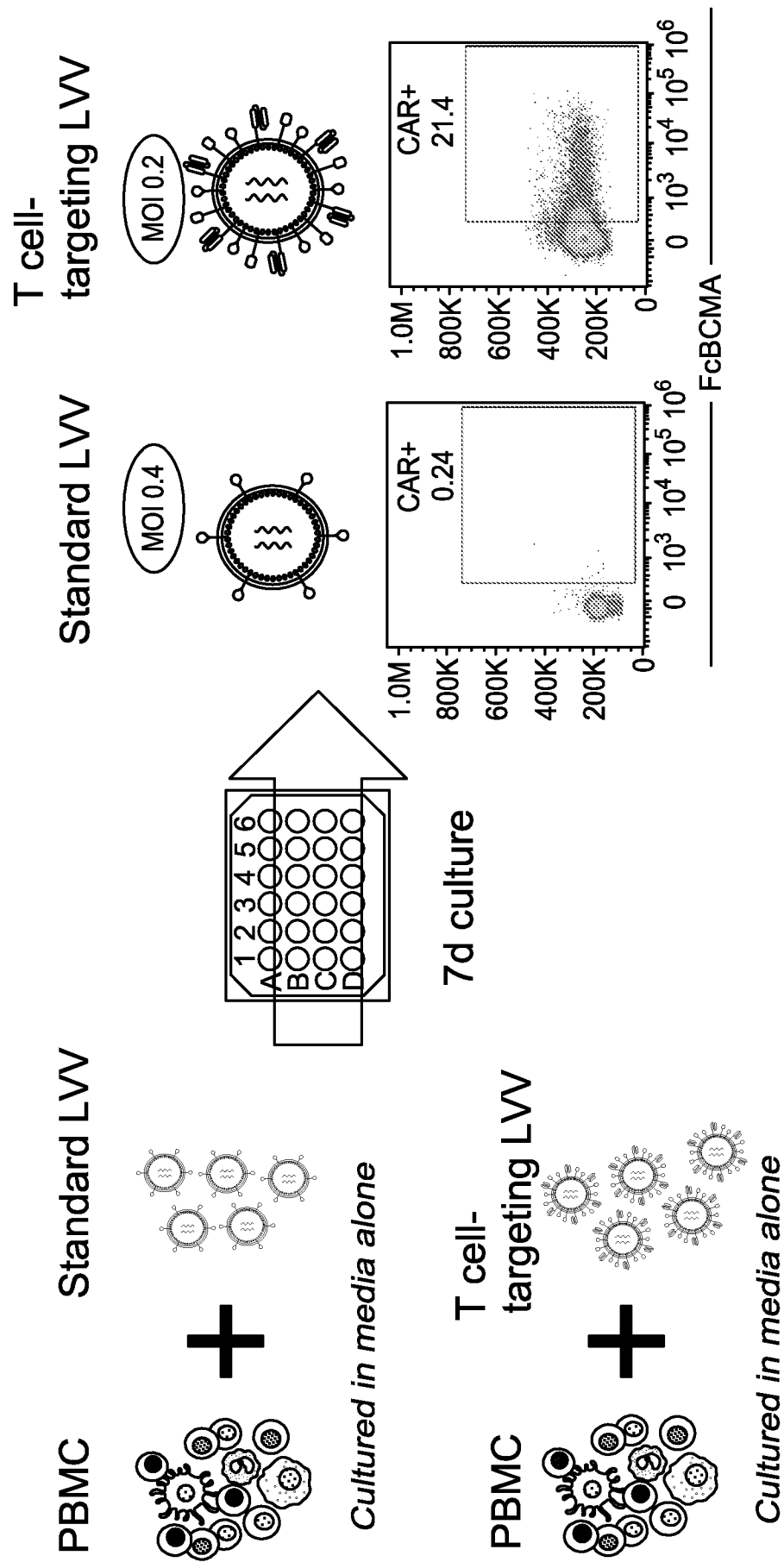


FIG. 6D



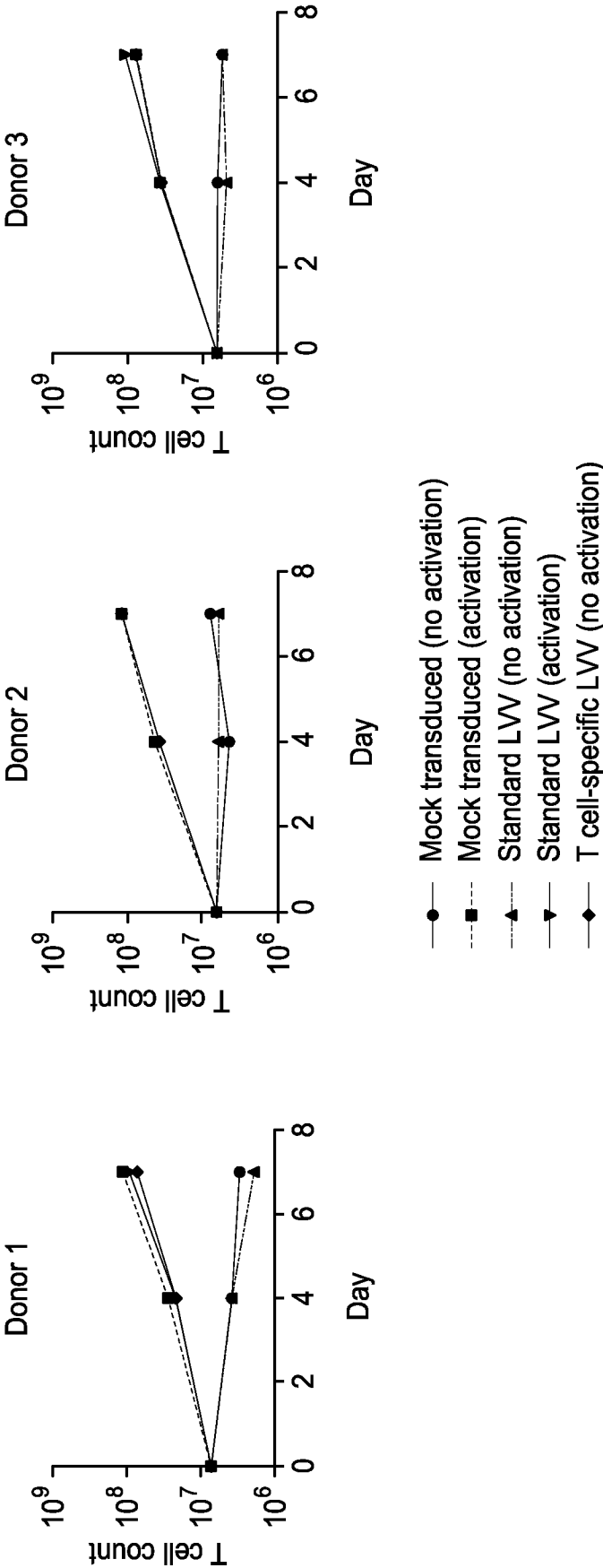


FIG. 8

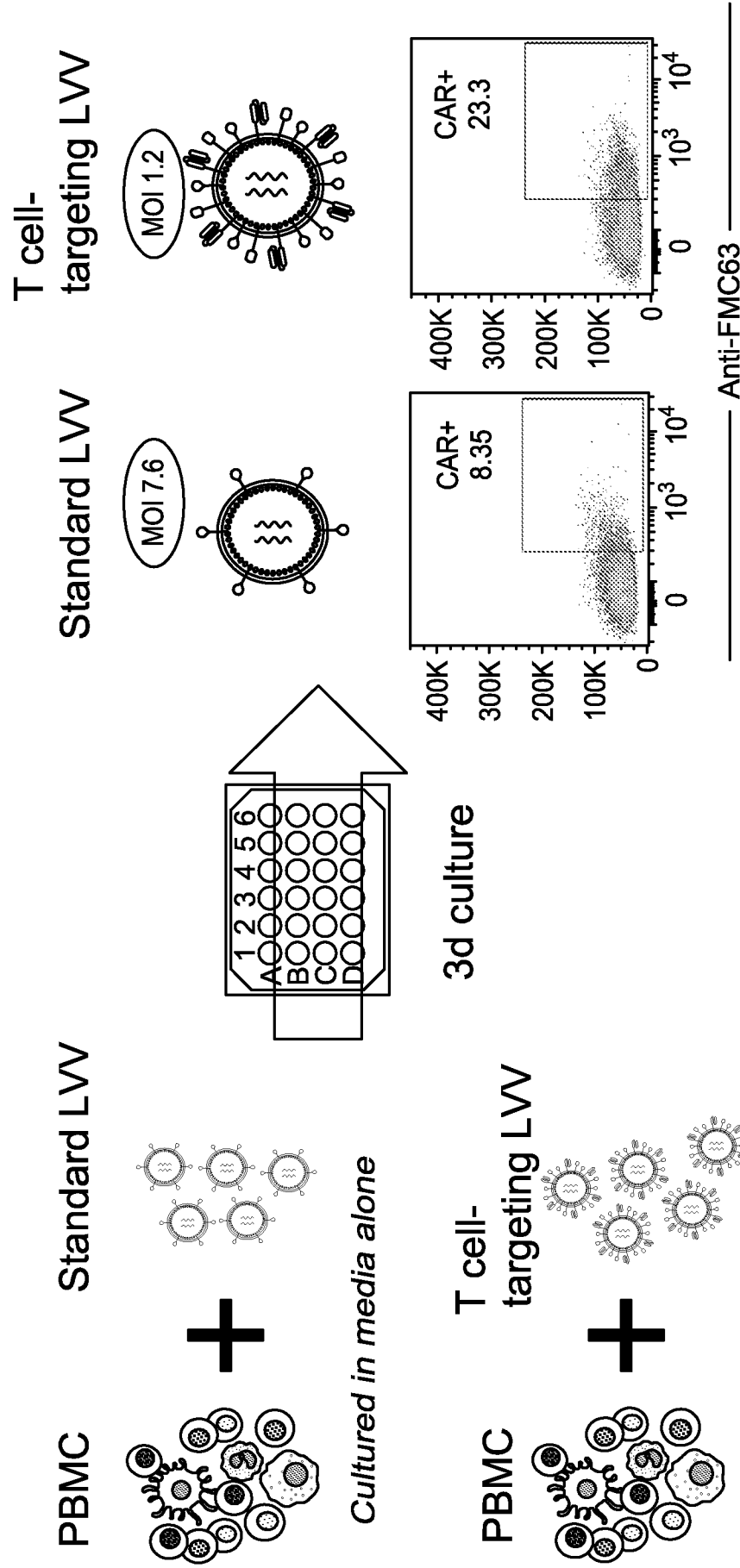


FIG. 9

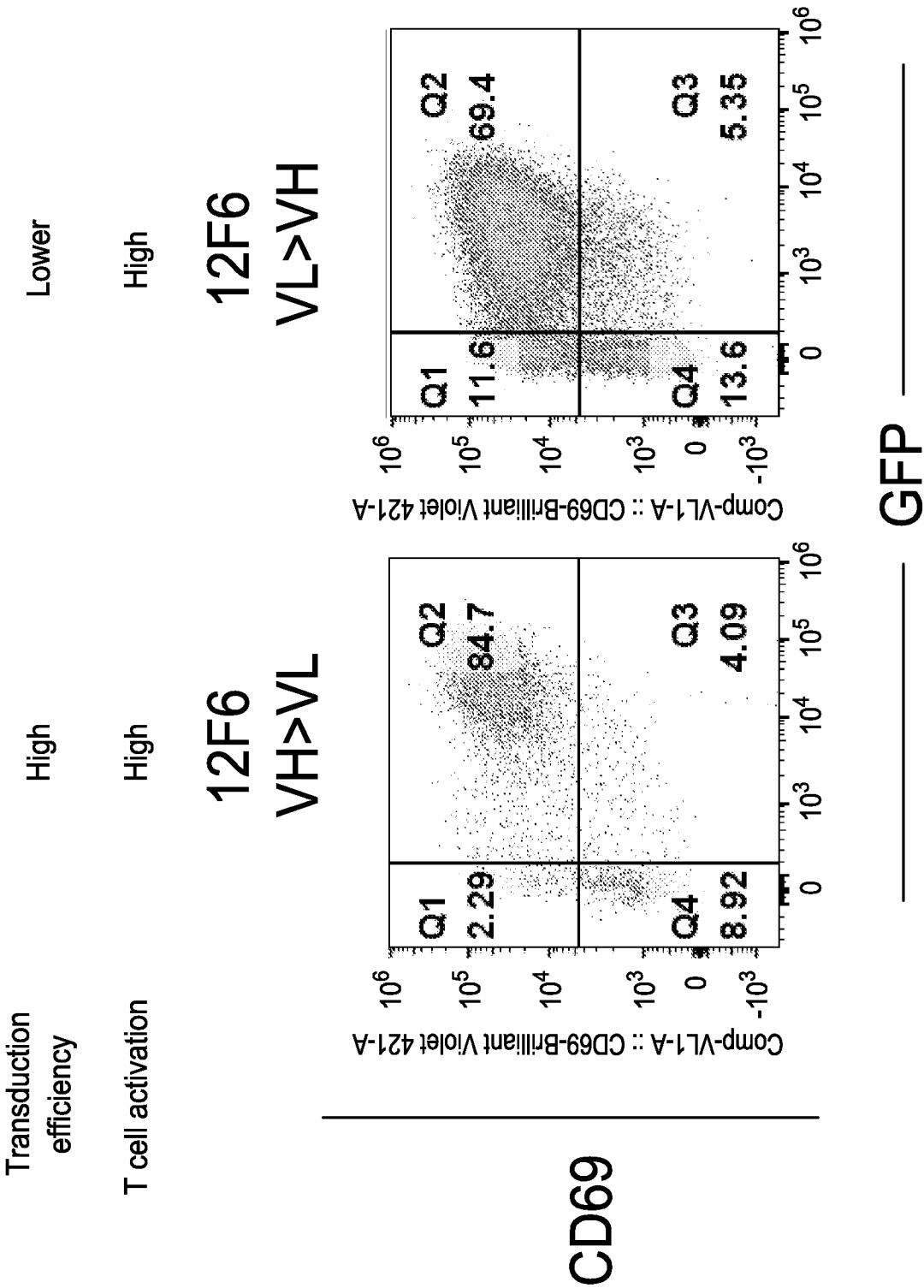


FIG. 10

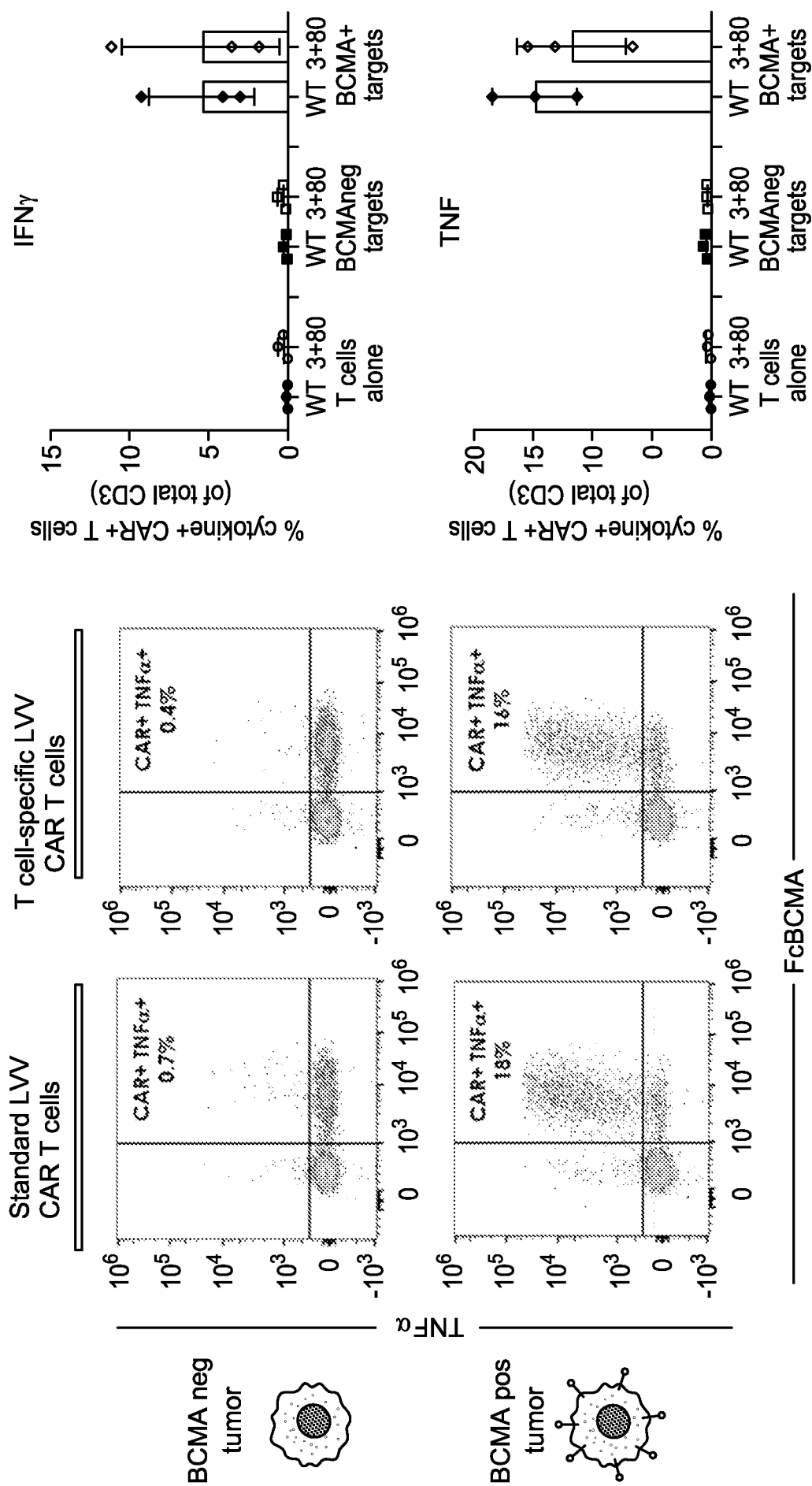


FIG. 11

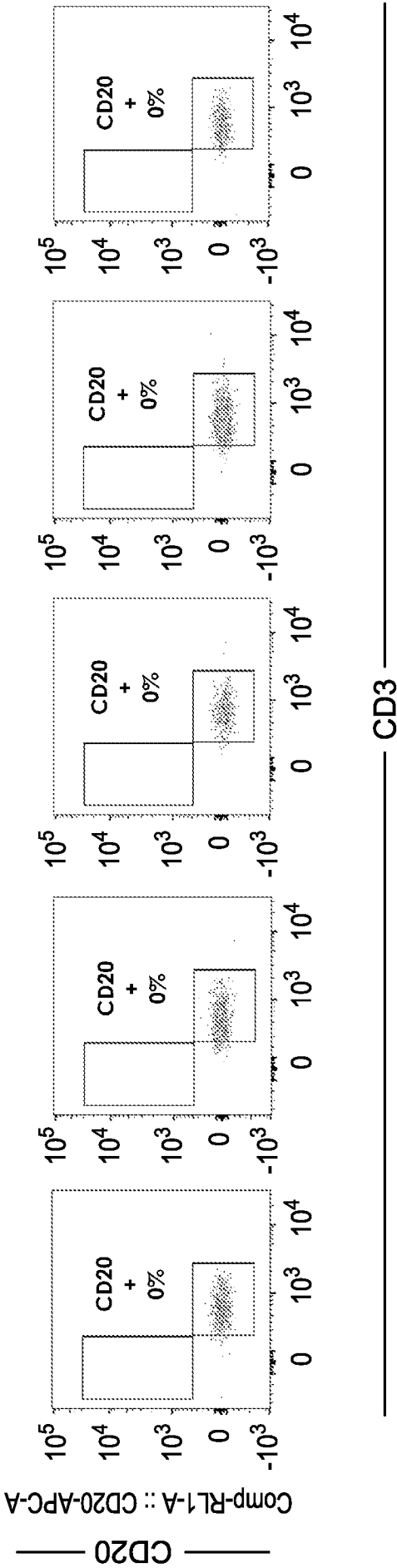


FIG. 12A

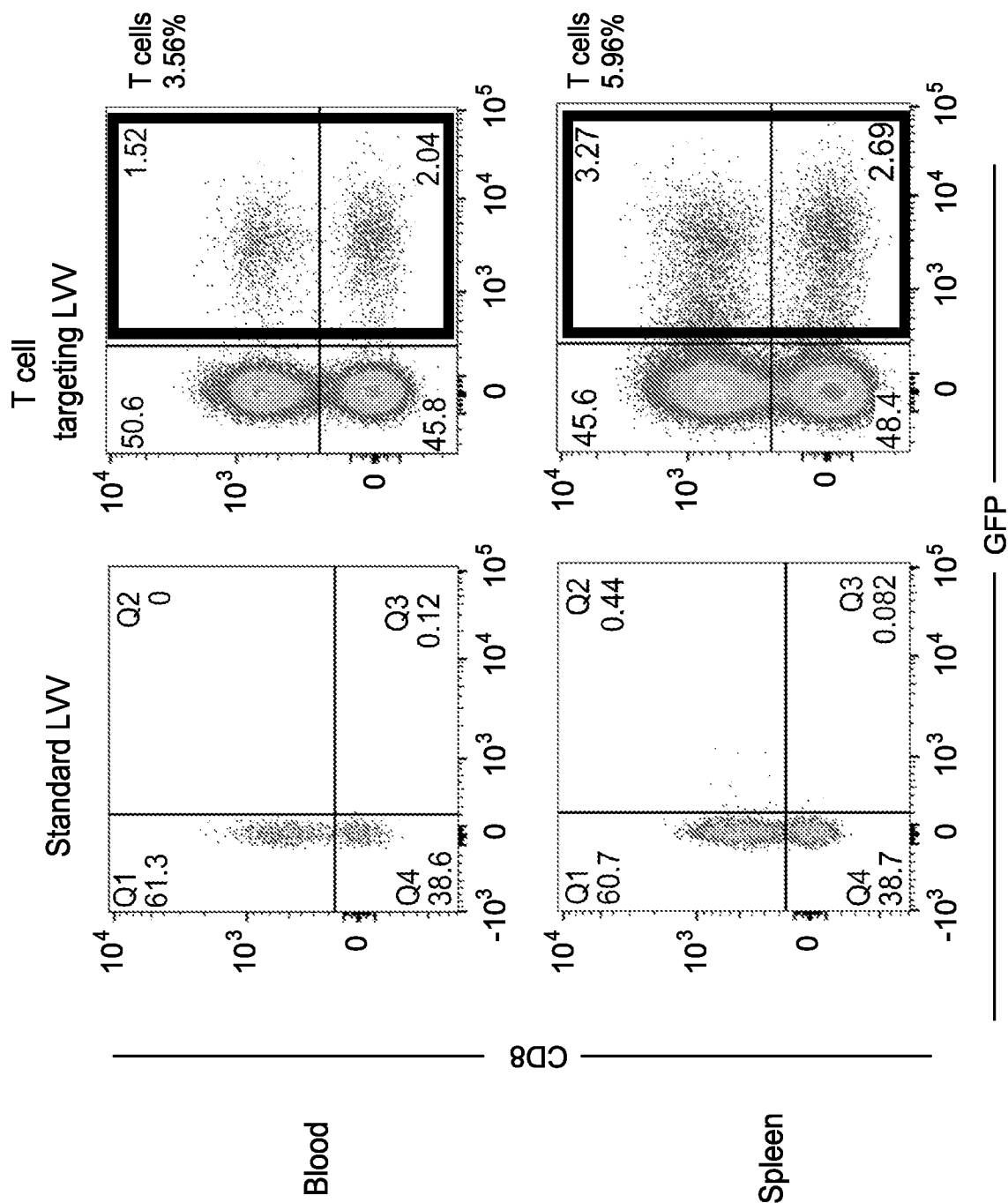


FIG. 12B

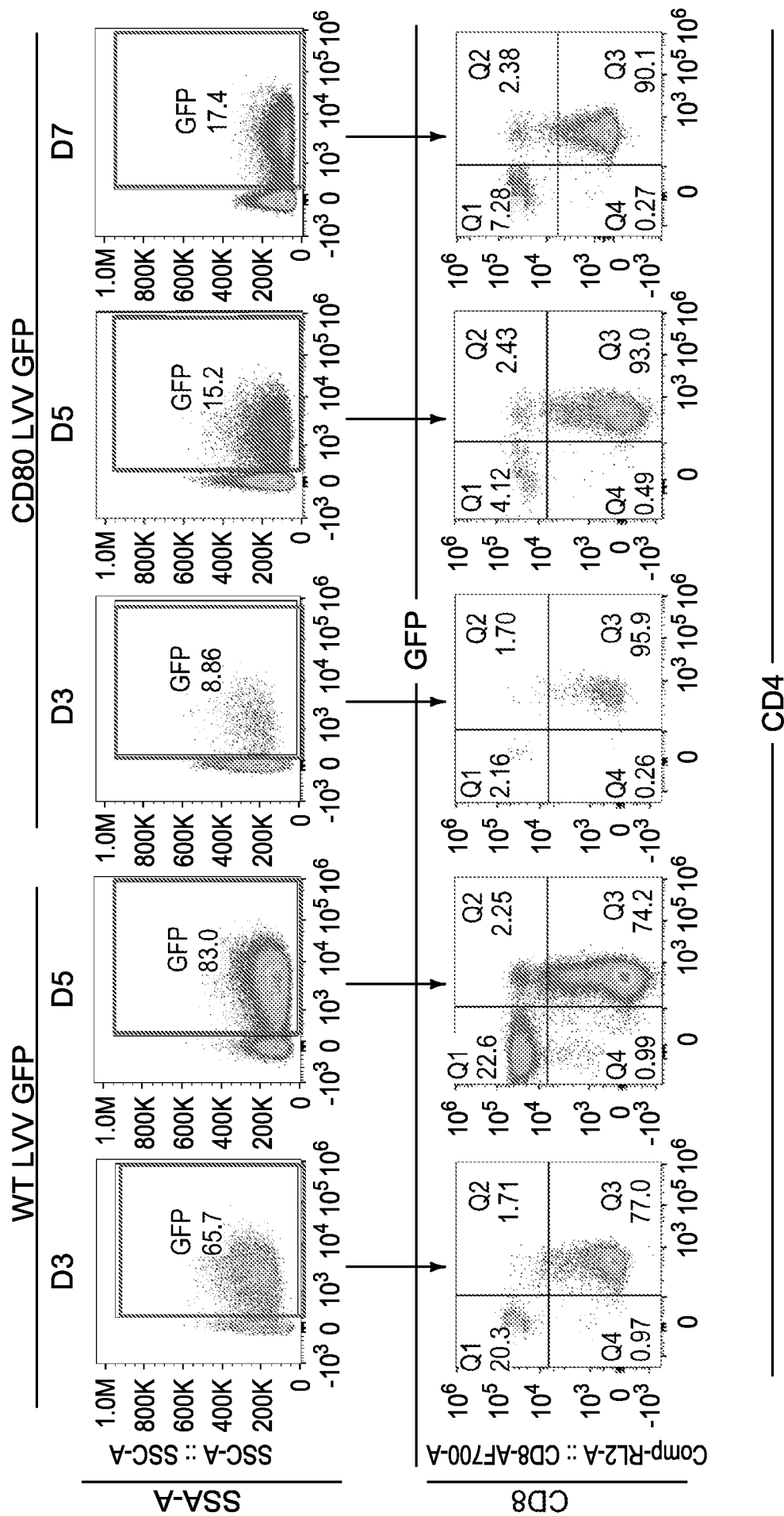


FIG. 13

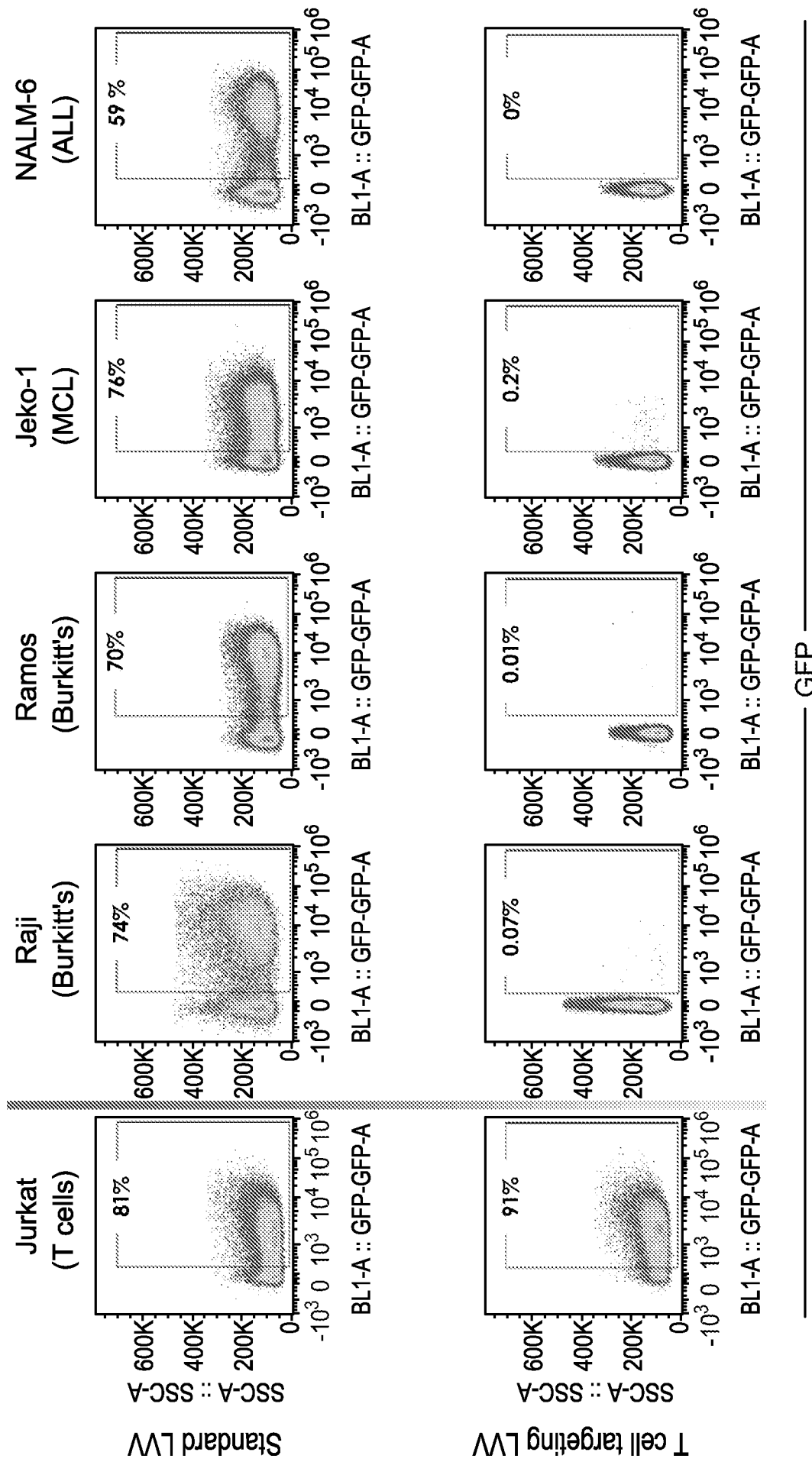


FIG. 14

International application No
PCT/US2022/018027

According to International Patent Classification (IPC) or to both national classification and IPC

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/371088 A1 (BIRNBAUM MICHAEL [US] ET AL) 26 November 2020 (2020-11-26)	1-18, 21-46, 49, 51-100
Y	abstract paragraphs [0004], [0006], [0009], [0010], [0013], [0014], [0019], [0055], [0057], [0064], [0067], [0069], [0097] examples 3-8, 10-12 figures 10, 17-22, 25, 27 ----- -/--	19, 20, 47, 48, 50

x See patent family annex.

"&" document member of the same patent family

Date of mailing of the international search report

21/06/2022

Authorized officer _____

Chavanne, Franz

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/018027

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOVAN NIKOLIC; BELOT LAURA; RAUX HÉLÈNE; LEGRAND PIERRE; GAUDIN YVES; ALBERTINI AURÉLIE A: "Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein", NATURE COMMUNICATIONS, vol. 9, no. 1, 12 March 2018 (2018-03-12), pages 1-12, XP055518690, DOI: 10.1038/s41467-018-03432-4 abstract pages 7-9 figures 4, 5 -----	19, 20, 47, 48, 50

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/018027

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/018027

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2020371088 A1	26-11-2020	AU 2020278470 A1	02-12-2021
		BR 112021023353 A2	01-02-2022
		CA 3140115 A1	26-11-2020
		CN 114127305 A	01-03-2022
		EP 3973065 A1	30-03-2022
		KR 20220012291 A	03-02-2022
		SG 11202112344V A	30-12-2021
		US 2020371088 A1	26-11-2020
		WO 2020236263 A1	26-11-2020
