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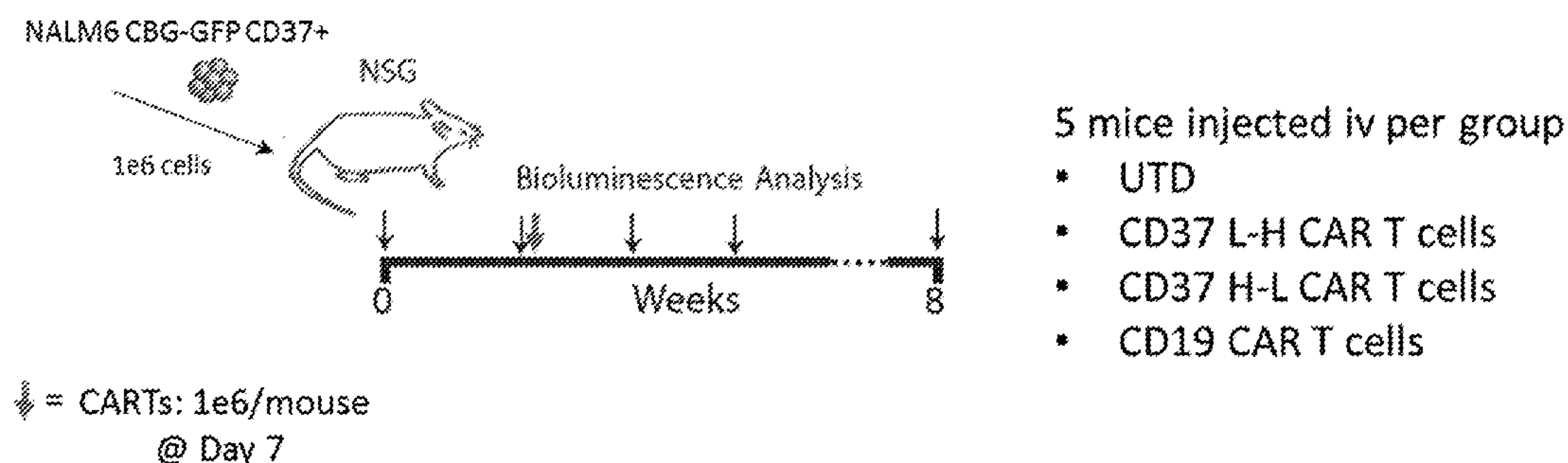
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(54) Titre : RECEPTEURS D'ANTIGENES CHIMERIQUES CIBLANT CD37
(54) Title: CHIMERIC ANTIGEN RECEPTORS TARGETING CD37

FIG. 8



(57) **Abrégé/Abstract:**

Described herein are chimeric antigen receptors (CARs) targeting CD37, as well as related molecules and methods.

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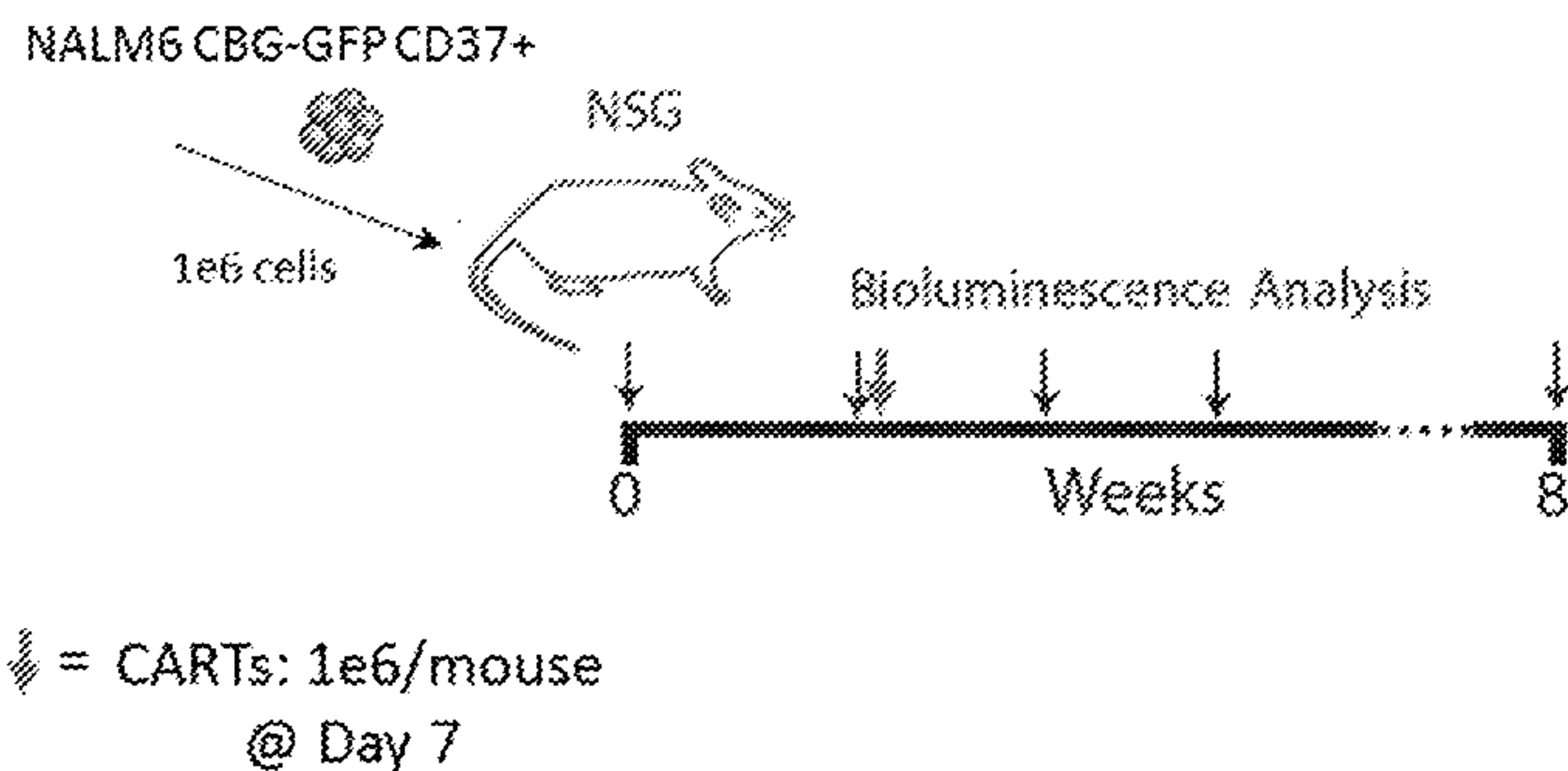
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(54) Title: CHIMERIC ANTIGEN RECEPTORS TARGETING CD37

FIG. 8



- 5 mice injected iv per group
- UTD
 - CD37 L-H CAR T cells
 - CD37 H-L CAR T cells
 - CD19 CAR T cells

(57) Abstract: Described herein are chimeric antigen receptors (CARs) targeting CD37, as well as related molecules and methods.

CHIMERIC ANTIGEN RECEPTORS TARGETING CD37**TECHNICAL FIELD**

[0001] The technology described herein relates to immunotherapy.

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BACKGROUND

[0002] Chimeric antigen receptor (CARs) provide a way to direct a cytotoxic T cell response to target cells expressing a selected target antigen, most often a tumor antigen or tumor-associated antigen. CARs are an adaptation of the T cell receptor, where the antigen binding domain is replaced with the antigen binding domain of an antibody that specifically binds the derived target antigen. Engagement of the target antigen on the surface of a target cell by a CAR expressed on a T cell ("CAR T cell" or "CAR-T") promotes killing of the target cell.

SUMMARY

15 [0003] The invention provides chimeric antigen receptor (CAR) polypeptides that each include: (a) an extracellular domain including a CD37-binding sequence; (b) a transmembrane domain; and (c) T cell intracellular signaling domain. The CAR polypeptides can each further include an optional co-stimulatory domain.

[0004] In various embodiments, the CD37-binding sequence includes an antibody reagent such as, for example, a single-chain antibody (scFv). The scFv can include an antibody light chain N-terminal to an antibody heavy chain, or the scFv can include an antibody heavy chain N-terminal to an antibody light chain. In specific examples, the antibody light chain includes the sequence of SEQ ID NO: 4 or 6, or a variant thereof, and/or the heavy chain includes the sequence of SEQ ID NO: 2 or 8, or a variant thereof. In other specific examples, the antibody reagent includes a sequence selected from SEQ ID NO: 1 or 5, or a variant thereof.

[0005] In various embodiments, transmembrane domain includes the transmembrane domain of CD8 or 4-1BB. In specific examples, the transmembrane domain includes the sequence of SEQ ID NO: 12 or 18, or a variant thereof. Other examples of transmembrane domains that can be included in the CAR polypeptides are provided below.

30 [0006] In various embodiments, the co-stimulatory domain includes the co-stimulatory domain of 4-1BB. In specific examples, the co-stimulatory domain includes the sequence of SEQ ID NO: 13 or 19, or a variant thereof. Other examples of co-stimulatory domains that can be included in the CAR polypeptides are provided below.

[0007] In various embodiments, the T cell intracellular domain includes a CD3 ζ intracellular signaling domain. In specific examples, the CD3 ζ intracellular signaling domain includes the sequence of SEQ ID NO: 14 or 20, or a variant thereof. In specific examples, the CD3 ζ intracellular signaling domain includes 1, 2, or 3 immunoreceptor tyrosine-based activation motifs (ITAMs), and the native tyrosine residues of the ITAM(s) are maintained.

[0008] In specific embodiments, the CAR polypeptide includes the sequence of SEQ ID NO: 9 or 15, or a variant thereof.

[0009] The invention also provides mammalian cells including: (a) a CAR polypeptide as described above or elsewhere herein, or (b) a nucleic acid encoding any one of the CAR polypeptides described herein or elsewhere herein.

[0010] In various embodiments, the cell is a T cell and/or a human cell. In various embodiments, the cell (e.g., a T cell) is obtained from an individual (e.g., a human) having or diagnosed as having cancer, a plasma cell disorder, or autoimmune disease.

[0011] The invention further provides methods of treating cancer, plasma cell disorders, or autoimmune diseases in subjects (e.g., human patients) in need thereof. These methods can include: (a) engineering a T cell to include or express a CAR polypeptide as described above or elsewhere herein on the T cell surface; and (b) administering the engineered T cell to the subject.

[0012] The invention additionally includes methods of treating cancer, plasma cell disorders, or autoimmune diseases in subjects (e.g., human patients) in need thereof. These methods can include administering a cell or cells as described above or elsewhere herein to the subject.

[0013] In various embodiments of the methods described above and elsewhere herein, the cancer is a CD37⁺ cancer. For example, the CD37⁺ cancer can be a lymphoma or a leukemia. In specific examples, the lymphoma is B-cell non-Hodgkin lymphoma (NHL), mantle cell lymphoma, Burkitt's lymphoma, B cell lymphoblastic lymphoma, or T cell lymphoma, or the leukemia is acute myeloid leukemia (AML). In another specific example, the T cell lymphoma is peripheral T cell lymphoma (PTCL), for example, cutaneous T-cell lymphoma (CTCL) or anaplastic large cell lymphoma (ALCL).

[0014] The invention also provides methods of treating cancer, plasma cell disorders, or autoimmune diseases in subjects (e.g., human patients) in need thereof. These methods include administering a cell as described above and elsewhere herein to the subject, wherein the cell includes a CAR including an extracellular domain including a CD37-binding sequence (e.g., as described herein) and the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[0015] Further, the invention provides methods of treating cancer, plasma cell disorders, or autoimmune diseases in a subject (e.g., a human patient) in need thereof. These methods

include: (a) selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy; (b) engineering a T cell to include a CAR polypeptide as described above or elsewhere herein; and (c) administering the engineered T cell to the subject; wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

5 [0016] The invention additionally provides methods of treating cancer, plasma cell disorders, or autoimmune diseases in subjects (e.g., human patients) in need thereof. These methods include (a) selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy; (b) administering a cell as described above or elsewhere herein to the subject, wherein the cell includes a CAR including an extracellular domain including a CD37-binding sequence (e.g., as
10 described herein); wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[0017] The invention further provides methods of treating cancer, plasma cell disorders, or autoimmune diseases in subjects (e.g., human patients) in need thereof. These methods include (a) engineering a T cell to include or express a CAR polypeptide described above or elsewhere herein on the T cell surface; and (b) administering the engineered T cell to the subject; wherein
15 the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

[0018] Also provided by the invention are methods of treating cancer, plasma cell disorders, or autoimmune diseases in subjects (e.g., human patients) in need thereof. These methods include administering a cell as described above or elsewhere herein to the subject, wherein the cell includes a CAR including an extracellular domain including a CD37-binding sequence (e.g.,
20 as described herein); wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

[0019] The invention also provides compositions including one or more of the CAR polypeptides, nucleic acid molecules, or cells (e.g., T cells) described above or elsewhere herein formulated for the treatment of cancer. The compositions can further include a pharmaceutically
25 acceptable carrier.

[0020] The invention further provides the use of the polypeptides, nucleic acid molecules, compositions, and cells described herein in the treatment or prevention of the diseases or conditions described herein, as well as the use of these polypeptides, nucleic acid molecules, compositions, and cells for the preparation of medicaments for preventing or treating such
30 diseases or conditions.

[0021] *Definitions*

[0022] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions

are provided to aid in describing particular embodiments, and are not intended to limit the claimed technology, because the scope of the technology is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[0023] Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter et al. (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of each of which are all incorporated by reference herein in their entireties.

[0024] The terms "decrease," "reduced," "reduction," or "inhibit" are all used herein to mean a decrease by a statistically significant amount. In some embodiments, "reduce," "reduction," or "decrease" or "inhibit" typically means a decrease by at least 10% as compared to a reference level (e.g., the absence of a given treatment or agent) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at

least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99% ,
or more. As used herein, “reduction” or “inhibition” does not encompass a complete inhibition
or reduction as compared to a reference level. “Complete inhibition” is a 100% inhibition as
compared to a reference level. Where applicable, a decrease can be preferably down to a level
5 accepted as within the range of normal for an individual without a given disorder.

[0025] The terms “increased,” “increase,” “enhance,” or “activate” are all used herein to
mean an increase by a statically significant amount. In some embodiments, the terms
“increased,” “increase,” “enhance,” or “activate” can mean an increase of at least 10% as
compared to a reference level, for example, an increase of at least about 20%, or at least about
10 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or
at least about 80%, or at least about 90% or up to and including a 100% increase or any increase
between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a
3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or
any increase between 2-fold and 10-fold or greater as compared to a reference level. In the
15 context of a marker or symptom, an “increase” is a statistically significant increase in such level.

[0026] As used herein, a “subject” means a human or animal. Usually the animal is a
vertebrate such as a primate, rodent, domestic animal or game animal. Primates include, for
example, chimpanzees, cynomolgous monkeys, spider monkeys, and macaques, e.g., rhesus.
Rodents include, for example, mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic
20 and game animals include, for example, cows, horses, pigs, deer, bison, buffalo, feline species,
e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich,
and fish, e.g., trout, catfish and salmon. In some embodiments, the subject is a mammal, e.g., a
primate, e.g., a human. The terms, “individual,” “patient,” and “subject” are used
interchangeably herein.

25 [0027] Preferably, the subject is a mammal. The mammal can be a human, non-human
primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals
other than humans can be advantageously used as subjects that represent animal models of
disease, e.g., cancer. A subject can be male or female.

[0028] A subject can be one who has been previously diagnosed with or identified as
30 suffering from or having a condition in need of treatment (e.g., leukemia or another type of
cancer, among others) or one or more complications related to such a condition, and optionally,
have already undergone treatment for the condition or the one or more complications related to
the condition. Alternatively, a subject can also be one who has not been previously diagnosed as
having such condition or related complications. For example, a subject can be one who exhibits

one or more risk factors for the condition or one or more complications related to the condition or a subject who does not exhibit risk factors.

[0029] A “subject in need” of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at risk of developing that condition.

5 [0030] A “disease” is a state of health of an animal, for example, a human, wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated, then the animal's health continues to deteriorate. In contrast, a “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not
10 necessarily cause a further decrease in the animal's state of health.

[0031] As used herein, the terms “tumor antigen” and “cancer antigen” are used interchangeably to refer to antigens that are differentially expressed by cancer cells and can thereby be exploited in order to target cancer cells. Cancer antigens are antigens that can potentially stimulate apparently tumor-specific immune responses. Some of these antigens are
15 encoded, although not necessarily expressed, by normal cells. These antigens can be characterized as those which are normally silent (i.e., not expressed) in normal cells, those that are expressed only at certain stages of differentiation and those that are temporally expressed such as embryonic and fetal antigens. Other cancer antigens are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant p53), and
20 fusion proteins resulting from internal deletions or chromosomal translocations. Still other cancer antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses. Many tumor antigens have been defined in terms of multiple solid tumors: MAGE 1, 2, & 3, defined by immunity; MART-1/Melan-A, gp100, carcinoembryonic antigen (CEA), HER2, mucins (i.e., MUC-1), prostate-specific antigen (PSA), and prostatic acid phosphatase (PAP). In
25 addition, viral proteins such as some encoded by hepatitis B (HBV), Epstein-Barr (EBV), and human papilloma (HPV) have been shown to be important in the development of hepatocellular carcinoma, lymphoma, and cervical cancer, respectively.

[0032] As used herein, the term “chimeric” refers to the product of the fusion of portions of at least two or more different polynucleotide molecules. In one embodiment, the term
30 “chimeric” refers to a gene expression element produced through the manipulation of known elements or other polynucleotide molecules

[0033] In some embodiments, “activation” can refer to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. In some embodiments activation can refer to induced cytokine production. In other embodiments, activation can refer

to detectable effector functions. At a minimum, an “activated T cell” as used herein is a proliferative T cell.

[0034] As used herein, the terms “specific binding” and “specifically binds” refer to a physical interaction between two molecules, compounds, cells and/or particles wherein the first
 5 entity binds to the second, target, entity with greater specificity and affinity than it binds to a third entity which is a non-target. In some embodiments, specific binding can refer to an affinity of the first entity for the second target, entity, which is at least 10 times, at least 50 times, at least 100 times, at least 500 times, at least 1000 times or more greater than the affinity for the third non-target entity under the same conditions. A reagent specific for a given target is one that
 10 exhibits specific binding for that target under the conditions of the assay being utilized. A non-limiting example includes an antibody, or a ligand, which recognizes and binds with a cognate binding partner (for example, a stimulatory and/or costimulatory molecule present on a T cell) protein.

[0035] A “stimulatory ligand,” as used herein, refers to a ligand that when present on an
 15 antigen presenting cell (APC, e.g., a macrophage, a dendritic cell, a B-cell, an artificial APC, and the like) can specifically bind with a cognate binding partner (referred to herein as a “stimulatory molecule” or “co-stimulatory molecule”) on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, proliferation, activation, initiation of an immune response, and the like. Stimulatory ligands are well-known in the art and encompass, inter alia,
 20 an MHC Class I molecule loaded with a peptide, an anti-CD3 antibody, a superagonist anti-CD28 antibody, and a superagonist anti-CD2 antibody.

[0036] A “stimulatory molecule,” as the term is used herein, means a molecule on a T cell that specifically binds with a cognate stimulatory ligand present on an antigen presenting cell.

[0037] “Co-stimulatory ligand,” as the term is used herein, includes a molecule on an APC
 25 that specifically binds a cognate co-stimulatory molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A co-stimulatory ligand can include, but is not limited to, 4-1BBL, OX40L, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-
 30 L2, inducible COStimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll-like receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also can include, but is not limited to, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited

to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83.

[0038] A “co-stimulatory molecule” refers to the cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the T cell, such as, but not limited to, proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA, a Toll-like receptor, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and CD83.

[0039] In one embodiment, the term “engineered” and its grammatical equivalents as used herein can refer to one or more human-designed alterations of a nucleic acid, e.g., the nucleic acid within an organism’s genome. In another embodiment, engineered can refer to alterations, additions, and/or deletion of genes. An “engineered cell” can refer to a cell with an added, deleted and/or altered gene. The term “cell” or “engineered cell” and their grammatical equivalents as used herein can refer to a cell of human or non-human animal origin.

[0040] As used herein, the term “operably linked” refers to a first polynucleotide molecule, such as a promoter, connected with a second transcribable polynucleotide molecule, such as a gene of interest, where the polynucleotide molecules are so arranged that the first polynucleotide molecule affects the function of the second polynucleotide molecule. The two polynucleotide molecules may or may not be part of a single contiguous polynucleotide molecule and may or may not be adjacent. For example, a promoter is operably linked to a gene of interest if the promoter regulates or mediates transcription of the gene of interest in a cell.

[0041] In the various embodiments described herein, it is further contemplated that variants (naturally occurring or otherwise), alleles, homologs, conservatively modified variants, and/or conservative substitution variants of any of the particular polypeptides described are encompassed. As to amino acid sequences, one of ordinary skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid and retains the desired activity of the polypeptide. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles consistent with the disclosure.

[0042] A given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala

for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g., ligand-mediated receptor activity and specificity of a native or reference polypeptide is retained.

[0043] Amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); (4) basic: Lys (K), Arg (R), His (H). Alternatively, naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Particular conservative substitutions include, for example; Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

[0044] In some embodiments, a polypeptide described herein (or a nucleic acid encoding such a polypeptide) can be a functional fragment of one of the amino acid sequences described herein. As used herein, a “functional fragment” is a fragment or segment of a peptide that retains at least 50% of the wildtype reference polypeptide’s activity according to an assay known in the art or described below herein. A functional fragment can comprise conservative substitutions of the sequences disclosed herein.

[0045] In some embodiments, a polypeptide described herein can be a variant of a polypeptide or molecule as described herein. In some embodiments, the variant is a conservatively modified variant. Conservative substitution variants can be obtained by mutations of native nucleotide sequences, for example. A “variant,” as referred to herein, is a polypeptide substantially homologous to a native or reference polypeptide, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions, or substitutions. Variant polypeptide-encoding DNA

sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that encode a variant protein or fragment thereof that retains activity of the non-variant polypeptide. A wide variety of PCR-based site-specific mutagenesis approaches are known in the art and can be applied by the
5 ordinarily skilled artisan.

[0046] A variant amino acid or DNA sequence can be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to a native or reference sequence. The degree of homology (percent identity) between a native and a mutant sequence can be determined, for
10 example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTp or BLASTn with default settings).

[0047] Alterations of the native amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example,
15 at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites permitting ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons
20 altered according to the substitution, deletion, or insertion required. Techniques for making such alterations are well established and include, for example, those disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Patent Nos. 4,518,584 and 4,737,462, which are herein incorporated by reference in their entireties.

25 Any cysteine residue not involved in maintaining the proper conformation of a polypeptide also can be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) can be added to a polypeptide to improve its stability or facilitate oligomerization.

[0048] As used herein, the term “DNA” is defined as deoxyribonucleic acid. The term
30 “polynucleotide” is used herein interchangeably with “nucleic acid” to indicate a polymer of nucleosides. Typically a polynucleotide is composed of nucleosides that are naturally found in DNA or RNA (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine) joined by phosphodiester bonds. However, the term encompasses molecules comprising nucleosides or nucleoside analogs

containing chemically or biologically modified bases, modified backbones, etc., whether or not found in naturally occurring nucleic acids, and such molecules may be preferred for certain applications. Where this application refers to a polynucleotide it is understood that both DNA, RNA, and in each case both single- and double-stranded forms (and complements of each single-stranded molecule) are provided. “Polynucleotide sequence” as used herein can refer to the polynucleotide material itself and/or to the sequence information (i.e., the succession of letters used as abbreviations for bases) that biochemically characterizes a specific nucleic acid. A polynucleotide sequence presented herein is presented in a 5' to 3' direction unless otherwise indicated.

10 [0049] The term “polypeptide” as used herein refers to a polymer of amino acids. The terms “protein” and “polypeptide” are used interchangeably herein. A peptide is a relatively short polypeptide, typically between about 2 and 60 amino acids in length. Polypeptides used herein typically contain amino acids such as the 20 L-amino acids that are most commonly found in proteins. However, other amino acids and/or amino acid analogs known in the art can be used.

15 One or more of the amino acids in a polypeptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a fatty acid group, a linker for conjugation, functionalization, etc. A polypeptide that has a nonpolypeptide moiety covalently or noncovalently associated therewith is still considered a “polypeptide.” Exemplary modifications include glycosylation and palmitoylation. Polypeptides can be purified from

20 natural sources, produced using recombinant DNA technology or synthesized through chemical means such as conventional solid phase peptide synthesis, etc. The term “polypeptide sequence” or “amino acid sequence” as used herein can refer to the polypeptide material itself and/or to the sequence information (i.e., the succession of letters or three letter codes used as abbreviations for amino acid names) that biochemically characterizes a polypeptide. A polypeptide sequence

25 presented herein is presented in an N-terminal to C-terminal direction unless otherwise indicated.

[0050] In some embodiments, a nucleic acid encoding a polypeptide as described herein (e.g., a CAR polypeptide) is comprised by a vector. In some of the aspects described herein, a nucleic acid sequence encoding a given polypeptide as described herein, or any module thereof, is operably linked to a vector. The term “vector,” as used herein, refers to a nucleic acid

30 construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, a cloning vector, an

expression vector, a plasmid, phage, transposon, cosmid, artificial chromosome, virus, virion, etc.

[0051] As used herein, the term “expression vector” refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example, in human cells for expression and in a prokaryotic host for cloning and amplification. The term “expression” refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. “Expression products” include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. The term “gene” means the nucleic acid sequence which is transcribed (DNA) to RNA *in vitro* or *in vivo* when operably linked to appropriate regulatory sequences. The gene may or may not include regions preceding and following the coding region, e.g. 5’ untranslated (5’UTR) or “leader” sequences and 3’ UTR or “trailer” sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[0052] As used herein, the term “viral vector” refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain a nucleic acid encoding a polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

[0053] By “recombinant vector” is meant a vector that includes a heterologous nucleic acid sequence or “transgene” that is capable of expression *in vivo*. It should be understood that the vectors described herein can, in some embodiments, be combined with other suitable compositions and therapies. In some embodiments, the vector is episomal. The use of a suitable episomal vector provides a means of maintaining the nucleotide of interest in the subject in high copy number extra-chromosomal DNA thereby eliminating potential effects of chromosomal integration.

[0054] As used herein, the terms “treat,” “treatment,” “treating,” or “amelioration” refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a condition associated with a disease or disorder, e.g. acute

lymphoblastic leukemia or other cancer, disease, or disorder. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition, disease or disorder. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disease is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation of, or at least slowing of, progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, remission (whether partial or total), and/or decreased mortality, whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side effects of the disease (including palliative treatment).

[0055] As used herein, the term “pharmaceutical composition” refers to the active agent in combination with a pharmaceutically acceptable carrier e.g. a carrier commonly used in the pharmaceutical industry. The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a carrier other than water. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be a cream, emulsion, gel, liposome, nanoparticle, and/or ointment. In some embodiments of any of the aspects, a pharmaceutically acceptable carrier can be an artificial or engineered carrier, e.g., a carrier in which the active ingredient would not be found to occur in nature.

[0056] As used herein, the term “administering,” refers to the placement of a therapeutic or pharmaceutical composition as disclosed herein into a subject by a method or route that results in at least partial delivery of the agent at a desired site. Pharmaceutical compositions comprising agents as disclosed herein can be administered by any appropriate route that results in an effective treatment in the subject.

[0057] The term “statistically significant” or “significantly” refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[0058] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as

modified in all instances by the term “about.” The term “about” when used in connection with percentages can mean $\pm 1\%$.

[0059] As used herein, the term “comprising” means that other elements can also be present in addition to the defined elements presented. The use of “comprising” indicates inclusion rather than limitation.

[0060] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0061] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the technology.

[0062] The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, “e.g.” is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example.”

[0063] In some embodiments of any of the aspects, the disclosure described herein does not concern a process for cloning human beings, processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes.

[0064] Other terms are defined within the description of the various aspects and embodiments of the technology of the following.

BRIEF DESCRIPTION OF THE DRAWINGS

[0065] FIG. 1 shows CD37 expression in the indicated cells.

[0066] FIG. 2 presents schematic diagrams of anti-CD37 CARs.

[0067] FIG. 3 presents plots and a graph showing anti-CD37 CAR expression

[0068] FIG. 4 presents a graph and plots showing expansion of anti-CD37 CAR T cells.

[0069] FIG. 5 presents graphs showing results of a cytotoxicity assay, demonstrating that anti-CD37 CAR T cells lyse CD37 positive T cells.

[0070] FIG. 6 presents graphs of cytotoxicity assays and expression analysis of target clones.

[0071] FIG. 7 shows cell proliferation following stimulation of anti-CD37 CAR T cells with CD37. The assay began on day 17 and with FACS gating for CAR+.

[0072] FIG. 8 presents a schematic of experimental design for anti-CD37 CAR T cells engraftment and tumor clearance in NSG mice.

[0073] FIG. 9 presents diagrams of CAR design. pMGH69 (top construct): anti CD37scFv with light-heavy chains configuration. pMGH70 (bottom construct): anti CD37scFv with heavy-light chains configuration.

[0074] FIG. 10 shows expression of CD37 on cancer cell lines by flow. Jeko-1 (MCL), RAJI (Burkitt lymphoma), and OSU-CLL (CLL) clearly express high level of CD37. NALM6 cells (ALL) do not express CD37.

[0075] FIG. 11 demonstrates the generation and expansion of anti-CD37 CAR T cells. The graph on the left shows the growth curve for anti-CD37-CAR T cells. Starting at day 0 the T cells are expanded *in vitro* using Dynabeads until day 10, then T cells are stimulated with antigen, in this case irradiated K562 cells expressing CD37 and CD19. The plots on the right show the transduction efficiency of this particular batch of CAR T cells at day 10 of culture (19.5% and 23.5 of the total cells are CAR Ts). Bottom left: transduction efficiency of three different normal donors.

[0076] FIG. 12 is a graph showing antigen specific activation. Jurkat-NFAT reporter cell line transduced with CD37-CAR constructs is activated in the presence of target cells (RAJI, OSU-CLL, Jeko-1, NALM-CD37), but not by NALM6 or media alone.

[0077] FIG. 13 depicts proliferation capacity of CAR T cells. Proliferation assay: CAR T cells are labeled with Cell Trace Violet and co-cultured in presence or absence of antigen. The figure depicts CAR T cells expansion in the presence of CD37+ cells but not with cells negative for CD37.

[0078] FIG. 14 demonstrates *in vitro* tumor cell killing. Cytotoxicity at 16 hours of CD37-CAR T, CD19-CAR, or control T cells (UTD) when co-cultured at different E:T ratios with tumor cells. Increasing concentration of either CD37-CAR T or CAR T-19 led to similar levels of killing of target cells, while no killing was observed in the control group (UTD).

[0079] FIG. 15 demonstrates that cytokine production by CD37-CAR, CD19-CAR, or UTD incubated with tumor cells for 16 hours at 1:1 E:T ratio was analyzed in the culture supernatants by LUMINEX FLEXMAP 3D® assay. Technical duplicates (N=1 biological).

[0080] FIG. 16 depicts an experimental schematic: NSG mice were injected with 1e6 tumor cells (Jeko-1 CBG-GFP, i.v.). After 1 week, mice were randomized according to tumor burden and injected with 1e6 (left) or 2e6 (right) positive CAR T cells or UTD cells. Mice were imaged every 7 days and tumor growth was analyzed measuring bioluminescence (represented in
5 graphs). In both experiment, mice treated with UTD showed disease progression (blue line). CAR T-19 cells are capable of inducing responses in both conditions.

[0081] FIG. 17 demonstrates *in vivo* CAR efficacy. Representative mice of the second experiment are shown in the figure. Presence of CAR T cells in peripheral blood 14 days after T-cell injection: cells from blood were stained and gated based on the expression of human CD3.
10 The percentage of mCherry positive cells (CAR+) is displayed in the graph.

[0082] FIGS. 18A and 18B show CD37 protein expression in normal cells (FIG. 18A) and tumor cells (FIG. 18B). FIG. 18A: CD37 protein expression on normal cells is restricted to lymphoid tissues. FIG. 18B: CD37 expression on tumor cell lines. CD37 is highly expressed in non-Hodgkin lymphomas (NHL), including mantle cell lymphoma (JEKO-1), Burkitt lymphoma
15 (RAJI), and B-cell chronic lymphocytic leukemia (OSU-CLL) but it is absent in acute lymphoblastic leukemia cell line (NALM6).

[0083] FIG. 19 shows exemplary CAR-37 T cells design. Design of anti-CD37 second-generation CAR, encoded by a lentiviral vector and bearing a 4-1BB costimulatory domain. Two different orientations of a humanized murine antibody-derived single-chain variable
20 fragment: V_L-V_H (CAR-37 L-H, top) or V_H-V_L (CAR-37 H-L, bottom).

[0084] FIGS. 20A-20D show generation and expansion of CAR-37 T cells. FIG. 20A. Representative flow plots of primary human T cells transduced efficiency after 10 days of activation with CD3/CD28 beads. FIG. 20B. Expanded T cells included variable CAR-37 expression with a mean of 38% (L-H) and 75% (H-L) (N=3). FIG. 20C. Ex vivo enrichment
25 /expansion of CD3/CD28 bead-activated T cells using static culture conditions in healthy donors for 38 days. FIG. 20D. Activation of Jurkat reporter (NFAT-Luc) T cells transduced with different CAR constructs and co-cultured with tumor cells. Luciferase activity was measured after 16 hours (CD3-CD28 beads; positive control.)

[0085] FIGS. 21A-21C show *in vitro* cytotoxic activity of CAR-37 T cells. Cytotoxicity at
30 16 hours of CAR T cells co-cultured at the indicated E:T ratios with JEKO-1, K562 expressing CD19 and CD37 (FIG. 21A), OSU-CLL (FIG. 21B), and RAJI (FIG. 21C). Increasing concentration of CAR-37 and CAR-19 T cells lead to specific killing while no killing was observed in the control group (UTD).

[0086] FIGS. 22A-22C show *in vitro* cytokine production of CAR-37 T cells. Cytokine production by CAR-37, CAR-19, or UTD T cells incubated with JEKO-1 (FIG. 22A), RAJI (FIG. 22B), and OSU-CLL (FIG. 22C) cells for 24 hours at 1:1 E:T ratio was analyzed in the culture supernatants by Luminex assay.

5 [0087] FIGS. 23A-23C show *in vitro* effector function of CAR-37 T cells. FIG. 23A. Experiment schematic: female NSG mice were injected i.v. with 1×10^6 JEKO-1 cells (CBG-GFP+). At day 7, mice were randomized based on tumor burden (BLI) to receive 2×10^6 control T cells (UTD), CAR-37, or CAR-19 (2 normal donors, N=10). FIG. 23B. Representative bioluminescent images of JEKO-1 growth over time. FIG. 23C. Tumor growth curve from each
10 treatment group is displayed over time. Statistical analysis: two-way Anova ($P < 0.001$).

[0088] FIG. 24 shows expression of CD37 in tumor cells. The top panel shows expression of CD37 and CD19 in NALM6, CD19 CD38 K562, JEKO-1, RAJI, and OSU-CLL cell lines as assessed by flow cytometry. The bottom panel shows expression of CD37 and CD17 in MCL patient-derived xenograft (PDX) cell lines PDX_44685, PDX_98848, and PDX_96069 as
15 assessed by flow cytometry. The graph on the bottom right panel shows the percent expression of CD19 and CD37 in MCL PDX cell lines.

[0089] FIGS. 25A-25C demonstrate *in vivo* efficacy of CAR-37 T cells against MCL PDX tumors. Fig. 25A depicts an experimental schematic. On Day -39, mice were administered 1×10^6 PDX_98848 MCL PDX cells intravenously. Flow cytometry was performed on days -28
20 and -14. BLI was performed on Day -1. On day 0, 3×10^6 UTD (control) or CAR T positive cells (CAR-37 H-L or CAR-19) were administered intravenously into the mice. Mice were imaged on days 3, 7, 10, 14, 17, 21, and 35, and tumor growth was analyzed measuring bioluminescence (Figs. 25B and 25C). CAR-37 T cells had strong anti-tumor efficacy against MCL PDX tumors *in vivo*.

25 [0090] FIG. 26A shows expression of CD37 in the peripheral T cell lymphoma (PTCL) cell lines HUT78 (cutaneous T-cell lymphoma (CTCL)) (left panel) and FECD (anaplastic large cell lymphoma (ALCL)) (right panel) as assessed by flow cytometry. FIG. 26B shows expression of CD69 in unstimulated (unstim) and stimulated (stim) CAR T cells as assessed by flow cytometry. The left panel shows expression of mCherry (CAR+) as assessed by flow cytometry.
30 FIG. 26C shows the percentage of CD69+ UTD (control) CAR-37 L-H, and CAR-37 H-L cells stimulated with media, FECD cells, HUT78 cells, or CDR-CD28 beads.

[0091] FIG. 27 shows *in vitro* effector function of UTD (control), CAR-37 L-H, and CAR-37 H-L T cells against HUT78 (CTCL) and FECD (ALCL) PTCL cell lines. The graphs plot percent specific lysis for the indicated E:T ratios.

[0092] FIG. 28 shows expression of CD37 in the AML cell lines TF1, MOM13, and THP1 as assessed by flow cytometry.

DETAILED DESCRIPTION

5 [0093] The invention provides chimeric antigen receptors (CARs) directed against CD37, as described herein. In addition, the invention provides cells that express these CARs, nucleic acid molecules that encode them, vectors including the nucleic acid molecules, methods of using and making these molecules, and kits that include them. The CARs and related molecules can be used, for example, in the treatment and prevention of diseases including, e.g., cancer and
10 autoimmune diseases. Specific examples of types of cancers and autoimmune diseases that can be treated or prevented using the CARs of the invention are provided herein below. Additional methods employing the CARs and related molecules of the invention include diagnostic and imaging methods. The CARs and related molecules and methods of the invention are described further below, after considerations for making and using these and other aspects of the
15 technology.

[0094] Chimeric Antigen Receptors

[0095] The technology described herein provides improved CARs for use in immunotherapy. The following discusses CARs and the various improvements.

[0096] The terms “chimeric antigen receptor” or “CAR” or “CARs” as used herein refer to
20 engineered T cell receptors, which graft a ligand or antigen specificity onto T cells (for example, naïve T cells, central memory T cells, effector memory T cells or combinations thereof). CARs are also known as artificial T-cell receptors, chimeric T-cell receptors or chimeric immunoreceptors.

[0097] A CAR places a chimeric extracellular target-binding domain that specifically binds a
25 target, e.g., a polypeptide, expressed on the surface of a cell to be targeted for a T cell response onto a construct including a transmembrane domain and intracellular domain(s) of a T cell receptor molecule. In one embodiment, the chimeric extracellular target-binding domain comprises the antigen-binding domain(s) of an antibody that specifically binds an antigen expressed on a cell to be targeted for a T cell response. The properties of the intracellular
30 signaling domain(s) of the CAR can vary as known in the art and as disclosed herein, but the chimeric target/antigen-binding domains(s) render the receptor sensitive to signaling activation when the chimeric target/antigen binding domain binds the target/antigen on the surface of a targeted cell.

[0098] With respect to intracellular signaling domains, so-called “first- generation” CARs include those that solely provide CD3zeta (CD3ζ) signals upon antigen binding. So-called “second-generation” CARs include those that provide both co-stimulation (e.g., CD28 or CD 137) and activation (CD3ζ) domains, and so-called “third-generation” CARs include those that provide multiple costimulatory (e.g., CD28 and CD 137) domains and activation domains (e.g., CD3ζ). In various embodiments, the CAR is selected to have high affinity or avidity for the target/antigen – for example, antibody-derived target or antigen binding domains will generally have higher affinity and/or avidity for the target antigen than would a naturally-occurring T cell receptor. This property, combined with the high specificity one can select for an antibody provides highly specific T cell targeting by CAR T cells.

[0099] As used herein, a “CAR T cell” or “CAR-T” refers to a T cell that expresses a CAR. When expressed in a T cell, CARs have the ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T-cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape.

[00100] As used herein, the term “extracellular target binding domain” refers to a polypeptide found on the outside of the cell that is sufficient to facilitate binding to a target. The extracellular target binding domain will specifically bind to its binding partner, i.e., the target. As non-limiting examples, the extracellular target-binding domain can include an antigen-binding domain of an antibody, or a ligand, which recognizes and binds with a cognate binding partner (for example, CD37) protein. In this context, a ligand is a molecule that binds specifically to a portion of a protein and/or receptor. The cognate binding partner of a ligand useful in the methods and compositions described herein can generally be found on the surface of a cell. Ligand:cognate partner binding can result in the alteration of the ligand-bearing receptor, or activate a physiological response, for example, the activation of a signaling pathway. In one embodiment, the ligand can be non-native to the genome. Optionally, the ligand has a conserved function across at least two species. In one embodiment, the extracellular target binding domain comprises a non-antibody ligand.

[00101] Antibody Reagents

[00102] In various embodiments, the CARs described herein comprise an antibody reagent or an antigen-binding domain thereof as an extracellular target-binding domain.

[00103] As used herein, the term “antibody reagent” refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and

which specifically binds a given antigen. An antibody reagent can comprise an antibody or a polypeptide comprising an antigen-binding domain of an antibody. In some embodiments of any of the aspects, an antibody reagent can comprise a monoclonal antibody or a polypeptide comprising an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as V_H), and a light (L) chain variable region (abbreviated herein as V_L). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term “antibody reagent” encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, CDRs, and domain antibody (dAb) fragments (see, e.g., de Wildt et al., Eur J. Immunol. 26(3):629-639, 1996; which is incorporated by reference herein in its entirety)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, or IgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and primatized antibodies. Antibodies also include midibodies, humanized antibodies, chimeric antibodies, and the like. Fully human antibody binding domains can be selected, for example, from phage display libraries using methods known to those of ordinary skill in the art.

[00104] The V_H and V_L regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” (“CDR”), interspersed with regions that are more conserved, termed “framework regions” (“FR”). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E. A. et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia et al., J. Mol. Biol. 196:901-917, 1987; each of which are incorporated by reference herein in their entireties). Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00105] In one embodiment, the antibody or antibody reagent is not a human antibody or antibody reagent, (i.e., the antibody or antibody reagent is mouse), but has been humanized. A “humanized antibody or antibody reagent” refers to a non-human antibody or antibody reagent that has been modified at the protein sequence level to increase its similarity to antibody or antibody reagent variants produced naturally in humans. One approach to humanizing antibodies employs the grafting of murine or other non-human CDRs onto human antibody frameworks.

[00106] In one embodiment, a CAR's extracellular target binding domain comprises or consists essentially of a single-chain Fv (scFv) fragment created by fusing the V_H and V_L

domains of an antibody, generally a monoclonal antibody, via a flexible linker peptide. In various embodiments, the scFv is fused to a transmembrane domain and to a T cell receptor intracellular signaling domain, e.g., an engineered intracellular signaling domain as described herein.

5 [00107] Antibody binding domains and ways to select and clone them are well-known to those of ordinary skill in the art. In another embodiment, the antibody reagent is an anti-CD37 antibody reagent and has the sequence selected from SEQ ID NO: 1 or 5. In one embodiment, the anti-CD37 antibody reagent corresponds to the sequence of SEQ ID NO: 1 or 5; or comprises the sequence of SEQ ID NO: 1 or 5; or comprises a sequence with at least 80%, at least 85%, at
10 least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or greater sequence identity to the sequence of SEQ ID NO: 1 or 5.

[00108] In one embodiment, the CARs useful in the technology described herein comprise at least two antigen-specific targeting regions, an extracellular domain, a transmembrane domain, and an intracellular signaling domain. In such embodiments, the two or more antigen-specific
15 targeting regions target at least two different antigens and may be arranged in tandem and separated by linker sequences. In another embodiment, the CAR is a bispecific CAR. A bispecific CAR is specific to two different antigens.

[00109] Target/Antigen

[00110] Any cell-surface moiety can be targeted by a CAR. Most often, the target will be a
20 cell-surface polypeptide differentially or preferentially expressed on a cell one wishes to target for a T cell response. In this regard, tumor antigens or tumor-associated antigens provide attractive targets, providing a means to target tumor cells while avoiding or at least limiting collateral damage to non-tumor cells or tissues. Non-limiting examples of tumor antigens or tumor-associated antigens include CD37, BCMA (tumor necrosis factor receptor superfamily
25 member 17 (TNFRSF17); NCBI Gene ID: 608; NCBI Ref Seq NP_001183.2) and mRNA (e.g., NCBI Ref Seq NM_001192.2), CEA, Immature laminin receptor, TAG-72, HPV E6 and E7, BING-4, Calcium-activated chloride channel 2, Cyclin B1, 9D7, Ep-CAM, EphA3, Her2/neu, Telomerase, Mesotheliun, SAP-1, Survivin, BAGE family, CAGE family, GAGE family, MAGE family, SAGE family, XAGE family, NY-ESO-1/LAGE-1, PRAME, SSX-2, Melan-
30 A/MART-1, Gp100/pmel17, Tyrosinase, TRP-1/-2, MC1R, BRCA1/2, CDK4, MART-2, p53, Ras, MUC1, and TGF- β RII.

[00111] As noted above, the target of the CAR molecules of the present invention is CD37. CD37 is cell surface protein that contains four hydrophobic transmembrane domains. CD37 is expressed exclusively on immune cells. It is highly expressed on mature B cells, and is

moderately expressed on T cells and myeloid cells. CD37 sequences are known for a number of species, e.g., human CD37 (NCBI Gene ID: 951) polypeptide (e.g., NCBI Ref Seq NP_001035120.1) and mRNA (e.g., NCBI Ref Seq NM_001040031.1). CD37 can refer to human CD37, including naturally occurring variants, molecules, and alleles thereof. In some
 5 embodiments of any of the aspects, e.g., in veterinary applications, CD37 can refer to the CD37 of, e.g., dog, cat, cow, horse, pig, and the like. Homologs and/or orthologs of human CD37 are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search function or searching available sequence data for a given species for sequence similar to a reference CD37 sequence.

10 **[00112]** In one embodiment, the CD37-binding sequence comprises a ligand of CD37 or an antibody reagent that specifically binds CD37.

[00113] Transmembrane Domain

[00114] Each CAR as described herein necessarily includes a transmembrane domain that joins the extracellular target-binding domain to the intracellular signaling domain.

15 **[00115]** As used herein, “transmembrane domain” (TM domain) refers to the generally hydrophobic region of the CAR which crosses the plasma membrane of a cell. The TM domain can be the transmembrane region or fragment thereof of a transmembrane protein (for example a Type I transmembrane protein or other transmembrane protein), an artificial hydrophobic sequence, or a combination thereof. While specific examples are provided herein and used in the
 20 Examples, other transmembrane domains will be apparent to those of skill in the art and can be used in connection with alternate embodiments of the technology. A selected transmembrane region or fragment thereof would preferably not interfere with the intended function of the CAR. As used in relation to a transmembrane domain of a protein or polypeptide, “fragment thereof” refers to a portion of a transmembrane domain that is sufficient to anchor or attach a protein to a
 25 cell surface.

[00116] In one embodiment, a CAR’s transmembrane domain or fragment thereof is derived from or comprises the transmembrane domain of CD8. In an alternate embodiment, the transmembrane domain or fragment thereof of the CAR described herein comprises a transmembrane domain selected from the transmembrane domain of an alpha, beta or zeta chain
 30 of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFl), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103,

ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1(CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162),
 5 LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

[00117] CD8 is an antigen preferentially found on the cell surface of cytotoxic T lymphocytes. CD8 mediates cell-cell interactions within the immune system, and acts as a T cell coreceptor. CD8 consists of an alpha (CD8 α) and beta (CD8 β) chain. CD8a sequences are known for a number of species, e.g., human CD8a, (NCBI Gene ID: 925) polypeptide (e.g., NCBI Ref Seq
 10 NP_001139345.1) and mRNA (e.g., NCBI Ref Seq NM_000002.12). CD8 can refer to human CD8, including naturally occurring variants, molecules, and alleles thereof. In some embodiments of any of the aspects, e.g., in veterinary applications, CD8 can refer to the CD8 of, e.g., dog, cat, cow, horse, pig, and the like. Homologs and/or orthologs of human CD8 are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search
 15 function or searching available sequence data for a given species for sequence similar to a reference CD8 sequence.

[00118] Co-stimulatory Domain

[00119] Each CAR described herein optionally comprises an intracellular domain of a co-stimulatory molecule, or co-stimulatory domain. As used herein, the term “co-stimulatory
 20 domain” refers to an intracellular signaling domain of a co-stimulatory molecule. Co-stimulatory molecules are cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. Illustrative examples of such co-stimulatory molecules include CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-
 25 1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70. In one embodiment, the intracellular domain is the intracellular domain of 4-1BB.

[00120] 4-1BBL is a type 2 transmembrane glycoprotein belonging to the TNF superfamily. 4-1BBL is expressed on activated T lymphocytes. 4-1BBL sequences are known for a number of
 30 species, e.g., human 4-1BBL, also known as TNFSF9 (NCBI Gene ID: 8744) polypeptide (e.g., NCBI Ref Seq NP_003802.1) and mRNA (e.g., NCBI Ref Seq NM_003811.3). 4-1BBL can refer to human 4-1BBL, including naturally occurring variants, molecules, and alleles thereof. In some embodiments of any of the aspects, e.g., in veterinary applications, 4-1BBL can refer to the 4-1BBL of, e.g., dog, cat, cow, horse, pig, and the like. Homologs and/or orthologs of human 4-

1BBL are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search function or searching available sequence data for a given species for sequence similar to a reference 4-1BBL sequence.

[00121] Intracellular Signaling Domain

5 **[00122]** CARs as described herein comprise an intracellular signaling domain. An “intracellular signaling domain,” refers to the part of a CAR polypeptide that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector cell to elicit effector cell function, e.g., activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound
10 target cell, or other cellular responses elicited following antigen binding to the extracellular CAR domain. Non-limiting examples of ITAM-containing intracellular signaling domains that are of particular use in the technology include those derived from TCR ζ , FcR γ , FcR β , CD3 γ , CD3 θ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and CD66d.

[00123] CD3 is a T cell co-receptor that facilitates T lymphocytes activation when
15 simultaneously engaged with the appropriate co-stimulation (e.g., binding of a co-stimulatory molecule). A CD3 complex consists of 4 distinct chains; mammal CD3 consists of a CD3 γ chain, a CD3 δ chain, and two CD3 ϵ chains. These chains associate with a molecule known as the T cell receptor (TCR) and the CD3 ζ to generate an activation signal in T lymphocytes. A complete TCR complex comprises a TCR, CD3 ζ , and the complete CD3 complex.

20 **[00124]** In some embodiments of any aspect, a CAR polypeptide described herein comprises an intracellular signaling domain that comprises an Immunoreceptor Tyrosine-based Activation Motif or ITAM from CD3 zeta (CD3 ζ). In some embodiments of any aspect, the ITAM comprises three motifs of ITAM of CD3 ζ (ITAM3). In some embodiments of any aspect, the three motifs of ITAM of CD3 ζ are not mutated and, therefore, include native or wild-type
25 sequences. In some embodiments, the CD3 ζ sequence comprises the sequence of SEQ ID NO: 14 or 20, as set forth below.

[00125] A more detailed description of CARs and CAR T cells can be found in Maus et al. Blood 2014 123:2624-35; Reardon et al. Neuro-Oncology 2014 16:1441-1458; Hoyos et al. Haematologica 2012 97:1622; Byrd et al. J Clin Oncol 2014 32:3039-47; Maher et al. Cancer
30 Res 2009 69:4559-4562; and Tamada et al. Clin Cancer Res 2012 18:6436-6445; each of which is incorporated by reference herein in its entirety.

[00126] In one embodiment, the CAR further comprises a linker domain. As used herein “linker domain” refers to an oligo- or polypeptide region from about 2 to 100 amino acids in length, which links together any of the domains/regions of the CAR as described herein. In some

embodiment, linkers can include or be composed of flexible residues such as glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers may be cleavable or non-cleavable. Examples of cleavable linkers include
 5 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In one embodiment, the linker region is T2A derived from *Thosea asigna* virus. Non-limiting examples of linkers that can be used in this technology include P2A and F2A.

[00127] In one embodiment, a CAR as described herein further comprises a reporter molecule,
 10 e.g., to permit for non-invasive imaging (e.g., positron-emission tomography PET scan). In a bispecific CAR that includes a reporter molecule, the first extracellular binding domain and the second extracellular binding domain can include different or the same reporter molecule. In a bispecific CAR T cell, the first CAR and the second CAR can express different or the same reporter molecule. In another embodiment, a CAR as described herein further comprises a
 15 reporter molecule (for example hygromycin phosphotransferase (hph)) that can be imaged alone or in combination with a substrate or chemical (for example 9-[4-[¹⁸F]fluoro-3-(hydroxymethyl)butyl]guanine ([¹⁸F]FHBG)). In another embodiment, a CAR as described herein further comprises nanoparticles at can be readily imaged using non-invasive techniques (e.g., gold nanoparticles (GNP) functionalized with ⁶⁴Cu²⁺). Labeling of CAR T cells for non-
 20 invasive imaging is reviewed, for example in Bhatnagar et al., *Integr Biol. (Camb)*. 5(1):231-238, 2013, and Keu et al., *Sci Transl. Med.* 18; 9(373), 2017, which are incorporated herein by reference in their entireties.

[00128] GFP and mCherry are demonstrated herein as fluorescent tags useful for imaging a CAR expressed on a T cell (e.g., a CAR T cell). It is expected that essentially any fluorescent
 25 protein known in the art can be used as a fluorescent tag for this purpose. For clinical applications, the CAR need not include a fluorescent tag or fluorescent protein.

[00129] In one embodiment, the CAR polypeptide sequence corresponds to, or comprises, or comprises a sequence with at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or
 30 greater sequence identity of a sequence selected from SEQ ID NO: 9 or 15. In various embodiments, the CD3ζ sequence of such CAR polypeptides is a native or wild-type sequence.

[00130] One aspect of the technology described herein relates to a mammalian cell comprising any of the CAR polypeptides described herein; or a nucleic acid encoding any of the CAR polypeptides described herein. In one embodiment, the mammalian cell comprises an antibody,

antibody reagent, antigen-binding portion thereof, or any of the CARs described herein, or a nucleic acid encoding such an antibody, antibody reagent, antigen-binding portion thereof, or any of the CARs described herein. The mammalian cell or tissue can be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used. In a preferred embodiment of any aspect, the mammalian cell is human.

[00131] In one embodiment, the cell is a T cell. In alternate embodiments of any aspect, the cell is an immune cell. As used herein, “immune cell” refers to a cell that plays a role in the immune response. Immune cells are of hematopoietic origin, and include lymphocytes, such as B cells and T cells; natural killer cells; myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes. In some embodiments, the cell is a T cell; a NK cell; a NKT cell; lymphocytes, such as B cells and T cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes.

[00132] In one embodiment, the cell is obtained from an individual having or diagnosed as having cancer, a plasma cell disorder, or autoimmune disease.

[00133] “Cancer” as used herein can refer to a hyperproliferation of cells whose unique trait, loss of normal cellular control, results in unregulated growth, lack of differentiation, local tissue invasion, and metastasis, and can be leukemia, lymphoma, multiple myeloma, or a solid tumor. Non-limiting examples of leukemia include acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia (CLL).

In one embodiment, the cancer is ALL or CLL. Non-limiting examples of lymphoma include diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), marginal zone lymphomas, Burkitt lymphoma, hairy cell leukemia (HCL), and T cell lymphoma (e.g., peripheral T cell lymphoma (PTCL), including cutaneous T cell lymphoma (CTCL) and anaplastic large cell lymphoma (ALCL)). In one embodiment, the cancer is DLBCL or follicular lymphoma. Non-limiting examples of solid tumors include adrenocortical tumor, alveolar soft part sarcoma, carcinoma, chondrosarcoma, colorectal carcinoma, desmoid tumors, desmoplastic small round cell tumor, endocrine tumors, endodermal sinus tumor, epithelioid hemangioendothelioma, Ewing sarcoma, germ cell tumors (solid tumor), giant cell tumor of bone and soft tissue, hepatoblastoma, hepatocellular carcinoma, melanoma, nephroma, neuroblastoma, non-rhabdomyosarcoma soft tissue sarcoma (NRSTS), osteosarcoma, paraspinal sarcoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, synovial sarcoma, and Wilms tumor. Solid tumors can be found in bones, muscles, or organs, and can be sarcomas or carcinomas. It is contemplated that any aspect of the technology described herein can be used to treat all types of

cancers, including cancers not listed in the instant application. As used herein, the term “tumor” refers to an abnormal growth of cells or tissues, e.g., of malignant type or benign type.

[00134] As used herein, an “autoimmune disease or disorder” is characterized by the inability of one’s immune system to distinguish between a foreign cell and a healthy cell. This results in one’s immune system targeting one’s healthy cells for programmed cell death. Non-limiting examples of an autoimmune disease or disorder include inflammatory arthritis, type 1 diabetes mellitus, multiples sclerosis, psoriasis, inflammatory bowel diseases, SLE, and vasculitis, allergic inflammation, such as allergic asthma, atopic dermatitis, and contact hypersensitivity. Other examples of auto-immune-related disease or disorder, but should not be construed to be limited to, include rheumatoid arthritis, multiple sclerosis (MS), systemic lupus erythematosus, Graves’ disease (overactive thyroid), Hashimoto’s thyroiditis (underactive thyroid), celiac disease, Crohn’s disease and ulcerative colitis, Guillain-Barre syndrome, primary biliary sclerosis/cirrhosis, sclerosing cholangitis, autoimmune hepatitis, Raynaud’s phenomenon, scleroderma, Sjogren’s syndrome, Goodpasture’s syndrome, Wegener’s granulomatosis, polymyalgia rheumatica, temporal arteritis/giant cell arteritis, chronic fatigue syndrome CFS), psoriasis, autoimmune Addison’s Disease, ankylosing spondylitis, acute disseminated encephalomyelitis, antiphospholipid antibody syndrome, aplastic anemia, idiopathic thrombocytopenic purpura, myasthenia gravis, opsoclonus myoclonus syndrome, optic neuritis, Ord’s thyroiditis, pemphigus, pernicious anaemia, polyarthritis in dogs, Reiter’s syndrome, Takayasu’s arteritis, warm autoimmune hemolytic anemia, Wegener’s granulomatosis and fibromyalgia (FM).

[00135] In one embodiment, the mammalian cell is obtained for a patient having an immune system disorder that results in abnormally low activity of the immune system, or immune deficiency disorders, which hinders one’s ability to fight a foreign cell, (i.e., a virus or bacterial cell).

[00136] A plasma cell is a white blood cell produces from B lymphocytes which function to generate and release antibodies needed to fight infections. As used herein, a “plasma cell disorder or disease” is characterized by abnormal multiplication of a plasma cell. Abnormal plasma cells are capable of “crowding out” healthy plasma cells, which results in a decreased capacity to fight a foreign object, such as a virus or bacterial cell. Non-limiting examples of plasma cell disorders include amyloidosis, Waldenstrom’s macroglobulinemia, osteosclerotic myeloma (POEMS syndrome), monoclonal gammopathy of unknown significance (MGUS), and plasma cell myeloma.

[00137] T cells can be obtained from a subject using standard techniques known in the field. For example, T cells can be isolated from peripheral blood taken from a donor or patient. T cells can be isolated from a mammal. Preferably, T cells are isolated from a human.

[00138] A cell, for example a T cell, can be engineered to comprise any of the CAR polypeptides described herein; or a nucleic acid encoding any of the CAR polypeptides described herein. In one embodiment, the any of the CAR polypeptides described herein are comprised in a lentiviral vector. The lentiviral vector is used to express the CAR polypeptide in a cell using infection standard techniques.

[00139] Retroviruses, such as lentiviruses, provide a convenient platform for delivery of nucleic acid sequences encoding a gene, or chimeric gene of interest. A selected nucleic acid sequence can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells, e.g., *in vitro* or *ex vivo*. Retroviral systems are well known in the art and are described in, for example, U.S. Patent No. 5,219,740; Kurth and Bannert (2010) "Retroviruses: Molecular Biology, Genomics and Pathogenesis" Calster Academic Press (ISBN:978-1-90455-55-4); and Hu et al., Pharmacological Reviews 52:493-512, 2000; which are all incorporated by reference herein in their entireties. Lentiviral system for efficient DNA delivery can be purchased from OriGene; Rockville, MD. In alternative embodiments, the CAR polypeptide of any of the CARS described herein are expressed in the mammalian cell via transfection or electroporation of an expression vector comprising nucleic acid encoding the CAR. Transfection or electroporation methods are known in the art.

[00140] Efficient expression of the CAR polypeptide of any of the CAR polypeptides described herein can be assessed using standard assays that detect the mRNA, DNA, or gene product of the nucleic acid encoding the CAR. For example, RT-PCR, FACS, northern blotting, western blotting, ELISA, or immunohistochemistry.

[00141] In one embodiment, the CAR polypeptide described herein is constitutively expressed. In one embodiment, the CAR polypeptide described herein is encoded by recombinant nucleic acid sequence.

[00142] One aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising: engineering a T cell to comprise any of the CAR polypeptides described herein on the T cell surface; and administering the engineered T cell to the subject.

[00143] One aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method

comprising: administering the cell of any of the mammalian cells comprising the any of the CAR polypeptides described herein.

[00144] Cluster differentiation (CD) molecules are cell surface markers present on leukocytes. As a leukocyte differentiates and matures its CD profile changes. In the case that a leukocytes
 5 turns into a cancer cell, (i.e., a lymphoma), its CD profile is important in diagnosing the disease. The treatment and prognosis of certain types of cancers is reliant on determining the CD profile of the cancer cell. “CDX+”, wherein “X” is a CD marker, indicates the CD marker is present in the cancer cell, while “CDX-” indicates the marker is not present. One skilled in the art will be capable of assessing the CD molecules present on a cancer cell using standard techniques, for
 10 example using immunofluorescence to detect commercially available antibodies bound to the CD molecules.

[00145] In one embodiment, the cancer expresses a CD and tumor antigen. In one embodiment, the cancer is a CD37+ or BCMA+ cancer. In one embodiment, the CD37+ cancer is lymphoma or leukemia. In one embodiment, lymphoma is B-cell non-Hodgkin lymphoma
 15 (NHL), mantle cell lymphoma, Burkitt’s lymphoma, B cell lymphoblastic lymphoma or T cell lymphoma (e.g., peripheral T cell lymphoma (PTCL), including cutaneous T-cell lymphoma (CTCL) and anaplastic large cell lymphoma (ALCL)).

[00146] One aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method
 20 comprising: engineering a T cell to comprise any of the CAR polypeptides described herein on the T cell surface; administering the engineered T cell to the subject; wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[00147] Cancer cells can evolve in response to treatment to alter its CD profile in order to evade said treatment. For example, a patient with CD19+ leukemia can be treated with an anti-
 25 CD19 therapy. Following treatment, the cancer cell can relapse, or come back after treatment, and no longer express the CD19 marker, resulting in CD19- leukemia. This cancer cell will no longer be targetable by an anti-CD19 therapy. In one embodiment, the subject is non-responsive, or refractory to anti-CD19 and/or anti-CD20 therapy. In one embodiment, the subject has a cancer that is CD19- and/or CD20-. In one embodiment, the subject has a cancer that is relapsed
 30 and no longer expresses CD19 or CD20.

[00148] Another aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering any of the mammalian cells comprising the any of the CAR polypeptides described herein to the subject, wherein the cell comprises CAR comprising an

extracellular domain comprising a CD37-binding sequence; wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[00149] Another aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising: selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy; engineering a T cell to comprise any of the CAR polypeptides described herein on the T cell surface; administering the engineered CAR T cell to the subject; wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[00150] Another aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising: selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy; administering any of the mammalian cells comprising the any of the CAR polypeptides described herein to the subject, wherein the cell comprises CAR comprising an extracellular domain comprising a CD37-binding sequence; wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[00151] Another aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising: engineering a T cell to comprise any of the CAR polypeptides described herein on the T cell surface; administering the engineered CAR T cell to the subject; wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

[00152] Another aspect of the technology described herein relates to a method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering any of the mammalian cells comprising the any of the CAR polypeptides described herein to the subject, wherein the cell comprises CAR comprising an extracellular domain comprising a CD37-binding sequence; wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

[00153] In some embodiments of any of the aspect, the engineered CAR-T cell is stimulated and/or activated prior to administration to the subject.

[00154] *Administration*

[00155] In some embodiments, the methods described herein relate to treating a subject having or diagnosed as having cancer, a plasma cell disease or disorder, or an autoimmune disease or disorder with a mammalian cell comprising any of the CAR polypeptides described herein, or a nucleic acid encoding any of the CAR polypeptides described herein. As used herein, a “CAR T cells as described herein” refers to a mammalian cell comprising any of the

CAR polypeptides described herein, or a nucleic acid encoding any of the CAR polypeptides described herein. As used herein, a “condition” refers to a cancer, a plasma cell disease or disorder, or an autoimmune disease or disorder. Subjects having a condition can be identified by a physician using current methods of diagnosing the condition. Symptoms and/or complications of the condition, which characterize these conditions and aid in diagnosis are well known in the art and include but are not limited to, fatigue, persistent infections, and persistent bleeding. Tests that may aid in a diagnosis of, e.g., the condition, but are not limited to, blood screening and bone marrow testing, and are known in the art for a given condition. A family history for a condition, or exposure to risk factors for a condition can also aid in determining if a subject is likely to have the condition or in making a diagnosis of the condition.

[00156] The compositions described herein can be administered to a subject having or diagnosed as having a condition. In some embodiments, the methods described herein comprise administering an effective amount of activated CAR T cells described herein to a subject in order to alleviate a symptom of the condition. As used herein, “alleviating a symptom of the condition” is ameliorating any condition or symptom associated with the condition. As compared with an equivalent untreated control, such reduction is by at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, 99% or more as measured by any standard technique. A variety of means for administering the compositions described herein to subjects are known to those of skill in the art. In one embodiment, the compositions described herein are administered systemically or locally. In a preferred embodiment, the compositions described herein are administered intravenously. In another embodiment, the compositions described herein are administered at the site of a tumor.

[00157] The term “effective amount” as used herein refers to the amount of activated CAR T cells needed to alleviate at least one or more symptom of the disease or disorder, and relates to a sufficient amount of the cell preparation or composition to provide the desired effect. The term “therapeutically effective amount” therefore refers to an amount of activated CAR T cells that is sufficient to provide a particular anti-condition effect when administered to a typical subject. An effective amount as used herein, in various contexts, would also include an amount sufficient to delay the development of a symptom of the disease, alter the course of a symptom disease (for example but not limited to, slowing the progression of a condition), or reverse a symptom of the condition. Thus, it is not generally practicable to specify an exact “effective amount.” However, for any given case, an appropriate “effective amount” can be determined by one of ordinary skill in the art using only routine experimentation.

[00158] Effective amounts, toxicity, and therapeutic efficacy can be evaluated by standard pharmaceutical procedures in cell cultures or experimental animals. The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from cell culture assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of activated CAR T cells, which achieves a half-maximal inhibition of symptoms) as determined in cell culture, or in an appropriate animal model. Levels in plasma can be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay, e.g., assay for bone marrow testing, among others. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

[00159] In one aspect of the technology, the technology described herein relates to a pharmaceutical composition comprising activated CAR T cells as described herein, and optionally a pharmaceutically acceptable carrier. The active ingredients of the pharmaceutical composition at a minimum comprise activated CAR T cells as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist essentially of activated CAR T cells as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist of activated CAR T cells as described herein.

Pharmaceutically acceptable carriers for cell-based therapeutic formulation include saline and aqueous buffer solutions, Ringer's solution, and serum component, such as serum albumin, HDL and LDL. The terms such as "excipient," "carrier," "pharmaceutically acceptable carrier" or the like are used interchangeably herein.

[00160] In some embodiments, the pharmaceutical composition comprising activated CAR T cells as described herein can be a parenteral dose form. Since administration of parenteral dosage forms typically bypasses the patient's natural defenses against contaminants, the components apart from the CAR T cells themselves are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions. Any of these can be added to the activated CAR T cells preparation prior to administration.

[00161] Suitable vehicles that can be used to provide parenteral dosage forms of activated CAR T cells as disclosed within are well known to those skilled in the art. Examples include, without limitation: saline solution; glucose solution; aqueous vehicles including but not limited to, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, and lactated Ringer's injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and propylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

[00162] *Dosage*

[00163] "Unit dosage form" as the term is used herein refers to a dosage for suitable one administration. By way of example, a unit dosage form can be an amount of therapeutic disposed in a delivery device, e.g., a syringe or intravenous drip bag. In one embodiment, a unit dosage form is administered in a single administration. In another, embodiment more than one unit dosage form can be administered simultaneously.

[00164] In some embodiments, the activated CAR T cells described herein are administered as a monotherapy, i.e., another treatment for the condition is not concurrently administered to the subject.

[00165] A pharmaceutical composition comprising the T cells described herein can generally be administered at a dosage of 10^4 to 10^9 cells/kg body weight, in some instances 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. If necessary, T cell compositions can also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. Med. 319:1676, 1988).

[00166] In certain aspects, it may be desired to administer activated CAR T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom as described herein, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain aspects, T cells can be activated from blood draws of from 10 cc to 400 cc. In certain aspects, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60 cc, 70 cc, 80 cc, 90 cc, or 100 cc.

[00167] Modes of administration can include, for example intravenous (i.v.) injection or infusion. The compositions described herein can be administered to a patient transarterially, intratumorally, intranodally, or intramedullary. In some embodiments, the compositions of T cells may be injected directly into a tumor, lymph node, or site of infection. In one embodiment,

the compositions described herein are administered into a body cavity or body fluid (e.g., ascites, pleural fluid, peritoneal fluid, or cerebrospinal fluid).

[00168] In a particular exemplary aspect, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted *ex vivo* to select and/or isolate the cells of interest, e.g., T cells. These T cell isolates can be expanded by contact with an aAPC as described herein, e.g., an aAPC expressing anti-CD28 and anti-CD3 CDRs, and treated such that one or more CAR constructs of the technology may be introduced, thereby creating a CAR T cell. Subjects in need thereof can subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. Following or concurrent with the transplant, subjects can receive an infusion of the expanded CAR T cells. In one embodiment, expanded cells are administered before or following surgery.

[00169] In some embodiments, lymphodepletion is performed on a subject prior to administering one or more CAR T cell as described herein. In such embodiments, the lymphodepletion can comprise administering one or more of melphalan, cytoxan, cyclophosphamide, and fludarabine.

[00170] The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices.

[00171] In some embodiments, a single treatment regimen is required. In others, administration of one or more subsequent doses or treatment regimens can be performed. For example, after treatment biweekly for three months, treatment can be repeated once per month, for six months or a year or longer. In some embodiments, no additional treatments are administered following the initial treatment.

[00172] The dosage of a composition as described herein can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. With respect to duration and frequency of treatment, it is typical for skilled clinicians to monitor subjects in order to determine when the treatment is providing therapeutic benefit, and to determine whether to administer further cells, discontinue treatment, resume treatment, or make other alterations to the treatment regimen. The dosage should not be so large as to cause adverse side effects, such as cytokine release syndrome. Generally, the dosage will vary with the age, condition, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication.

[00173] ***Combinational therapy***

[00174] The activated CAR T cells described herein can be used in combination with other known agents and therapies. In one embodiment, the subject is further administered an anti-

BCMA therapy. In one embodiment, the subject is resistant to anti-BCMA therapies.

Administered “in combination,” as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject’s affliction with the disorder, e.g., the

two or more treatments are delivered after the subject has been diagnosed with the disorder and

5 before the disorder has been cured or eliminated or treatment has ceased for other reasons. In

some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to

herein as “simultaneous” or “concurrent delivery.” In other embodiments, the delivery of one

treatment ends before the delivery of the other treatment begins. In some embodiments of either

10 case, the treatment is more effective because of combined administration. For example, the

second treatment is more effective, e.g., an equivalent effect is seen with less of the second

treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if

the second treatment were administered in the absence of the first treatment, or the analogous

situation is seen with the first treatment. In some embodiments, delivery is such that the

15 reduction in a symptom, or other parameter related to the disorder is greater than what would be

observed with one treatment delivered in the absence of the other. The effect of the two

treatments can be partially additive, wholly additive, or greater than additive. The delivery can

be such that an effect of the first treatment delivered is still detectable when the second is

delivered. The activated CAR T cells described herein and the at least one additional therapeutic

20 agent can be administered simultaneously, in the same or in separate compositions, or

sequentially. For sequential administration, the CAR-expressing cell described herein can be

administered first, and the additional agent can be administered second, or the order of

administration can be reversed. The CAR T therapy and/or other therapeutic agents, procedures

or modalities can be administered during periods of active disorder, or during a period of

25 remission or less active disease. The CAR T therapy can be administered before another

treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

[00175] When administered in combination, the activated CAR T cells and the additional agent (e.g., second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same as the amount or dosage of each agent used individually, e.g., as a

30 monotherapy. In certain embodiments, the administered amount or dosage of the activated CAR

T cells, the additional agent (e.g., second or third agent), or all, is lower (e.g., at least 20%, at

least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used

individually. In other embodiments, the amount or dosage of the activated CAR T cells, the

additional agent (e.g., second or third agent), or all, that results in a desired effect (e.g., treatment

of cancer) is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent individually required to achieve the same therapeutic effect. In further embodiments, the activated CAR T cells described herein can be used in a treatment regimen in combination with surgery, chemotherapy, radiation, an mTOR pathway inhibitor, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, or a peptide vaccine, such as that described in Izumoto et al., J. Neurosurg. 108:963- 971, 2008.

- 10 **[00176]** In one embodiment, the activated CAR T cells described herein can be used in combination with a checkpoint inhibitor. Exemplary checkpoint inhibitors include anti-PD-1 inhibitors (Nivolumab, MK-3475, Pembrolizumab, Pidilizumab, AMP-224, AMP-514), anti-CTLA4 inhibitors (Ipilimumab and Tremelimumab), anti-PDL1 inhibitors (Atezolizumab, Avelomab, MSB0010718C, MEDI4736, and MPDL3280A), and anti-TIM3 inhibitors.
- 15 **[00177]** In one embodiment, the activated CAR T cells described herein can be used in combination with a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzamab, gemtuzumab, rituximab, tositumomab), an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (e.g., fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide). General chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (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
- 20
25
30

(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®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®). Exemplary alkylating agents include, without limitation, 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 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 (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPAs and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytosan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HC1 (Treanda®). Exemplary mTOR inhibitors include, e.g., temsirolimus; ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-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-1,3,6-dioxo-4-

azatricyclo[30.3.1.04'9] 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 RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(35,)-3-methylmorpholin-4-yl]pyrido[2,3-(i)pyrimidin-7-yl]-2- methoxyphenyl)methanol (AZD8055); 2-Amino-8-[iraw5,-4-(2-hydroxyethoxy)cyclohexyl]-6- (6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-JJpyrimidin-7(8H)-one (PF04691502, CAS 1013101-36-4); and N2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4H-1-benzopyran-2- yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-a-aspartyl-L-serine-, inner salt (SF1126, CAS 936487-67-1), and XL765. Exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ , CAS 951209-71-5, available from IRX Therapeutics). Exemplary anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylavidomycin. Exemplary vinca alkaloids include, e.g., vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®). Exemplary proteasome inhibitors include bortezomib (Velcade®); carfilzomib (PX- 171-007, (5)-4-Methyl-N-((5)-1-(((5)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2- yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((5,)-2-(2-morpholinoacetamido)-4- phenylbutanamido)-pentanamide); marizomib (NPT0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and O-Methyl-N-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-O- methyl-N-[(11S')-2-[(2R)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]- L-serinamide (ONX-0912).

[00178] One of skill in the art can readily identify a chemotherapeutic agent of use (*e.g.* see Physicians' Cancer Chemotherapy Drug Manual 2014, Edward Chu, Vincent T. DeVita Jr., Jones & Bartlett Learning; Principles of Cancer Therapy, Chapter 85 in Harrison's Principles of Internal Medicine, 18th edition; Therapeutic Targeting of Cancer Cells: Era of Molecularly Targeted Agents and Cancer Pharmacology, Chs. 28-29 in Abeloff's Clinical Oncology, 2013 Elsevier; and Fischer D S (ed): The Cancer Chemotherapy Handbook, 4th ed. St. Louis, Mosby-Year Book, 2003).

[00179] In an embodiment, activated CAR T cells described herein are administered to a subject in combination with a molecule that decreases the level and/or activity of a molecule targeting GITR and/or modulating GITR functions, a molecule that decreases the Treg cell population, an mTOR inhibitor, a GITR agonist, a kinase inhibitor, a non-receptor tyrosine
5 kinase inhibitor, a CDK4 inhibitor, and/or a BTK inhibitor.

[00180] *Efficacy*

[00181] The efficacy of activated CAR T cells in, e.g. the treatment of a condition described herein, or to induce a response as described herein (e.g. a reduction in cancer cells) can be determined by the skilled clinician. However, a treatment is considered "effective treatment," as
10 the term is used herein, if one or more of the signs or symptoms of a condition described herein is altered in a beneficial manner, other clinically accepted symptoms are improved, or even ameliorated, or a desired response is induced, e.g., by at least 10% following treatment according to the methods described herein. Efficacy can be assessed, for example, by measuring a marker, indicator, symptom, and/or the incidence of a condition treated according to the methods
15 described herein or any other measurable parameter appropriate. Treatment according to the methods described herein can reduce levels of a marker or symptom of a condition, e.g. by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80 % or at least 90% or more.

[00182] Efficacy can also be measured by a failure of an individual to worsen as assessed by
20 hospitalization, or need for medical interventions (i.e., progression of the disease is halted). Methods of measuring these indicators are known to those of skill in the art and/or are described herein.

[00183] Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human or an animal) and includes: (1) inhibiting the disease, e.g.,
25 preventing a worsening of symptoms (e.g., pain or inflammation); or (2) relieving the severity of the disease, e.g., causing regression of symptoms. An effective amount for the treatment of a disease means that amount which, when administered to a subject in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease. Efficacy of an agent can be determined by assessing physical indicators of a condition or desired response. It is well
30 within the ability of one skilled in the art to monitor efficacy of administration and/or treatment by measuring any one of such parameters, or any combination of parameters. Efficacy of a given approach can be assessed in animal models of a condition described herein, for example treatment of ALL. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant change in a marker is observed.

[00184] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior technology or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00185] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

[00186] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[00187] The technology described herein is further illustrated by the following examples, which in no way should be construed as being further limiting.

EXAMPLES

EXAMPLE 1

[00188] Described herein is the use of genetically modified T cells expressing anti-CD37 chimeric antigen receptors (CARs) to treat CD37 positive malignancies including, e.g., B cell-
 5 NHL. These exemplary CARs include a CD37 binding domain, a CD8 transmembrane domain, a 4-1BB co-stimulatory signaling region, and a CD3 ζ signaling domain. In other embodiments, the CD8 transmembrane domain, the 4-1BB co-stimulatory signaling region, and/or the CD3 ζ domain can be replaced another, corresponding sequence, e.g., those as described herein. CAR T cells can be generated by introducing a lentiviral vector comprising CD37-CAR in primary
 10 human T cells. *In vitro* data described herein demonstrates the specific activation, proliferation, and killing of CD37 positive cancer cells by anti-CD37 CAR T cells.

[00189] CD37 is detectable in certain cancer-related cell lines (Fig. 1). Anti-CD37 CARs including the components shown in Fig. 2 were constructed. A difference between the two constructs is the order of the heavy and light chains in the single chain antibody component of
 15 the CAR. These constructs were expressed in cells, which were analyzed for CAR expression by detection of a marker protein, mCherry (Fig. 3). Expansion of transduced T cells was measured (Fig. 4), and their ability to lyse target cancer cells demonstrated (Fig. 5). Anti-CD37 CAR T cells were able to lyse mixed populations of CD37-transduced leukemia cells, as well as high-expressors of CD37 (clone 1) and lower expressors of CD37 (clone 2) (Fig. 6). Anti-CD37
 20 demonstrated proliferation in response to CD37 stimulation (Fig. 7). Anti-CD37 CAR T cells can be administered to mice to demonstrate tumor clearance *in vivo* (Fig. 8).

EXAMPLE 2

[00190] *Anti-CD37 chimeric antigen receptor T cells: a new potential therapeutic option for*
 25 *B-cell malignancies.*

[00191] CD37 is a tetraspanin expressed on mature B cells but absent on early progenitors or terminally differentiated plasma cells. CD37 is highly expressed on malignant B cells in non-Hodgkin lymphomas (NHL), including mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), Burkitt lymphoma and B-cell chronic
 30 lymphocytic leukemia (CLL). Thus, CD37 represents a promising target for B-cell malignancies, particularly for variants that escape existing therapies targeting the common B cell antigens CD19 and CD20.

[00192] Described herein is an anti-CD37 CAR (CAR-37) for the treatment of B-cell malignancies. Specifically, described herein is a second-generation CAR, encoded by a
 35 lentiviral vector and bearing a 4-1BB costimulatory domain. Two different orientations of a

humanized murine antibody-derived single-chain variable fragment (V_L-V_H or V_H-V_L) were tested and a pre-clinical data panel is provided herein.

[00193] **Results**

[00194] *In vitro* cytotoxic activity of CAR T-37 cells was evaluated by co-culturing CAR T-37 cells with CD37-expressing human tumor cell lines (RAJI, OSU-CLL, and JEKO-1) at different effector to target ratios. CD37-directed CAR T cells demonstrated antigen-specific activation, proliferation, cytokine production, and cytotoxic activity *in vitro* in multiple models of B cell malignancy. Next, the anti-lymphoma efficacy was assessed *in vivo* in a mantle cell lymphoma model. CAR-37 treatment eliminated the tumor cells within 2 weeks, and mice maintained durable remissions. CAR T cells were detectable in the blood of mice after 7 days of injection. Ongoing studies are evaluating the long-term persistence of CAR T cells in mice.

[00195] Taken together these results demonstrate that T cells expressing anti-CD37 CAR have substantial activity *in vitro* and *in vivo* against B cell malignancies. These findings indicate that CD37-CAR T cells are a novel potential therapeutic agent for the treatment of patients with CD37 expressing tumors.

EXAMPLE 3

[00196] CD37 is highly expressed in B cell malignancies (Fig. 10). Anti-CD37 CARs were constructed using the humanized anti-CD37 mAb BI836826 (Boehringer Ingelheim) and cloned into a lentivirus vector. Intracellular domains are 41BB (CD137) ICD linked to CD3ζ.

[00197] The anti-CD37 CAR T cells were demonstrated to expand upon stimulation (Fig. 11) and activate (Fig. 12). Anti-CD37 CAR T cells expanded upon stimulation with the target antigen (Fig. 13). The anti-CD37 CAR T cells were demonstrated to lyse tumor cells *in vitro* (Fig. 14) and the production of various cytokines was measured (Fig. 15). *In vivo* efficacy of the anti-CD37 CAR Ts was demonstrated (Figs. 16 and 17).

EXAMPLE 4

[00198] *scFv sequences*

[00199] CD37 scFv V_H-V_L (SEQ ID NO: 1) comprises a V_H chain (amino acids 1-116 (SEQ ID NO: 2)), a linker region (amino acids 117-136 (SEQ ID NO: 3)), and a V_L chain (amino acids 137-244 (SEQ ID NO: 4)).

AVQLVQSGAEVKKPGSSVKVSCKASGYSTGYNMNWVRQAPGQGLEWMGNIDPYYGGTTYNR
KFKGRVTLTVDKSSSTAYMELSSLRSEDTAVYYCARSVGPMDYWGQGTLLTVSSGGGGSGGG
SGGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRTSENVYSYLAWYQQKPGKAPKLLVSS

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AKTLAEGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQHHSDNPWTFGQGTKVEIKR
(SEQ ID NO: 1)

[00200] VH chain (SEQ ID NO: 2 (amino acids 1-116 of SEQ ID NO: 1))

AVQLVQSGAEVKKPGSSVKVSCASGYSTGYNMNWVRQAPGQGLEWMGNIDPYYGGTTYNR
5 KFKGRVTLTVDKSSSTAYMELSSLRSED TAVYYCAR SVGPMDYWGQGT LVT VSS (SEQ ID
NO: 2)

[00201] Linker region (SEQ ID NO: 3 (amino acids 117-136 of SEQ ID NO: 1))

GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 3)

[00202] VL chain (SEQ ID NO: 4 (amino acids 137-244 SEQ ID NO: 1))

10 DIQMTQSPSSLSASVGDRVTITCRTSENVYSYLA WYQQKPGKAPKLLVSSAKTLAEGVPSRF
SGSGSGTDFTLTISSLQPEDFATYFCQHHSDNPWTFGQGTKVEIKR (SEQ ID NO: 4)

[00203] CD37 scFv VL-VH (SEQ ID NO: 5) comprises a VL chain (amino acids 1-108 (SEQ ID NO: 6)), a linker region (amino acids 109-128 (SEQ ID NO: 7)), and a VH chain (amino acids 129-244 (SEQ ID NO: 8)).

15 DIQMTQSPSSLSASVGDRVTITCRTSENVYSYLA WYQQKPGKAPKLLVSSAKTLAEGVPSRF
SGSGSGTDFTLTISSLQPEDFATYFCQHHSDNPWTFGQGTKVEIKRGGGGSGGGGSGGGGSG
GGGSAVQLVQSGAEVKKPGSSVKVSCASGYSTGYNMNWVRQAPGQGLEWMGNIDPYYGGT
TYNRKFKGRVTLTVDKSSSTAYMELSSLRSED TAVYYCAR SVGPMDYWGQGT LVT VSS
(SEQ ID NO: 5)

20 [00204] VL chain (SEQ ID NO: 6 (amino acids 1-108 of SEQ ID NO: 5))

DIQMTQSPSSLSASVGDRVTITCRTSENVYSYLA WYQQKPGKAPKLLVSSAKTLAEGVPSRF
SGSGSGTDFTLTISSLQPEDFATYFCQHHSDNPWTFGQGTKVEIKR (SEQ ID NO: 6)

[00205] Linker region (SEQ ID NO: 7 (amino acids 109-128 of SEQ ID NO: 5))

GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 7)

25 [00206] VH chain (SEQ ID NO: 8 (amino acids 129-244 SEQ ID NO: 5))

AVQLVQSGAEVKKPGSSVKVSCASGYSTGYNMNWVRQAPGQGLEWMGNIDPYYGGTTYNR
KFKGRVTLTVDKSSSTAYMELSSLRSED TAVYYCAR SVGPMDYWGQGT LVT VSS (SEQ ID
NO: 8)

30 EXAMPLE 5

[00207] CAR sequences

[00208] pMGH8 - CD8Leader/anti-CD37 L-H/CD8 hinge + TM/4-1BB/CD3ζ (SEQ ID NO: 9) comprising CD8 leader sequence (amino acids 1-21 (SEQ ID NO: 10)); anti-CD37 L-H (amino acids 22-265 (SEQ ID NO: 11)); CD8 hinge and TM domain (amino acids 266-334 (SEQ

ID NO: 12)); 4-1BB (amino acids 335-376 (SEQ ID NO: 13)); and CD3 ζ (amino acids 377-488 (SEQ ID NO: 14)).

5 MALPVTALLLPLALLLHAARPDIQMTQSPSSLSASVGDRVTITCRTSENVYSYLAWEYQQKPG
KAPKLLVSSAKTLAEGVPSRFSGSGSGTDFTLTITSSLPEDFATYFCQHHSDNPWTFGQGTK
VEIKRGGGGSGGGGSGGGGSGGGGSAVQLVQSGAEVKKPGSSSVKVSCKASGYSFTGYNMNWV
RQAPGQGLEWMGNIDPYYGGTTYNRKFKGRVTLTVDKSSSTAYMELSSLRSEDTAVYYCARS
VGPM DYWGQGT LVTVSSTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACD
IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEG
10 YNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
NO: 9)

[00209] CD8 leader sequence (SEQ ID NO: 10 (amino acids 1-21 of SEQ ID NO: 9))

MALPVTALLLPLALLLHAARP (SEQ ID NO: 10)

[00210] anti-CD37 L-H (SEQ ID NO: 11 (amino acids 22-265 of SEQ ID NO: 9))

15 DIQMTQSPSSLSASVGDRVTITCRTSENVYSYLAWEYQQKPGKAPKLLVSSAKTLAEGVPSRF
SGSGSGTDFTLTITSSLPEDFATYFCQHHSDNPWTFGQGTKVEIKRGGGGSGGGGSGGGGSG
GGGSAVQLVQSGAEVKKPGSSSVKVSCKASGYSFTGYNMNWVRQAPGQGLEWMGNIDPYYGGT
TYNRKFKGRVTLTVDKSSSTAYMELSSLRSEDTAVYYCAR SVGPM DYWGQGT LVTVS
(SEQ ID NO: 11)

20 **[00211]** CD8 hinge and TM domain SEQ ID NO: 12 (amino acids 266-334 of SEQ ID NO: 9)

TTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYC (SEQ ID NO: 12)

[00212] 4-1BB (SEQ ID NO: 13 (amino acids 335-376 of SEQ ID NO: 9))

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 13)

25 **[00213]** CD3 ζ (SEQ ID NO: 14 (amino acids 377-488 of SEQ ID NO: 9))

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
14)

30 **[00214]** pMGH8 - CD8Leader/anti-CD37 H-L/CD8 hinge + TM/4-1BB/CD3 ζ (SEQ ID NO:
15) comprising CD8 leader sequence (amino acids 1-21 (SEQ ID NO: 16)); anti-CD37 H-L
(amino acids 22-265 (SEQ ID NO: 17)); CD8 hinge and TM domain (amino acids 266-334 (SEQ
ID NO: 18)); 4-1BB (amino acids 335-376 (SEQ ID NO: 19)); and CD3 ζ (amino acids 377-488
(SEQ ID NO: 20)).

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MALPVTALLLPLALLLHAARPAVQLVQSGAEVKKPGSSVKVSCKASGYSTGYNMNWVRQAP
 GQGLEWMGNIDPYYGGTTYNRKFKGRVTLTVDKSSSTAYMELSSLRSEDVAVYYCARSVGPM
 DYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRTSENV
 YSYLAWYQQKPGKAPKLLVSSAKTLAEGVPSRFSGSGSGTDFTLTITSSLPEDFATYFCQHH
 5 SDNPWTFGQGTKVEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
 IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
 GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEG
 YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
 NO: 15)

10 [00215] CD8 leader sequence (SEQ ID NO: 16 (amino acids 1-21 of SEQ ID NO: 15))

MALPVTALLLPLALLLHAARP (SEQ ID NO: 16)

[00216] anti-CD37 H-L (SEQ ID NO: 17 (amino acids 22-265 of SEQ ID NO: 15))

AVQLVQSGAEVKKPGSSVKVSCKASGYSTGYNMNWVRQAPGQGLEWMGNIDPYYGGTTYNR
 KFKGRVTLTVDKSSSTAYMELSSLRSEDVAVYYCARSVGPMDYWGQGTLLTVSSGGGGSGGG
 15 GSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRTSENVYSYLAWYQQKPGKAPKLLVSS
 AKTLAEGVPSRFSGSGSGTDFTLTITSSLPEDFATYFCQHHSDNPWTFGQGTKVEIKR
 (SEQ ID NO: 17)

[00217] CD8 hinge and TM domain SEQ ID NO: 18 (amino acids 266-334 of SEQ ID NO:
 15)

20 TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
 LVITLYC (SEQ ID NO: 18)

[00218] 4-1BB (SEQ ID NO: 19 (amino acids 335-376 of SEQ ID NO: 15))

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 19)

[00219] CD3 ζ (SEQ ID NO: 20 (amino acids 377-488 of SEQ ID NO: 15))

25 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNEL
 QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
 20)

EXAMPLE 6

30 *Anti-CD37 CAR-T Cells Effective Against B Cell Malignancies*

[00220] As noted above, CD37 is a tetraspanin expressed on mature B cells but absent on early progenitors or terminally differentiated plasma cells. It is highly expressed on malignant B cells and it represents a promising target for B-cell malignancies, particularly for variants that escape existing therapies targeting the common B-cell antigens CD19 and CD20. We designed

the first anti-CD37 CAR (CAR-37) for the treatment of B-cell malignancies. *In vitro* cytotoxic activity of CART-37 cells was evaluated by co-culturing CART-37 cells with CD 37-expressing human tumor cell lines at different effector to target ratios. CD37-directed CAR T cells demonstrated antigen-specific proliferation, cytokine production, and cytotoxic activity *in vitro* in multiple models of B-cell malignancy. We assessed the anti-lymphoma efficacy *in vivo* in a mantle cell lymphoma model. CAR-37 treatment eliminated the tumor cells within 2 weeks, and mice maintained durable remissions. Together these results show that T cells expressing anti-CD37 CAR have substantial activity *in vitro* and *in vivo* against B-cell malignancies. These findings indicated that CD37-CAR T cells are a novel potential therapeutic agent for the treatment of patients with CD37-expressing tumors.

[00221] In normal tissues, CD37 expression is restricted to lymphoid organs (FIG. 18A). However, it is highly expressed on B-cell leukemia and lymphoma cells (FIG. 18B). CAR-37 T cells were made based on the design shown in FIG. 19. CAR-37 T cells are able to proliferate and expand *ex vivo* (FIGS. 20A-20D). T cells expressing anti-CD37 CAR have substantial *in vitro* activity against mantle cell lymphoma, Burkitt lymphoma, and B-cell lymphoblastic leukemia tumor cells (FIGS. 21A-21D), and produce cytokines as shown in FIGS. 22A-22C. T cells expressing anti-CD37 CAR have a strong anti-tumor activity in a mantle cell lymphoma model *in vivo* (FIGS. 23A-23C)

EXAMPLE 7

CD37 and CD19 expression in human tumor cell lines

[00222] The expression of CD37 and CD19 in human tumor cell lines was evaluated. The top panel of FIG. 24 reproduces the data shown in FIG. 18A, and shows that CD37 is highly expressed in non-Hodgkin lymphomas including MCL (JEKO-1), Burkitt lymphoma (RAJI), and B-cell chronic lymphocytic leukemia (OSU-CLL), but is absent in the ALL cell line NALM6. The bottom panel of FIG. 24 shows the expression of CD37 and CD19 in the MCL patient-derived xenograft (PDX) lines PDX_44685, PDX_98848, and PDX_96069, as well as the percent expression of CD19 and CD37 in the PDX cell lines. The MCL PDX cell lines expressed both CD37 and CD19.

EXAMPLE 8

Anti-CD37 CAR-T Cells are Effective against MCL PDX Tumors

[00223] The efficacy of anti-CD37 CAR-T cells against MCL PDX cells was evaluated *in vivo*. FIG. 25A shows an experimental schematic; NOD/SCID mice were injected i.v. with

1x10⁶ PDX_98848 cells. On day 0, mice received 3x10⁶ control T cells (UTD), CAR-37 H-L, or CAR-19. Tumor growth was evaluated by BLI on day 3, day 7, day 10, day 14, day 17, day 21, and day 35. Representative bioluminescent images of the PDX growth over time is shown in FIG. 25B. T cells expressing anti-CD37 CAR have strong anti-tumor activity against MCL PDX
 5 *in vivo* (FIGS. 25A-25C).

EXAMPLE 9

CD37 Expression in Peripheral T Cell Lymphoma (PTCL)

[00224] The expression of CD37 was evaluated in PTCL cell lines, including HUT78
 10 (cutaneous T-cell lymphoma (CTCL)) and FEPD (anaplastic large cell lymphoma (ALCL)) (FIG. 26A). CD37 was expressed in both cell lines. Expression of the early activation marker CD69 was examined after CAR stimulation (FIGS. 26B and 26C).

EXAMPLE 10

Anti-CD37 CAR-T Cells have In Vitro Cytotoxic Activity against PTCL Cell Lines

[00225] The *in vitro* cytotoxic activity of anti-CD37 CAR-T cells against PTCL lines was evaluated (FIG. 27). T-cells expressing anti-CD37 CAR have substantial *in vitro* activity against PTCL lines, including CTCL and ALCL tumor models (FIG. 27). These findings demonstrated
 20 that CD37-CAR T cells are useful as therapeutic agents for the treatment of patients with PTCL, including CTCL and ALCL.

EXAMPLE 11

CD37 Expression in AML

[00226] CD37 expression has been detected in AML samples (Pereira et al., Mol. Cancer Ther. 14(7):1650-1660, 2015). The expression of CD37 in AML cell lines was evaluated in the AML cell lines TF1, MOLM13, and THP by flow cytometry (FIG. 28). All three AML cell lines expressed CD37 (FIG. 28). These findings demonstrated that CD37-CAR T cells are useful as
 25 therapeutic agents for the treatment of patients with AML.

30 The invention is further described in the following numbered paragraphs:

[00227] A chimeric antigen receptor (CAR) polypeptide comprising:

- a. an extracellular domain comprising a CD37-binding sequence;
- b. a transmembrane domain; and
- c. a T cell intracellular signaling domain.

35 [00228] The CAR polypeptide of paragraph 1, further comprising a co-stimulatory domain.

[00229] The CAR polypeptide of paragraph 1 or 2, wherein the CD37-binding sequence comprises an antibody reagent.

[00230] The CAR polypeptide of paragraph 3, wherein the antibody reagent comprises a single-chain antibody (scFv).

5 [00231] The CAR polypeptide of paragraph 4, wherein the scFv comprises an antibody light chain N-terminal to an antibody heavy chain.

[00232] The CAR polypeptide of paragraph 4, wherein the scFv comprises an antibody heavy chain N-terminal to an antibody light chain.

10 [00233] The CAR polypeptide of paragraph 5 or 6, wherein the antibody light chain comprises the sequence of SEQ ID NO: 4 or 6, or a variant thereof, and/or the heavy chain comprises the sequence of SEQ ID NO: 2 or 8, or a variant thereof.

[00234] The CAR polypeptide of any one of paragraphs 3 to 7, wherein the antibody reagent comprises a sequence selected from SEQ ID NO: 1 or 5, or a variant thereof.

15 [00235] The CAR polypeptide of any one of paragraphs 1 to 7, wherein the transmembrane domain comprises the transmembrane domain from CD8 or 4-1BB.

[00236] The CAR polypeptide of paragraph 8, wherein the transmembrane domain comprises the sequence of SEQ ID NO: 12 or 18, or a variant thereof.

[00237] The CAR polypeptide of any one of paragraphs 2 to 8, wherein the co-stimulatory domain comprises the co-stimulatory domain of 4-1BB.

20 [00238] The CAR polypeptide of paragraph 11, wherein the co-stimulatory domain comprises the sequence of SEQ ID NO: 13 or 19, or a variant thereof.

[00239] The CAR polypeptide of any one of paragraphs 1 to 12, wherein the T cell intracellular domain comprises a CD3 ζ intracellular signaling domain.

25 [00240] The CAR polypeptide of paragraph 13, wherein the CD3 ζ intracellular signaling domain comprises the sequence of SEQ ID NO: 14 or 20, or a variant thereof.

[00241] The CAR polypeptide of paragraph 14, wherein the CD3 ζ intracellular signaling domain comprises 1, 2, or 3 immunoreceptor tyrosine-based activation motifs (ITAMs), and the native tyrosine residues of the ITAM(s) are maintained.

30 [00242] The CAR polypeptide of paragraph 14, wherein the CD3 ζ intracellular signaling domain comprises the sequence of SEQ ID NO: 14 or 20.

[00243] The CAR polypeptide of any one of paragraphs 1 to 16, comprising a sequence of SEQ ID NO: 9 or 15, or a variant thereof.

[00244] A mammalian cell comprising:

- a. the CAR polypeptide of any one of paragraphs 1 to 16; or

- b. a nucleic acid encoding any one of the CAR polypeptides of any one of paragraphs 1 to 17.

[00245] The cell of paragraph 18, wherein the cell is a T cell.

[00246] The cell of paragraph 18 or 19, wherein the cell is a human cell.

- 5 [00247] The cell of any one of paragraphs 18 to 20, wherein the cell is obtained from an individual having or diagnosed as having cancer, a plasma cell disorder, or autoimmune disease.

[00248] A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:

- a. engineering a T cell to comprise a CAR polypeptide of any one of paragraphs 1 to 10 17 on the T cell surface;
- b. administering the engineered T cell to the subject.

[00249] A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering the cell of paragraph any one of paragraphs 18 to 21 to the subject.

- 15 [00250] The method of paragraph 22 or 23, wherein the cancer is a CD37+ cancer.

[00251] The method of paragraph 24, wherein the CD37+ cancer is lymphoma or leukemia.

[00252] The method of paragraph 25, wherein the lymphoma is B-cell Non-Hodgkin Lymphoma (NHL), mantle cell lymphoma, Burkitt's lymphoma, B cell lymphoblastic lymphoma, or T cell lymphoma, or the leukemia is acute myeloid leukemia (AML).

- 20 [00253] The method of paragraph 26, wherein the T cell lymphoma is peripheral T cell lymphoma (PTCL).

[00254] The method of paragraph 27, wherein the PTCL is cutaneous T-cell lymphoma (CTCL) or anaplastic large cell lymphoma (ALCL).

- 25 [00255] A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering a cell of any one of paragraphs 18 to 21 to the subject, wherein the cell comprises a CAR comprising an extracellular domain comprising a CD37-binding sequence and the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

- 30 [00256] A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:

- a. selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy;
- b. engineering a T cell to comprise a CAR polypeptide of any one of paragraphs 1 to 17 on the T cell surface;
- c. administering the engineered T cell to the subject;

wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[00257] A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:

- a. selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy;
- 5 b. administering a cell of any one of paragraphs 18 to 21 to the subject, wherein the cell comprises a CAR comprising an extracellular domain comprising a CD37-binding sequence and the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

[00258] A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:

- a. engineering a T cell to comprise a CAR polypeptide of any one of paragraphs 1 to 17 on the T cell surface;
- b. administering the engineered T cell to the subject;

wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

15 **[00259]** A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering a cell of any one of paragraphs 18 to 21 to the subject, wherein the cell comprises a CAR comprising an extracellular domain comprising a CD37-binding sequence;

wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

20 **[00260]** A composition comprising the CAR polypeptide of any one of paragraphs 1 to 17 or a cell of any one of paragraphs 18 to 21 formulated for the treatment of cancer.

[00261] The composition of paragraph 34, further comprising a pharmaceutically acceptable carrier.

[00262] Other embodiments are within the scope of the following claims.

What is claimed is:

1. A chimeric antigen receptor (CAR) polypeptide comprising:
 - a) an extracellular domain comprising a CD37-binding sequence;
 - b) a transmembrane domain; and
 - c) a T cell intracellular signaling domain.
2. The CAR polypeptide of claim 1, further comprising a co-stimulatory domain.
3. The CAR polypeptide of claim 1 or 2, wherein the CD37-binding sequence comprises an antibody reagent.
4. The CAR polypeptide of claim 3, wherein the antibody reagent comprises a single-chain antibody (scFv).
5. The CAR polypeptide of claim 4, wherein the scFv comprises an antibody light chain N-terminal to an antibody heavy chain.
6. The CAR polypeptide of claim 4, wherein the scFv comprises an antibody heavy chain N-terminal to an antibody light chain.
7. The CAR polypeptide of claim 5 or 6, wherein the antibody light chain comprises the sequence of SEQ ID NO: 4 or 6, or a variant thereof, and/or the heavy chain comprises the sequence of SEQ ID NO: 2 or 8, or a variant thereof.
8. The CAR polypeptide of any one of claims 3 to 7, wherein the antibody reagent comprises a sequence selected from SEQ ID NO: 1 or 5, or a variant thereof.
9. The CAR polypeptide of any one of claims 1 to 7, wherein the transmembrane domain comprises the transmembrane domain from CD8 or 4-1BB.
10. The CAR polypeptide of claim 8, wherein the transmembrane domain comprises the sequence of SEQ ID NO: 12 or 18, or a variant thereof.

11. The CAR polypeptide of any one of claims 2 to 8, wherein the co-stimulatory domain comprises the co-stimulatory domain of 4-1BB.
12. The CAR polypeptide of claim 11, wherein the co-stimulatory domain comprises the sequence of SEQ ID NO: 13 or 19, or a variant thereof.
13. The CAR polypeptide of any one of claims 1 to 12, wherein the T cell intracellular domain comprises a CD3 ζ intracellular signaling domain.
14. The CAR polypeptide of claim 13, wherein the CD3 ζ intracellular signaling domain comprises the sequence of SEQ ID NO: 14 or 20, or a variant thereof.
15. The CAR polypeptide of claim 14, wherein the CD3 ζ intracellular signaling domain comprises 1, 2, or 3 immunoreceptor tyrosine-based activation motifs (ITAMs), and the native tyrosine residues of the ITAM(s) are maintained.
16. The CAR polypeptide of claim 14, wherein the CD3 ζ intracellular signaling domain comprises the sequence of SEQ ID NO: 14 or 20.
17. The CAR polypeptide of any one of claims 1 to 16, comprising a sequence of SEQ ID NO: 9 or 15, or a variant thereof.
18. A mammalian cell comprising:
 - a) the CAR polypeptide of any one of claims 1 to 16; or
 - b) a nucleic acid encoding any one of the CAR polypeptides of any one of claims 1 to 17.
19. The cell of claim 18, wherein the cell is a T cell.
20. The cell of claim 18 or 19, wherein the cell is a human cell.
21. The cell of any one of claims 18 to 20, wherein the cell is obtained from an individual having or diagnosed as having cancer, a plasma cell disorder, or autoimmune disease.

22. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:
- a) engineering a T cell to comprise a CAR polypeptide of any one of claims 1 to 17 on the T cell surface;
 - b) administering the engineered T cell to the subject.
23. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering the cell of claim any one of claims 18 to 21 to the subject.
24. The method of claim 22 or 23, wherein the cancer is a CD37+ cancer.
25. The method of claim 24, wherein the CD37+ cancer is lymphoma or leukemia.
26. The method of claim 25, wherein the lymphoma is B-cell Non-Hodgkin Lymphoma (NHL), mantle cell lymphoma, Burkitt's lymphoma, B cell lymphoblastic lymphoma, or T cell lymphoma, or the leukemia is acute myeloid leukemia (AML).
27. The method of claim 26, wherein the T cell lymphoma is peripheral T cell lymphoma (PTCL).
28. The method of claim 27, wherein the PTCL is cutaneous T-cell lymphoma (CTCL) or anaplastic large cell lymphoma (ALCL).
29. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering a cell of any one of claims 18 to 21 to the subject, wherein the cell comprises a CAR comprising an extracellular domain comprising a CD37-binding sequence and the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.
30. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:
- a) selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy;

- b) engineering a T cell to comprise a CAR polypeptide of any one of claims 1 to 17 on the T cell surface;
 - c) administering the engineered T cell to the subject;
- wherein the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

31. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:

- a) selecting a subject who is non-responsive to anti-CD19 and/or anti-CD20 therapy;
- b) administering a cell of any one of claims 18 to 21 to the subject, wherein the cell comprises a CAR comprising an extracellular domain comprising a CD37-binding sequence and the subject is non-responsive to anti-CD19 and/or anti-CD20 therapy.

32. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising:

- a) engineering a T cell to comprise a CAR polypeptide of any one of claims 1 to 17 on the T cell surface;
- b) administering the engineered T cell to the subject;

wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

33. A method of treating cancer, a plasma cell disorder, or an autoimmune disease in a subject in need thereof, the method comprising administering a cell of any one of claims

18 to 21 to the subject, wherein the cell comprises a CAR comprising an extracellular domain comprising a CD37-binding sequence;

wherein the subject is concurrently administered an anti-CD19 and/or anti-CD20 therapy.

34. A composition comprising the CAR polypeptide of any one of claims 1 to 17 or a cell of any one of claims 18 to 21 formulated for the treatment of cancer.

35. The composition of claim 34, further comprising a pharmaceutically acceptable carrier.

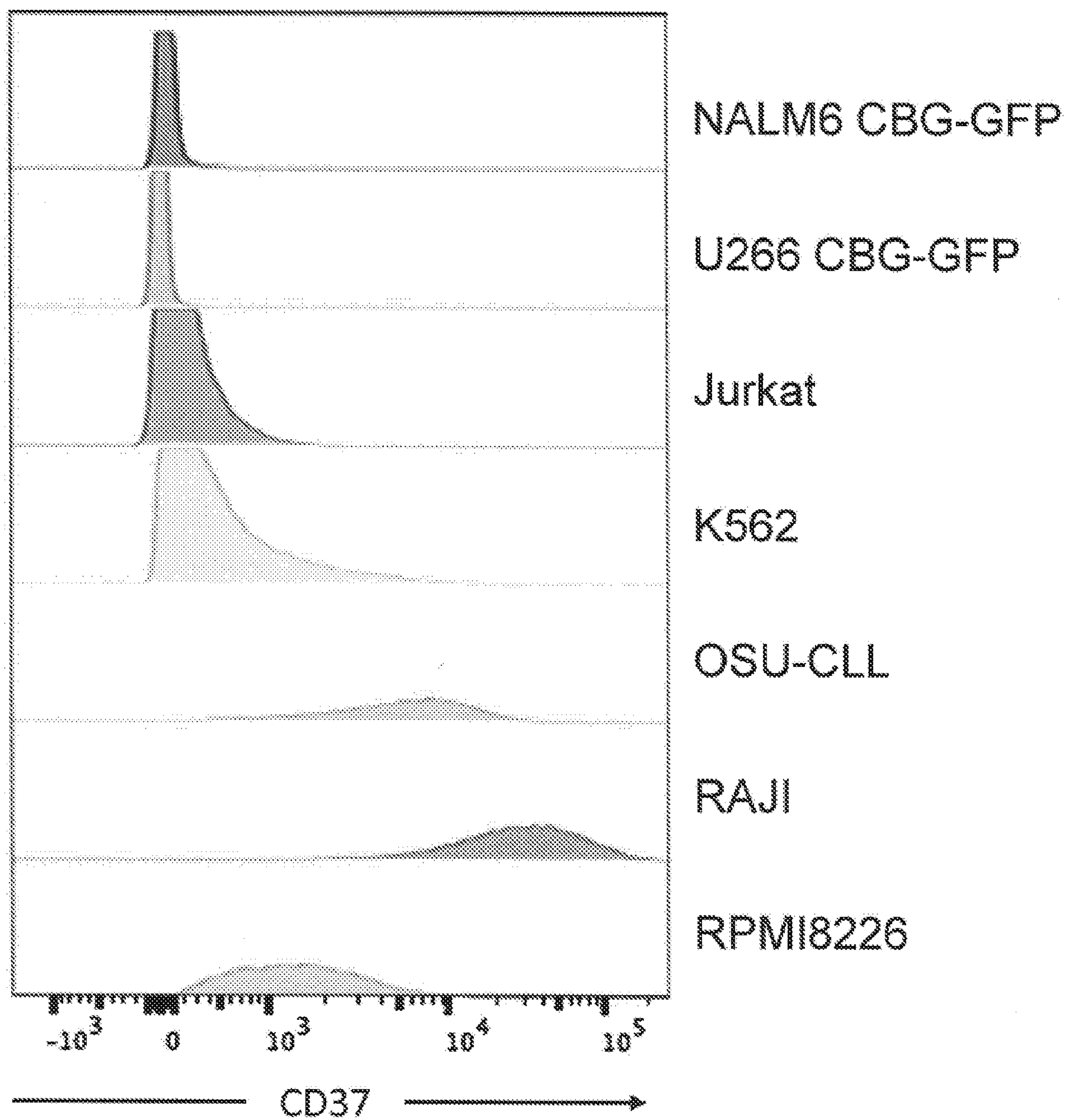


FIG. 1

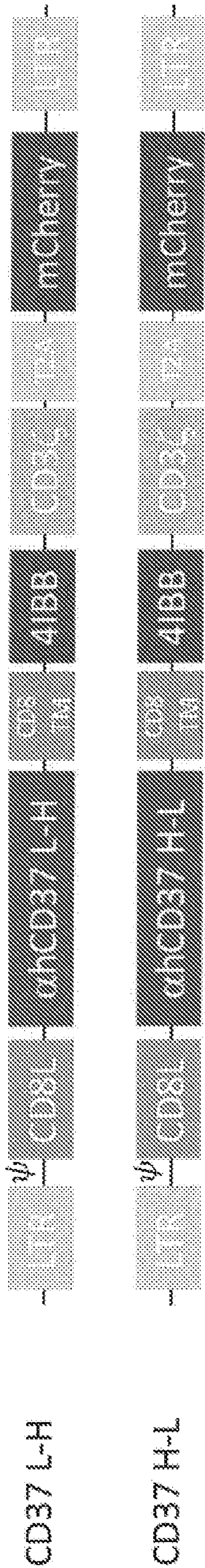


FIG. 2

FIG. 3

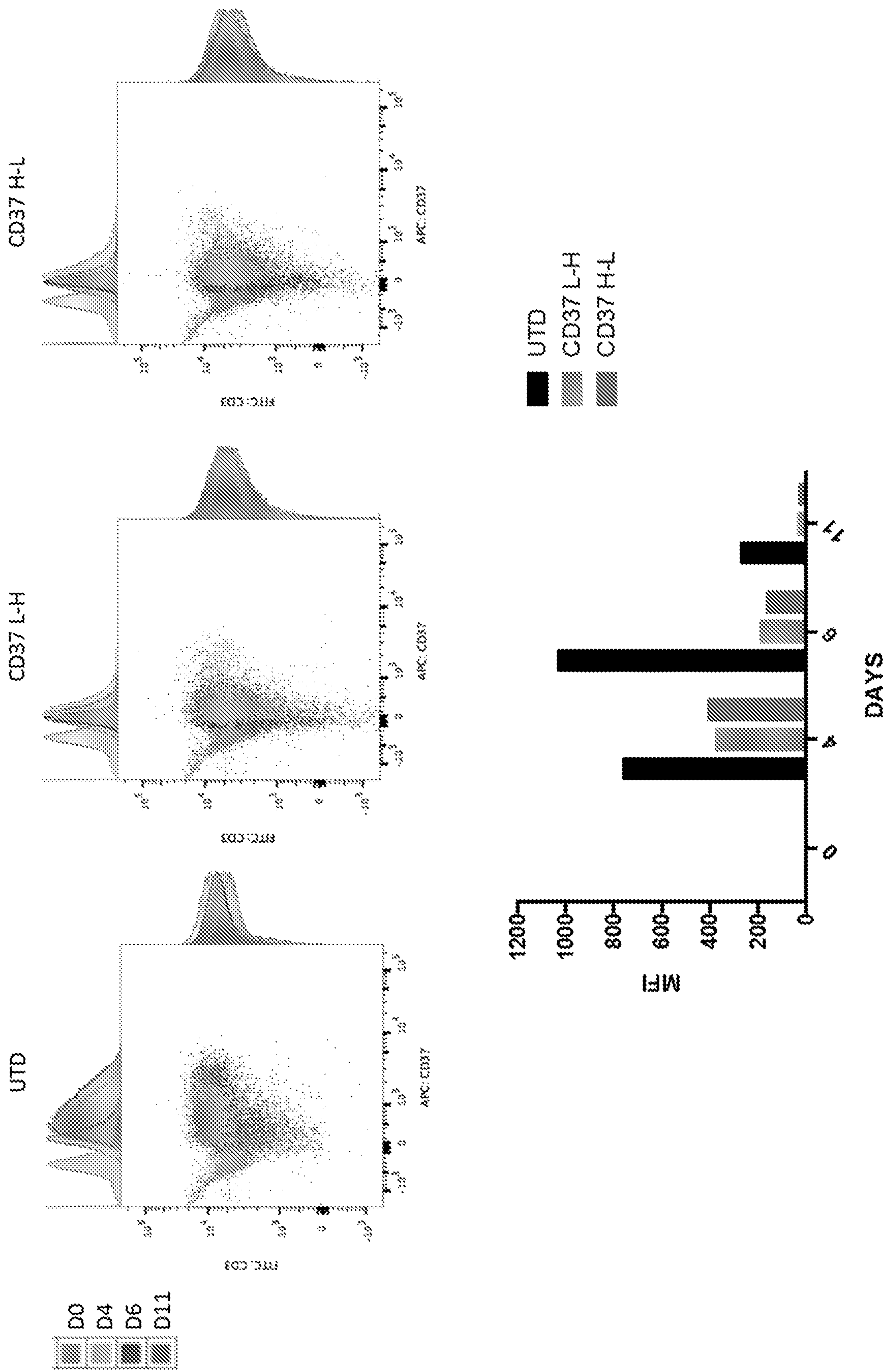
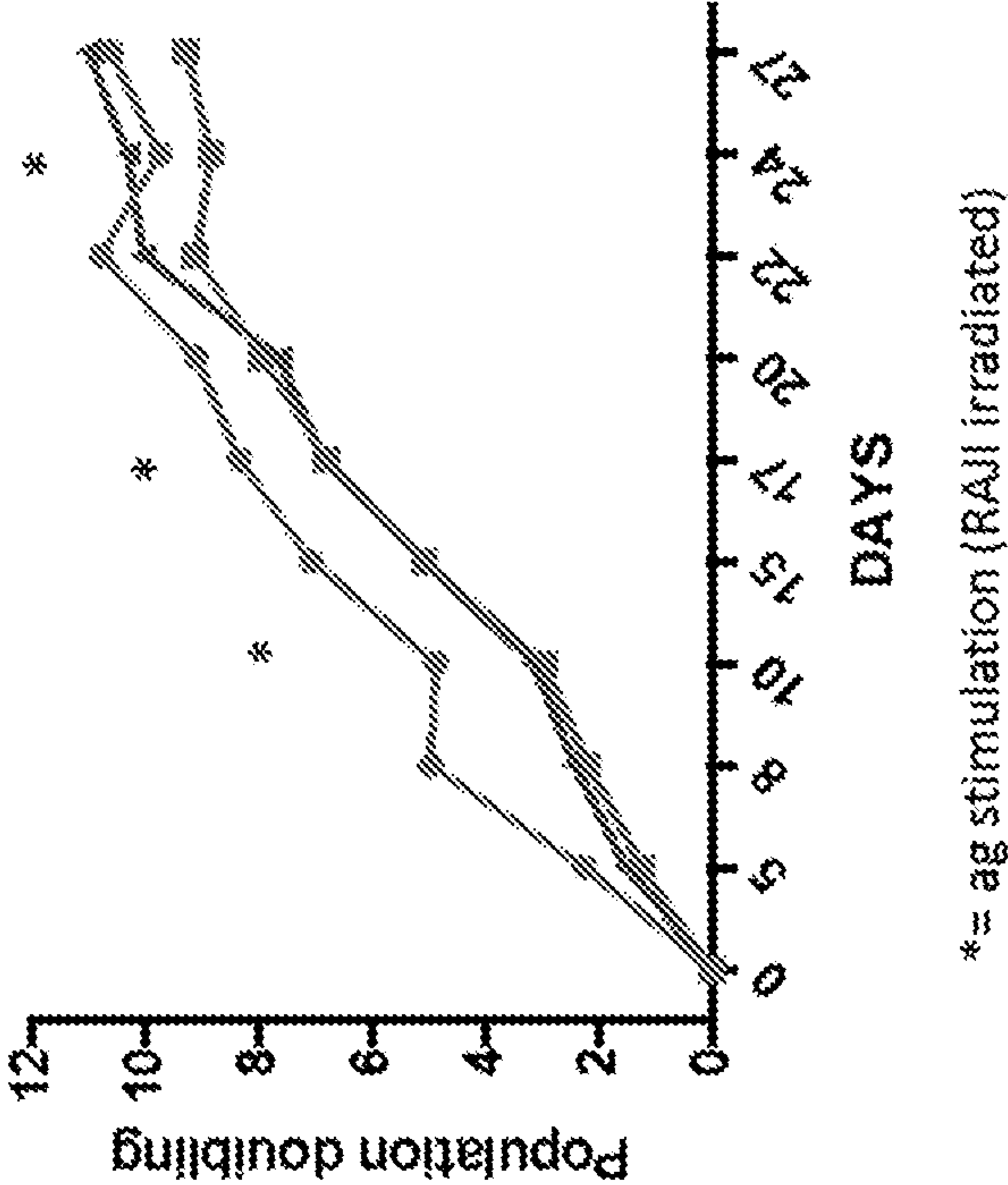
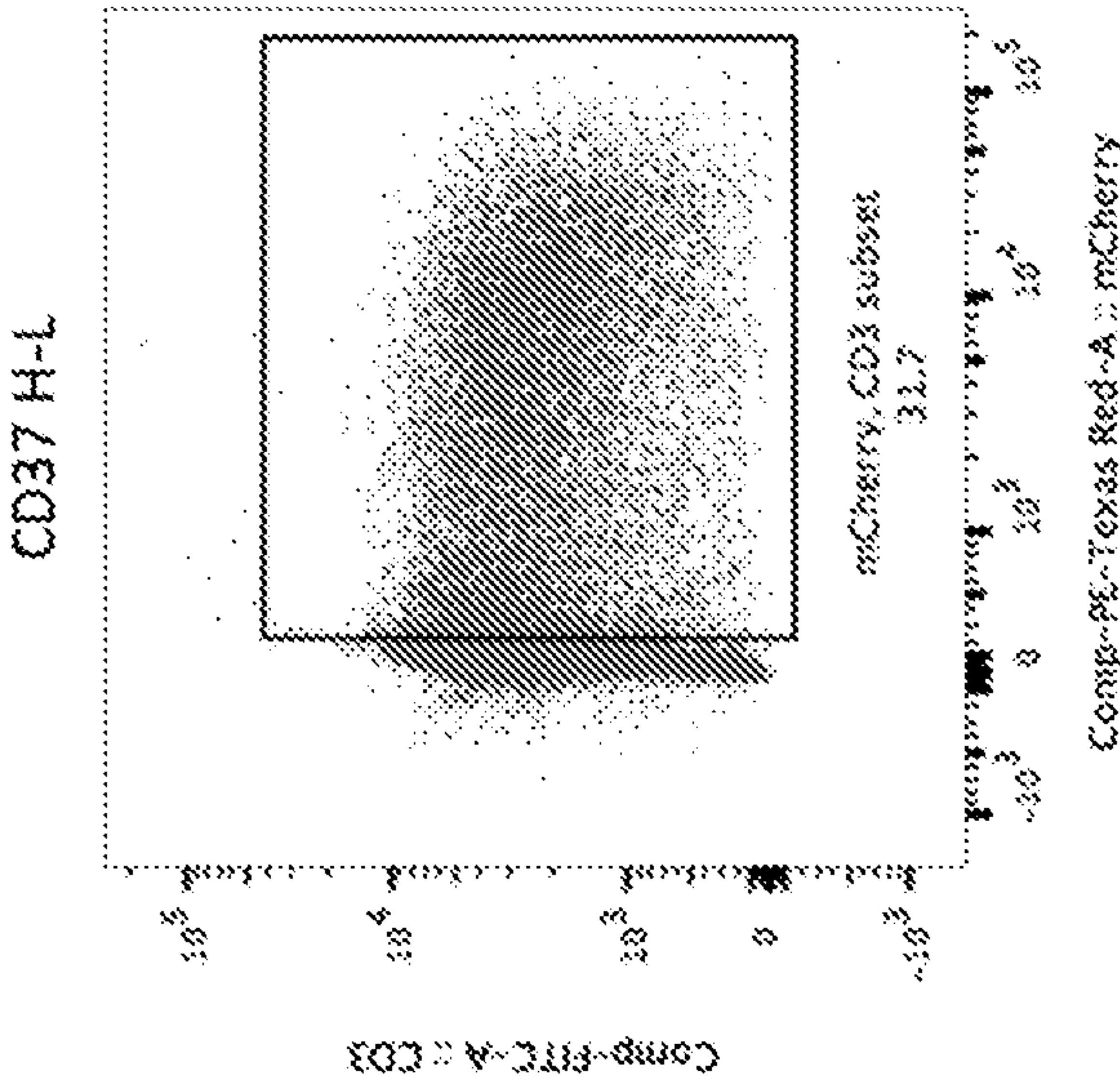
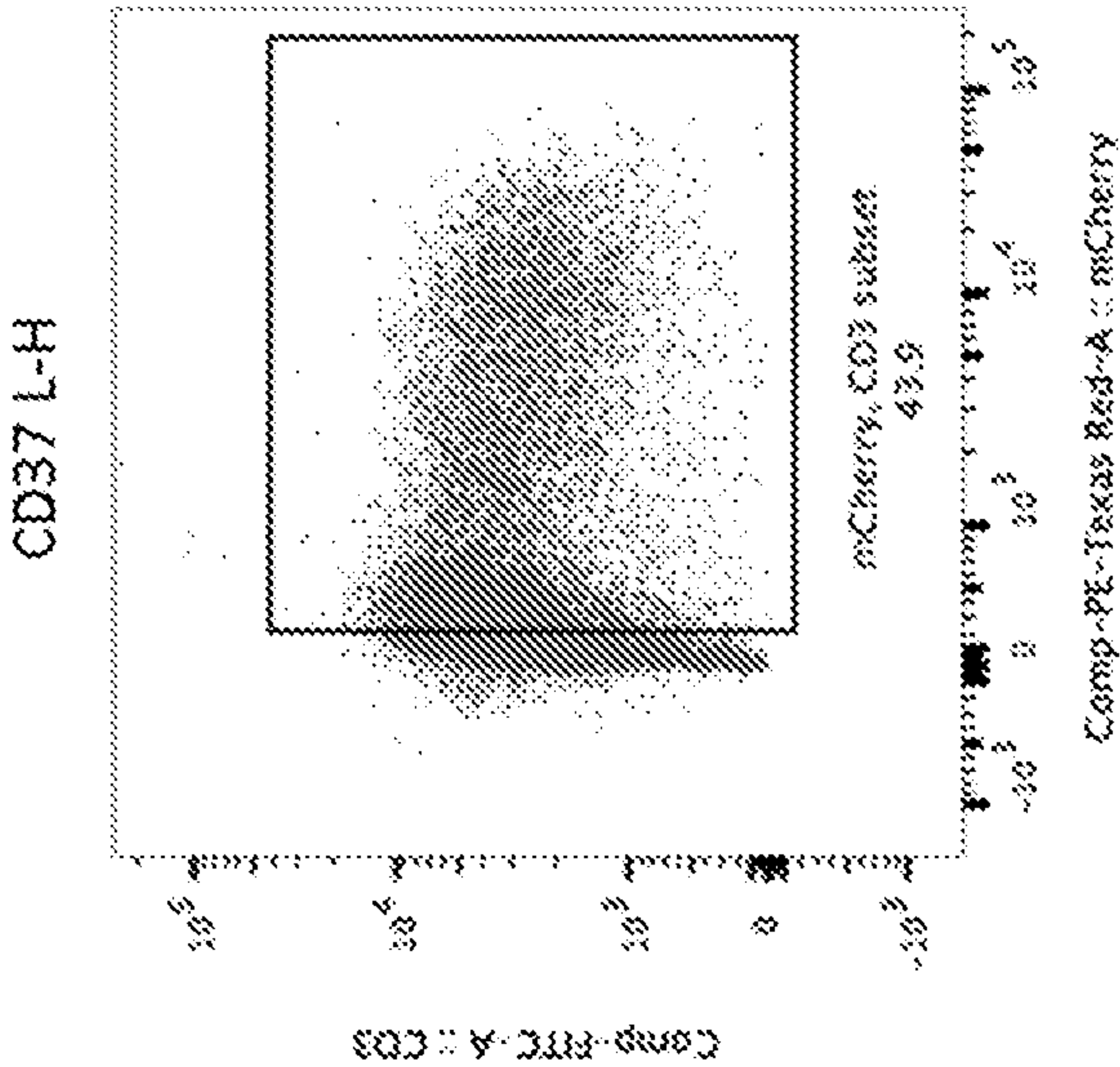


FIG. 4



UTD
CD37 L-H
CD37 H-L



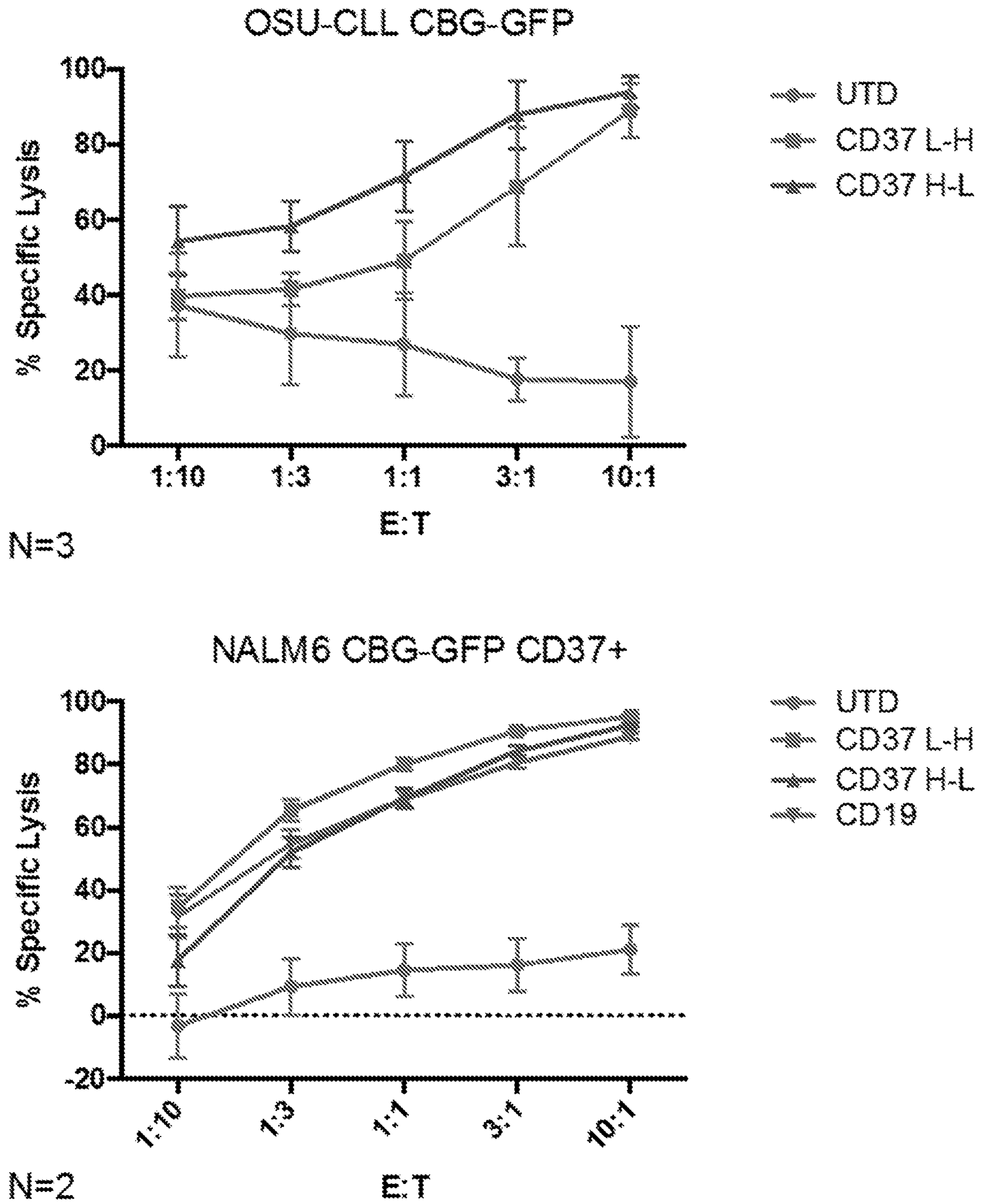


FIG. 5

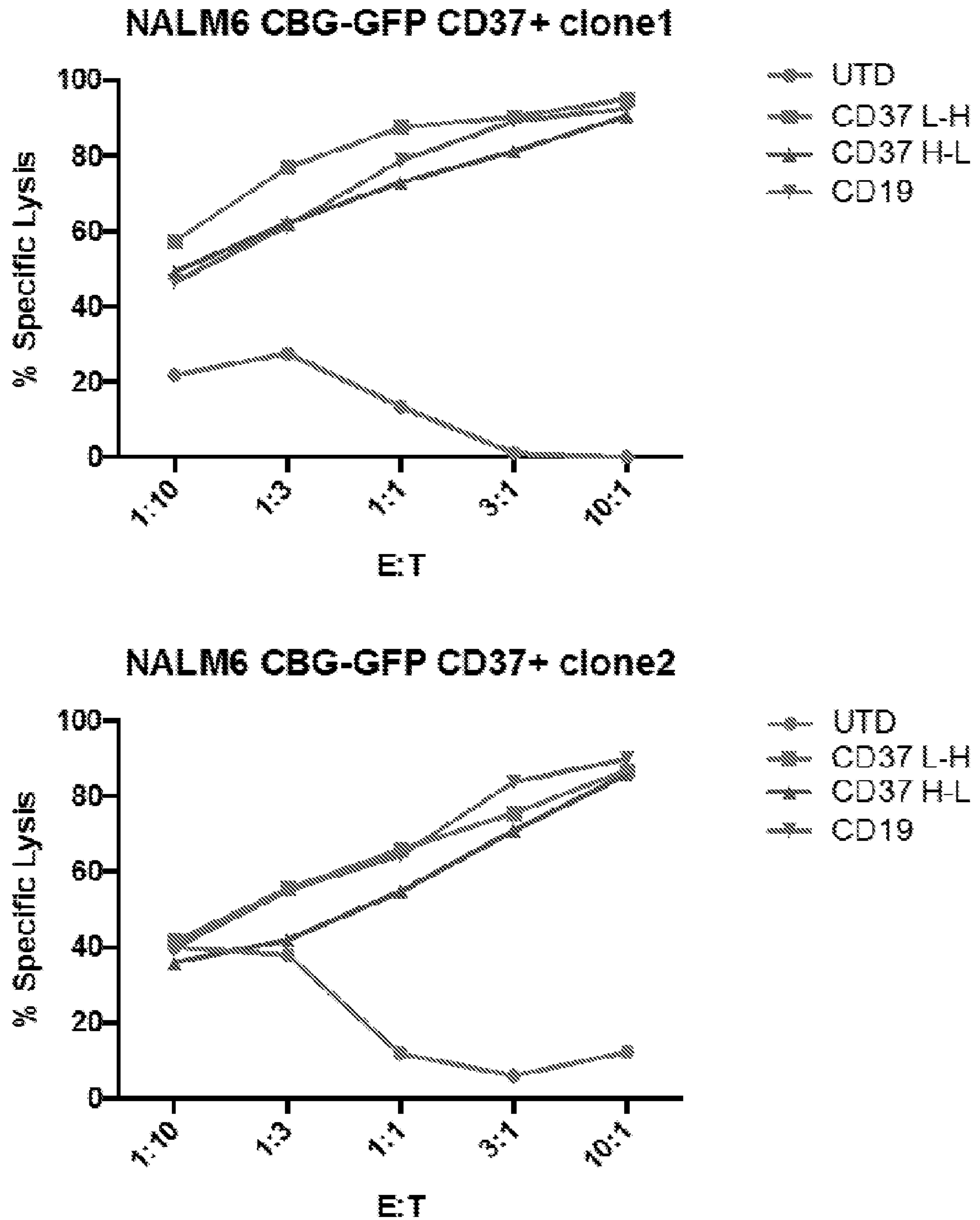


FIG. 5 (CONT.)

FIG. 6

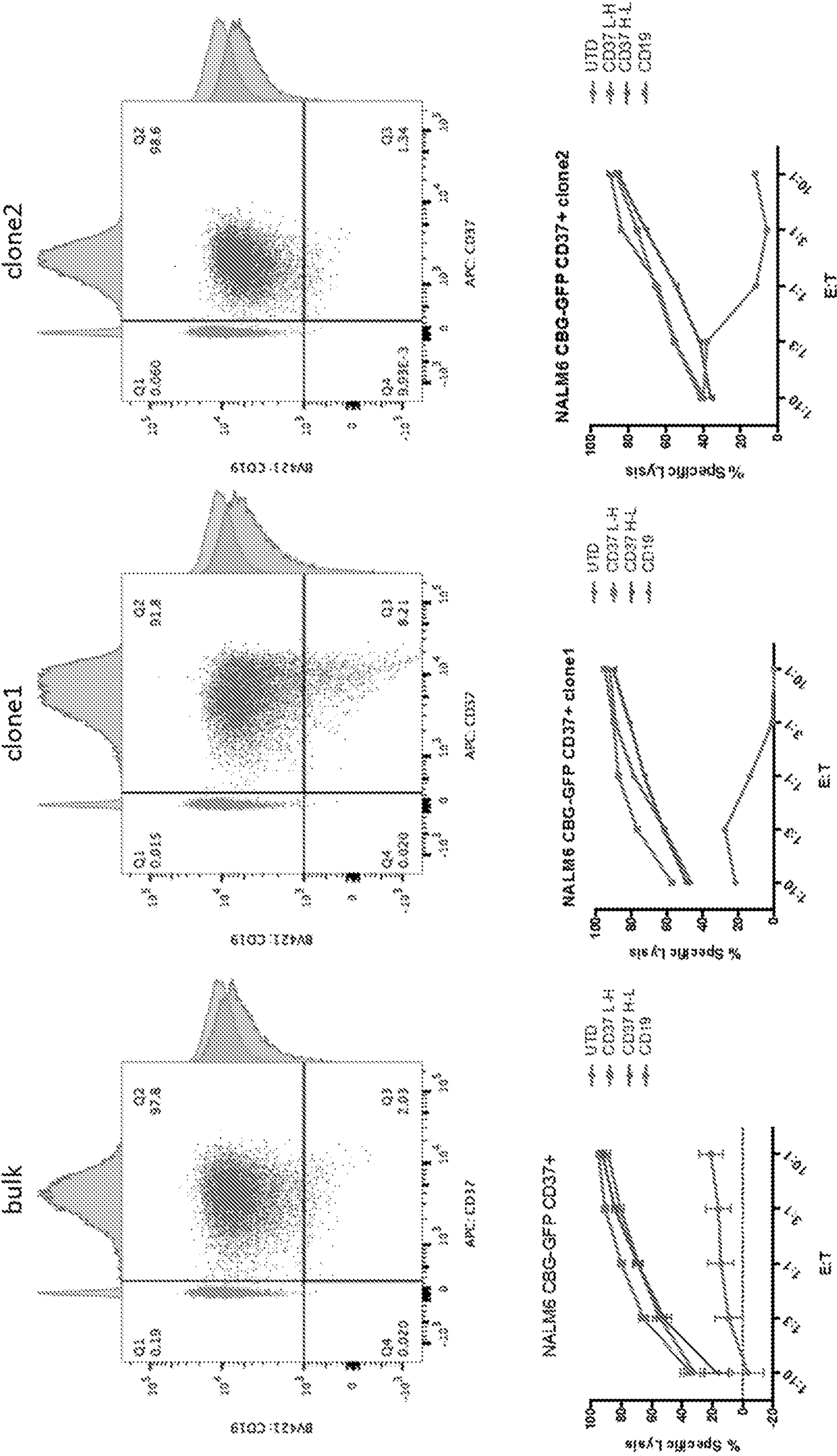


FIG. 7

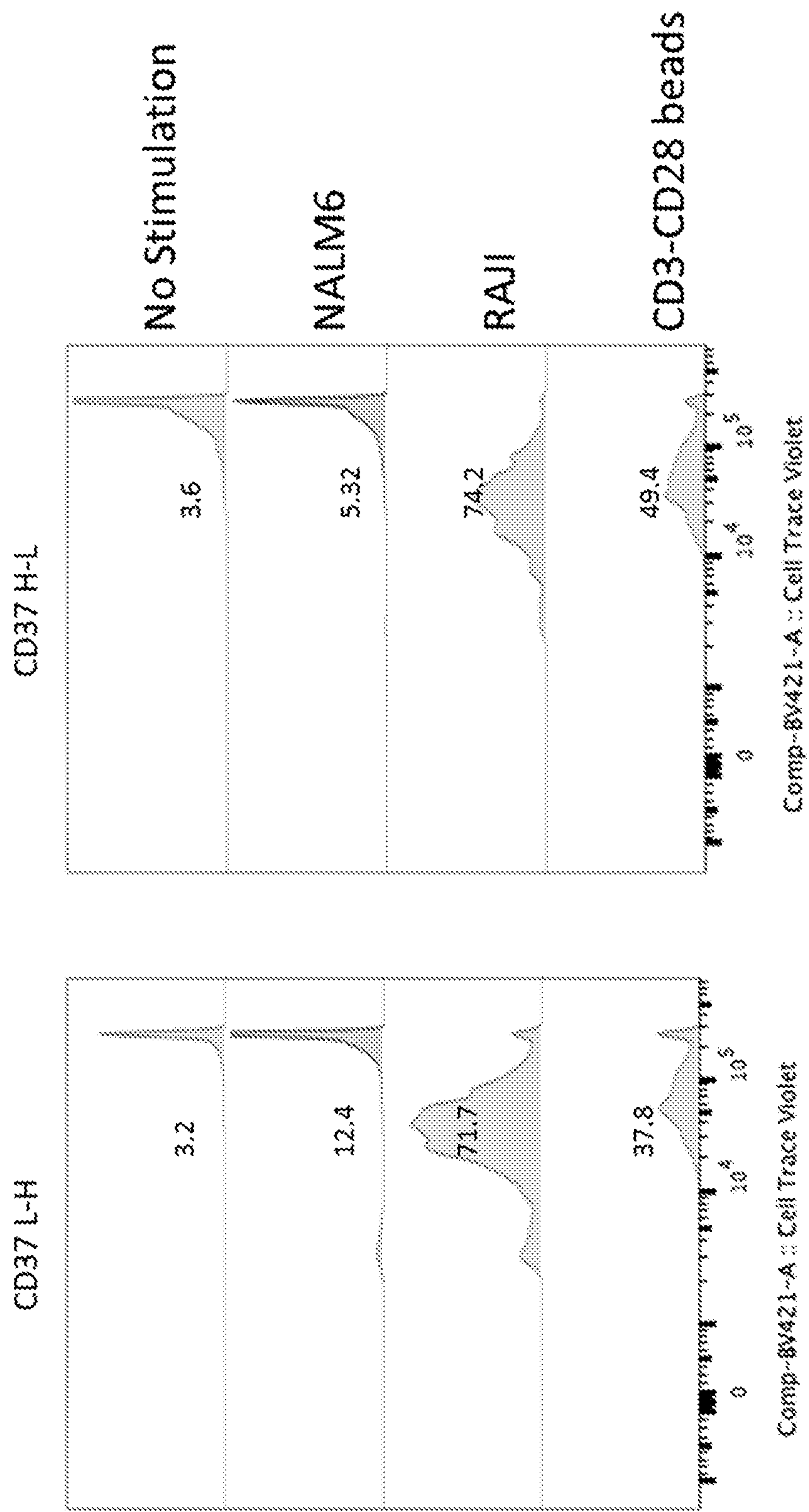


FIG. 7 (Cont'd)

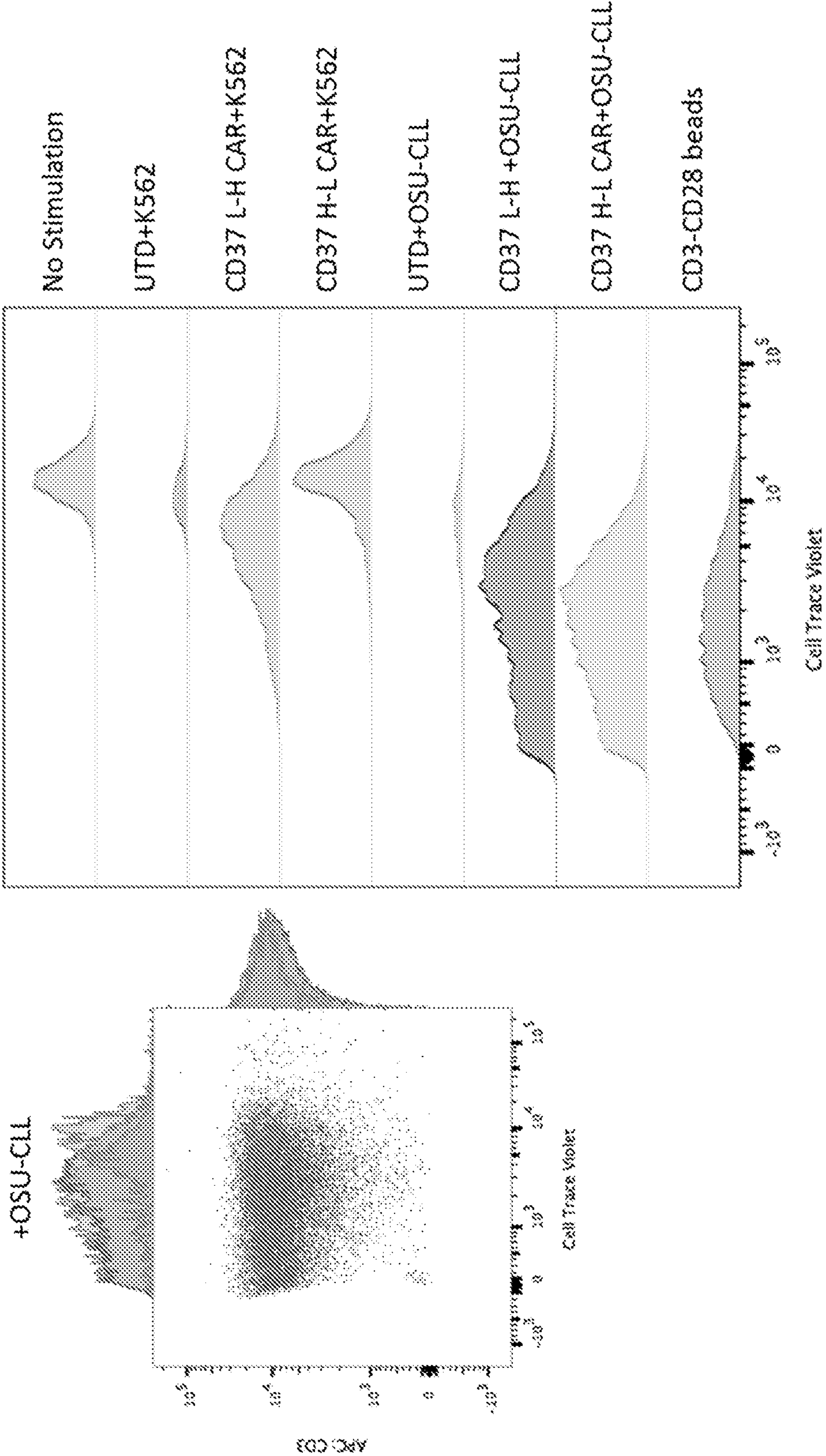


FIG. 8

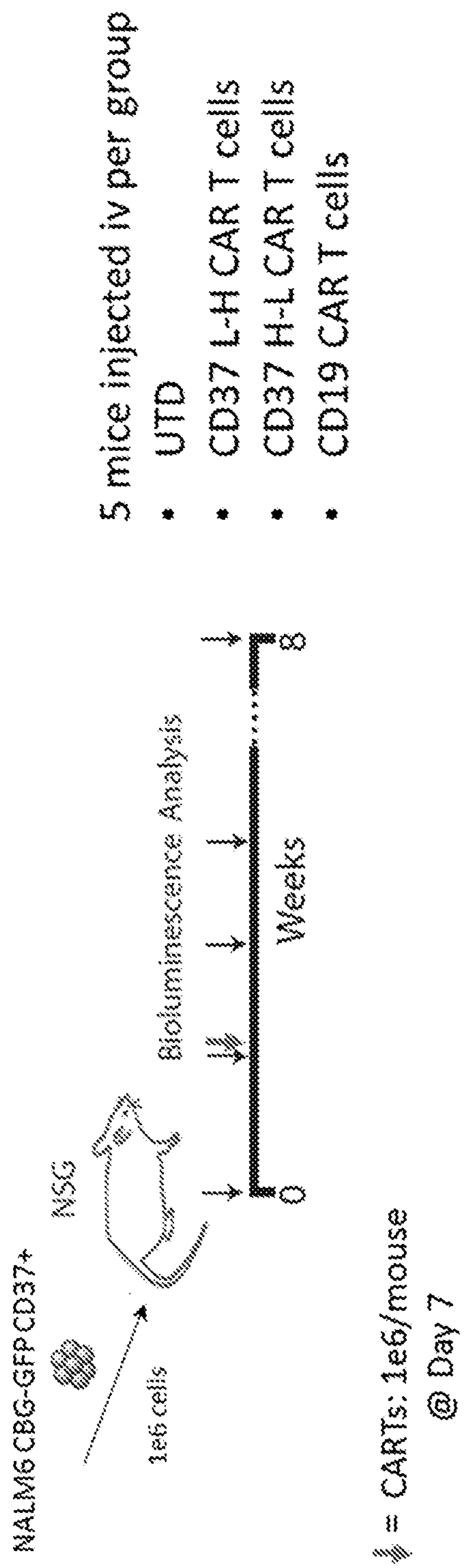
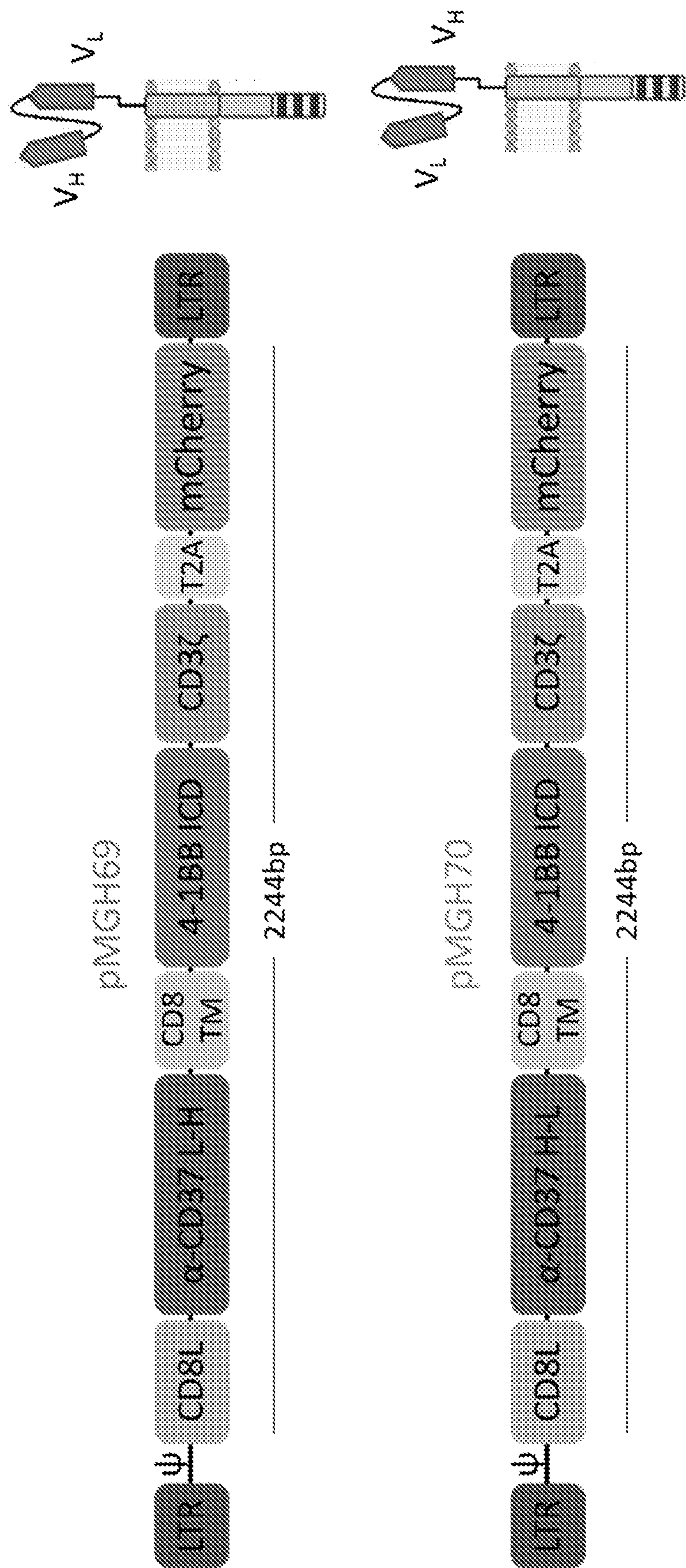


Fig. 9



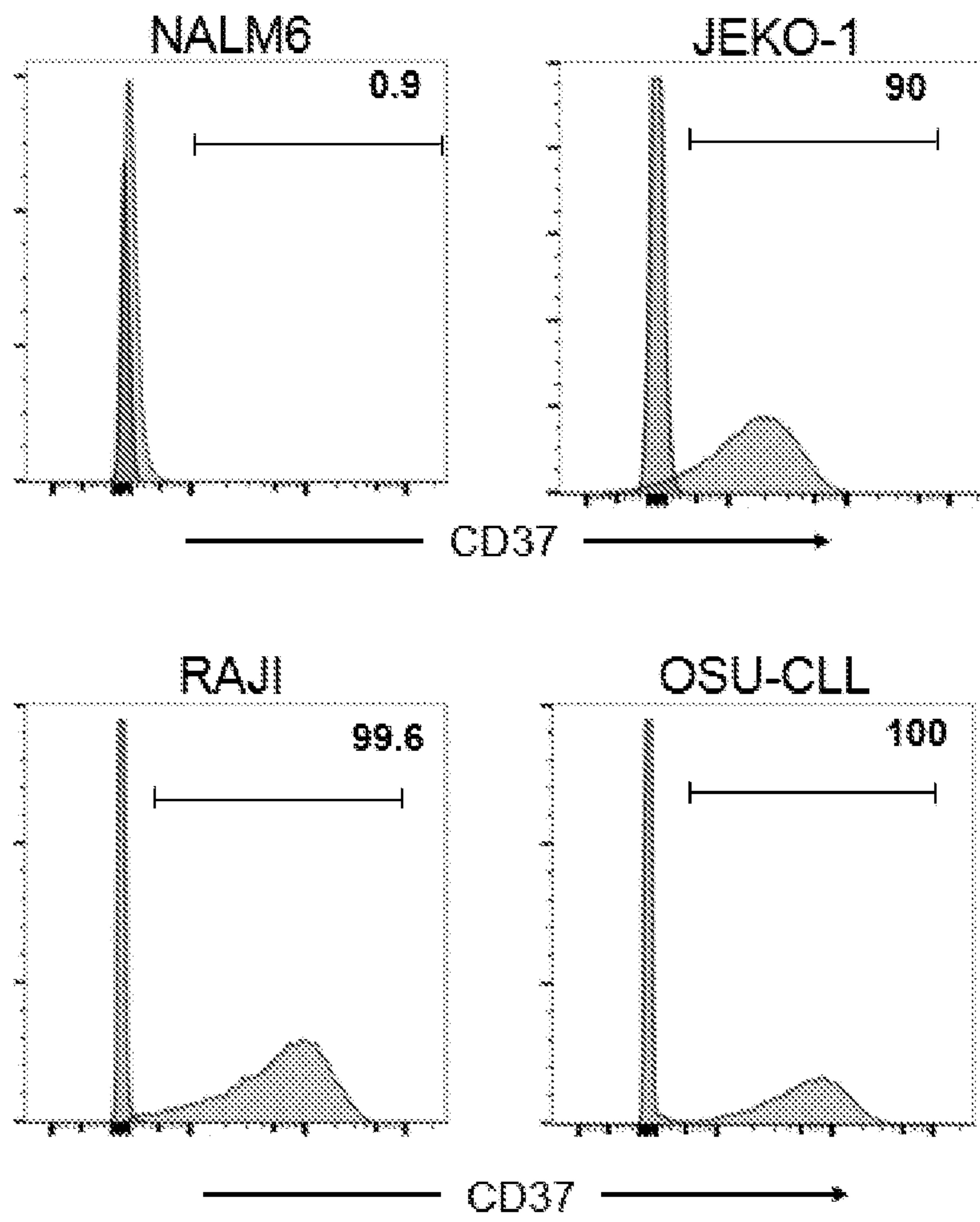
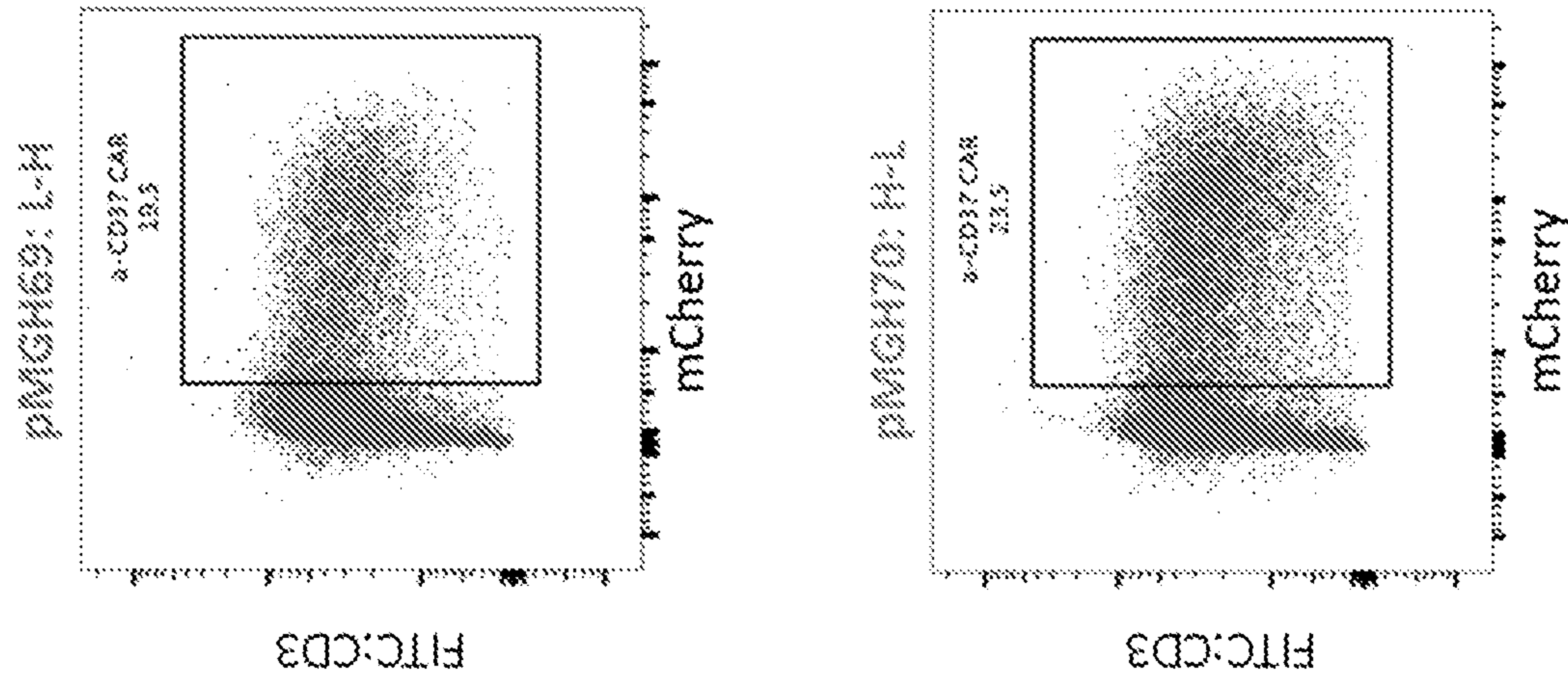
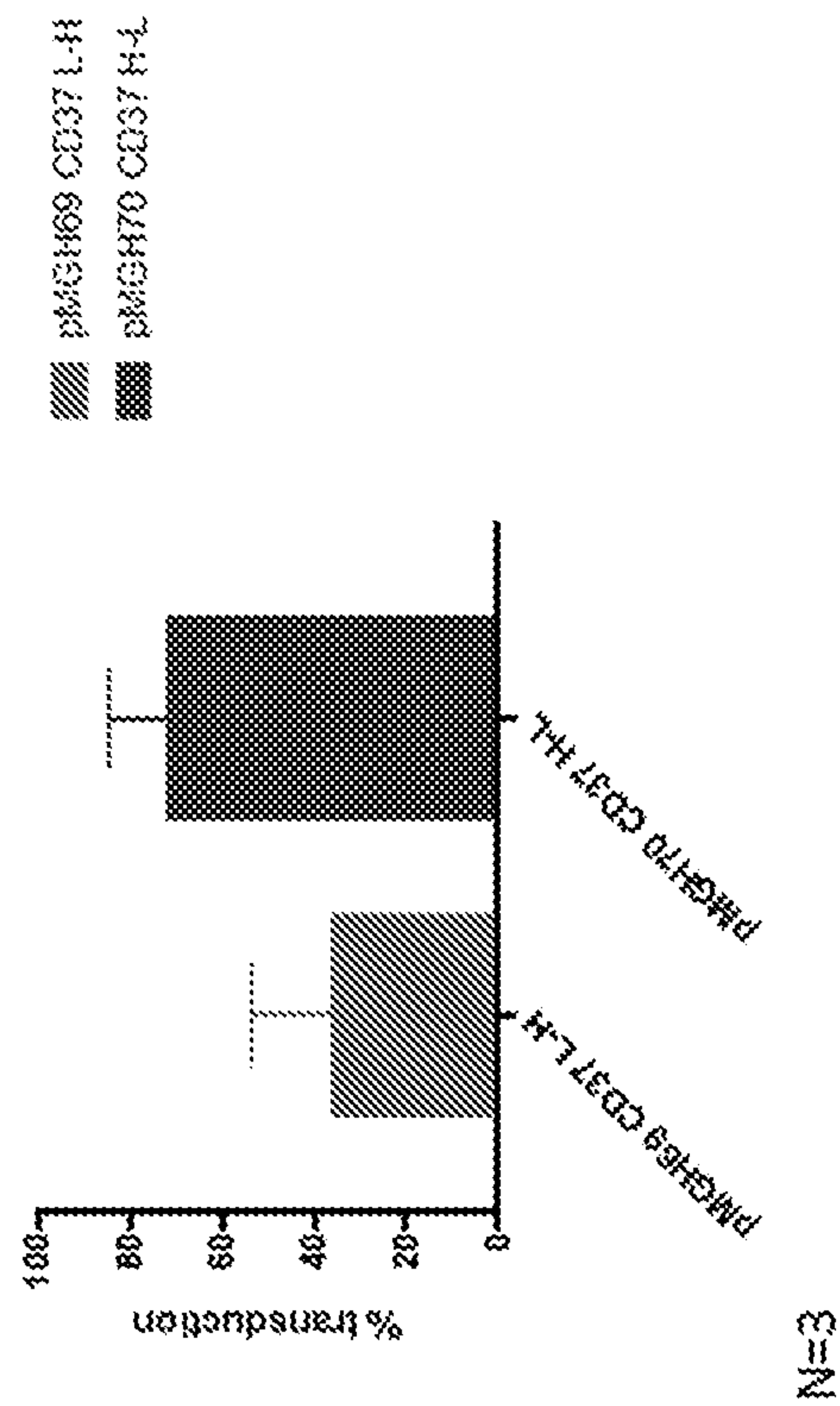
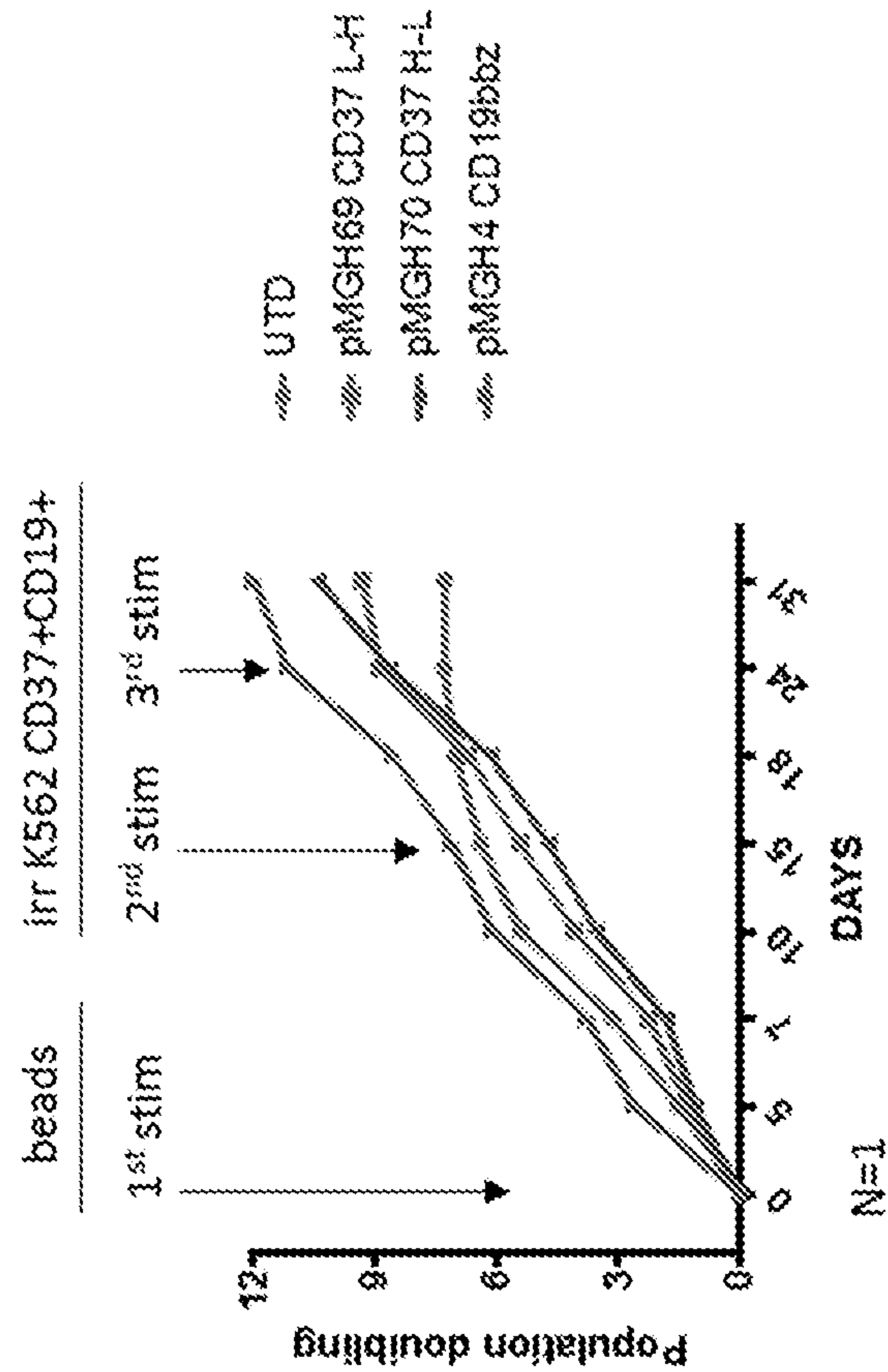


FIG. 10

FIG. 11

Growth Curve α -CD37 CAR T cells



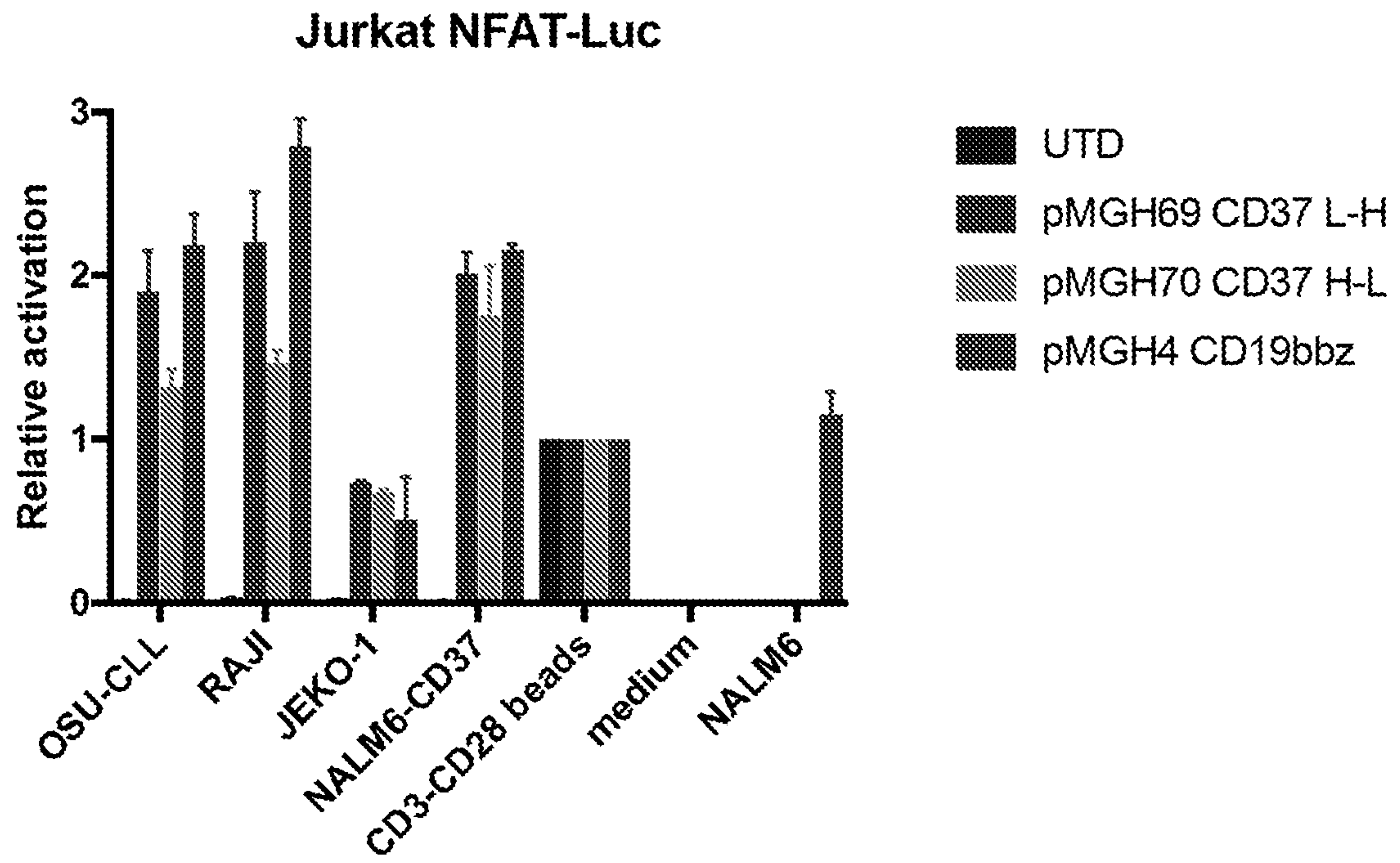


FIG. 12

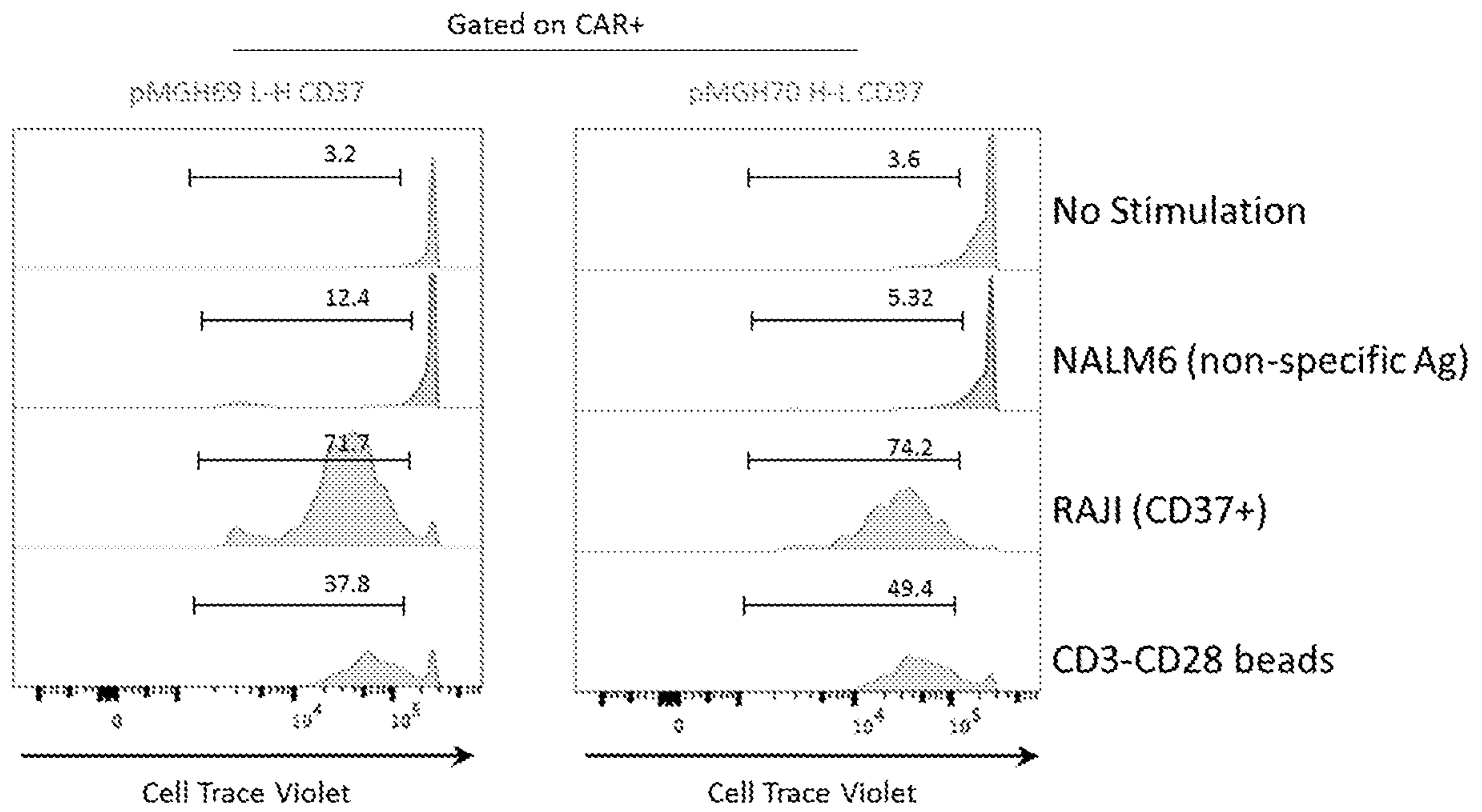


FIG. 13

FIG. 14

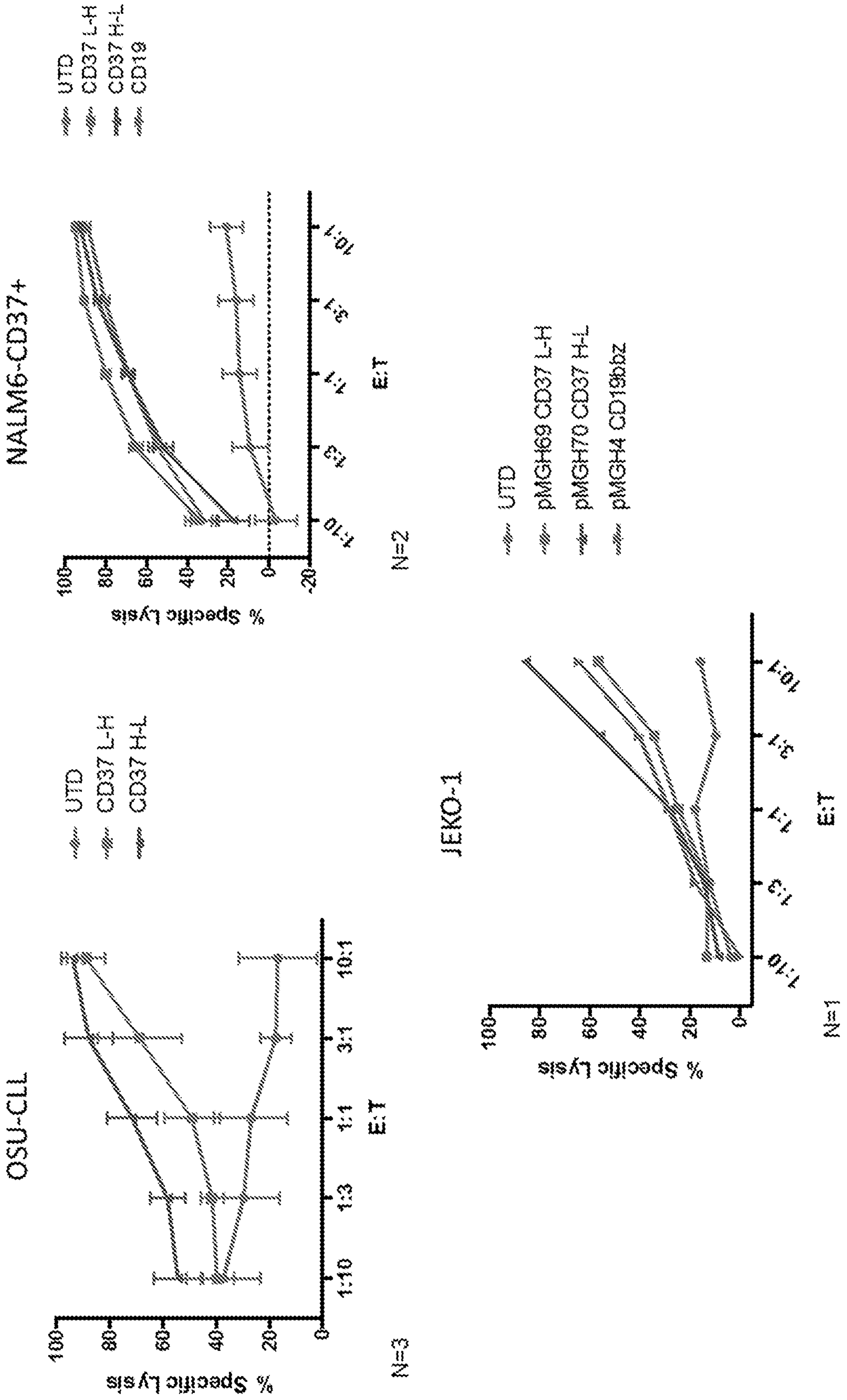


FIG. 15

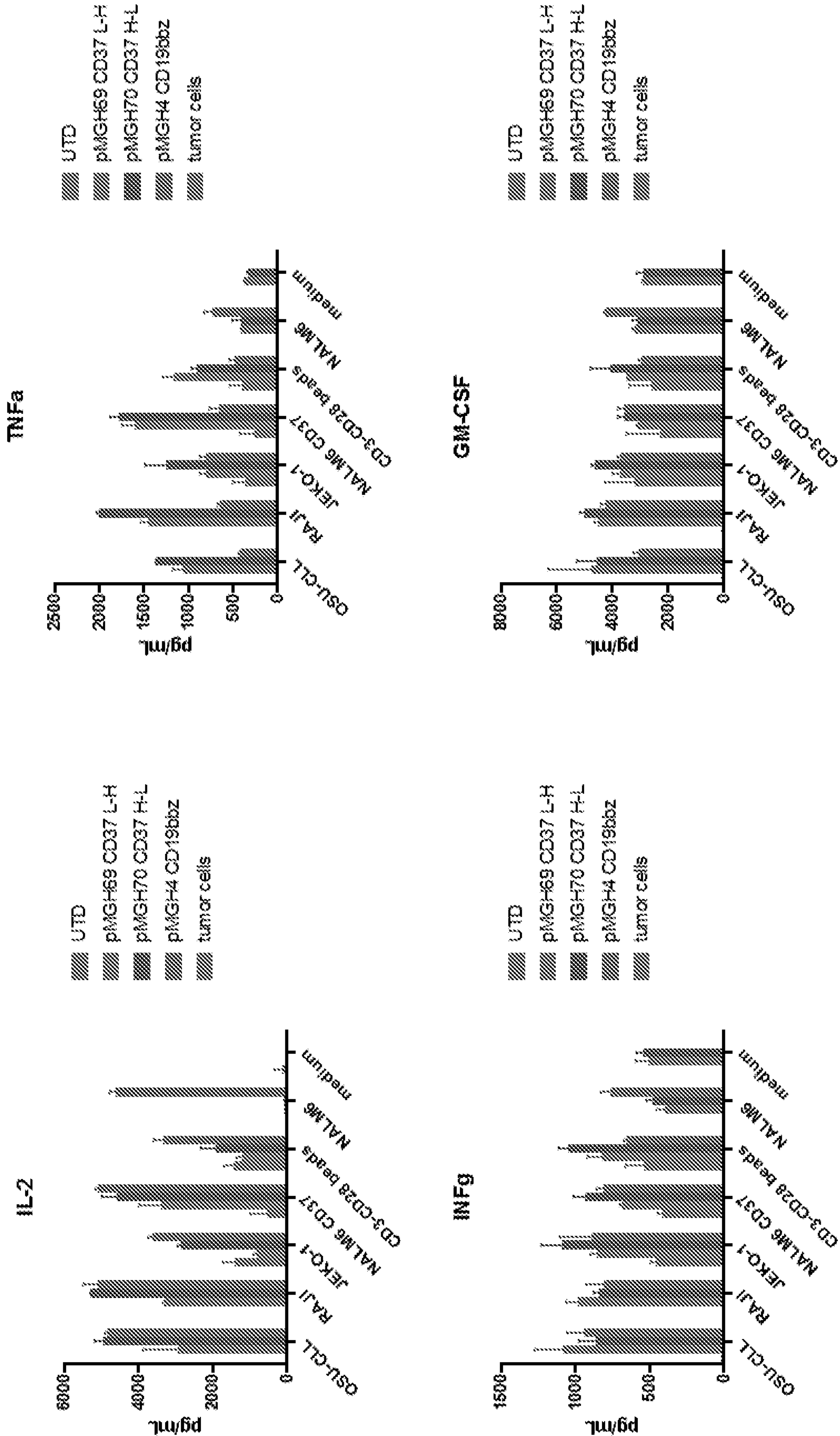


FIG. 15 (Cont'd)

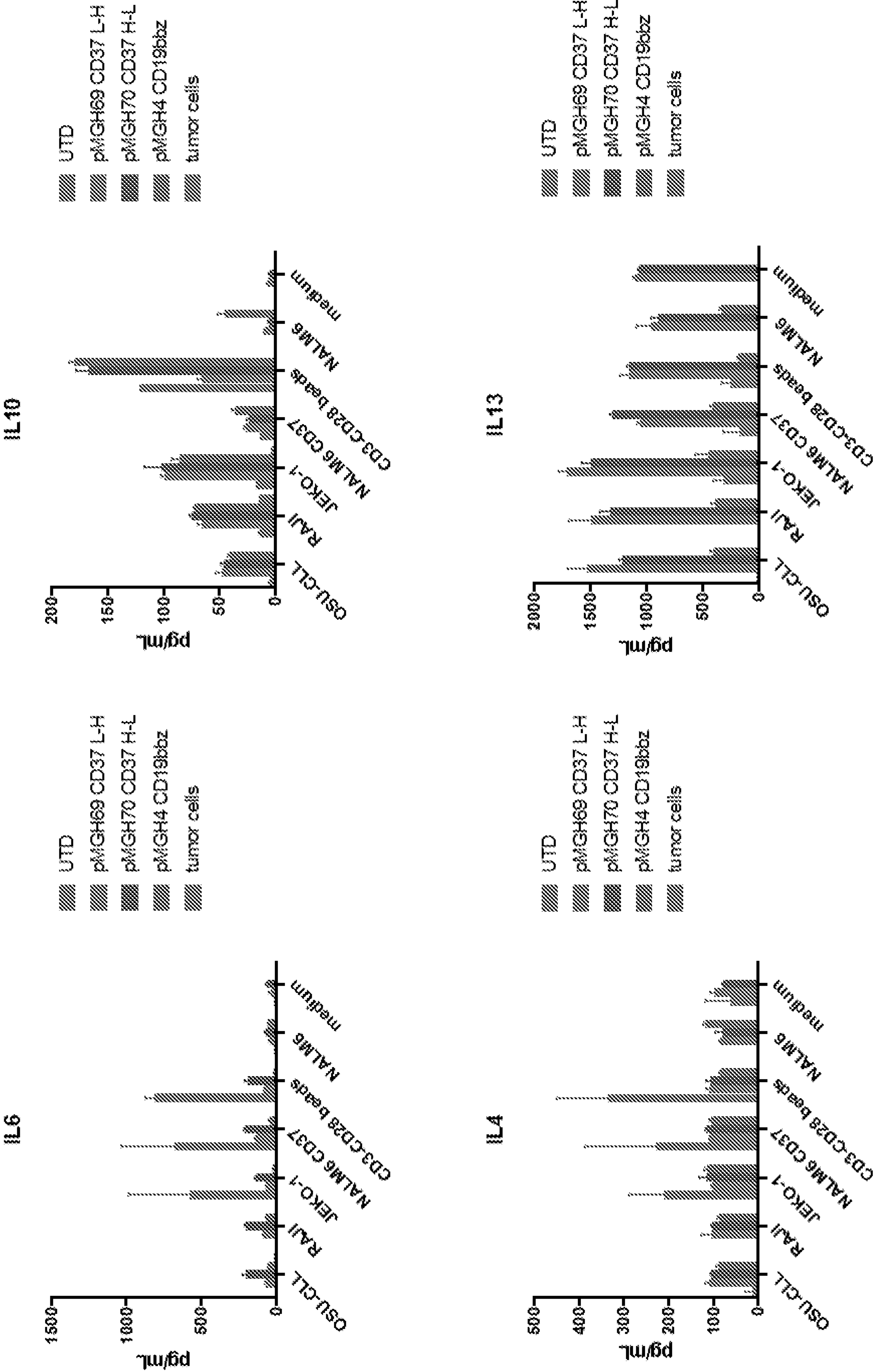


FIG. 16

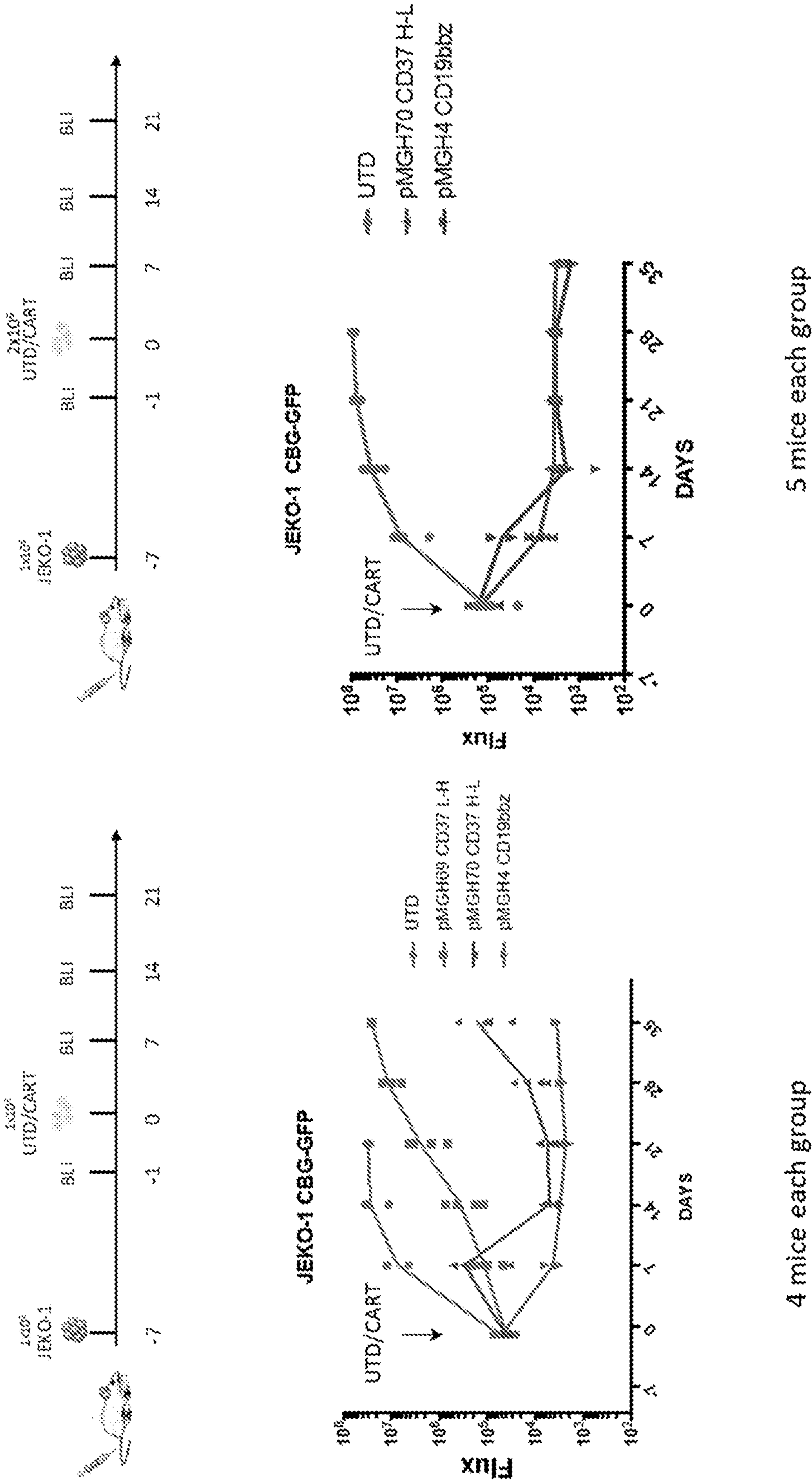
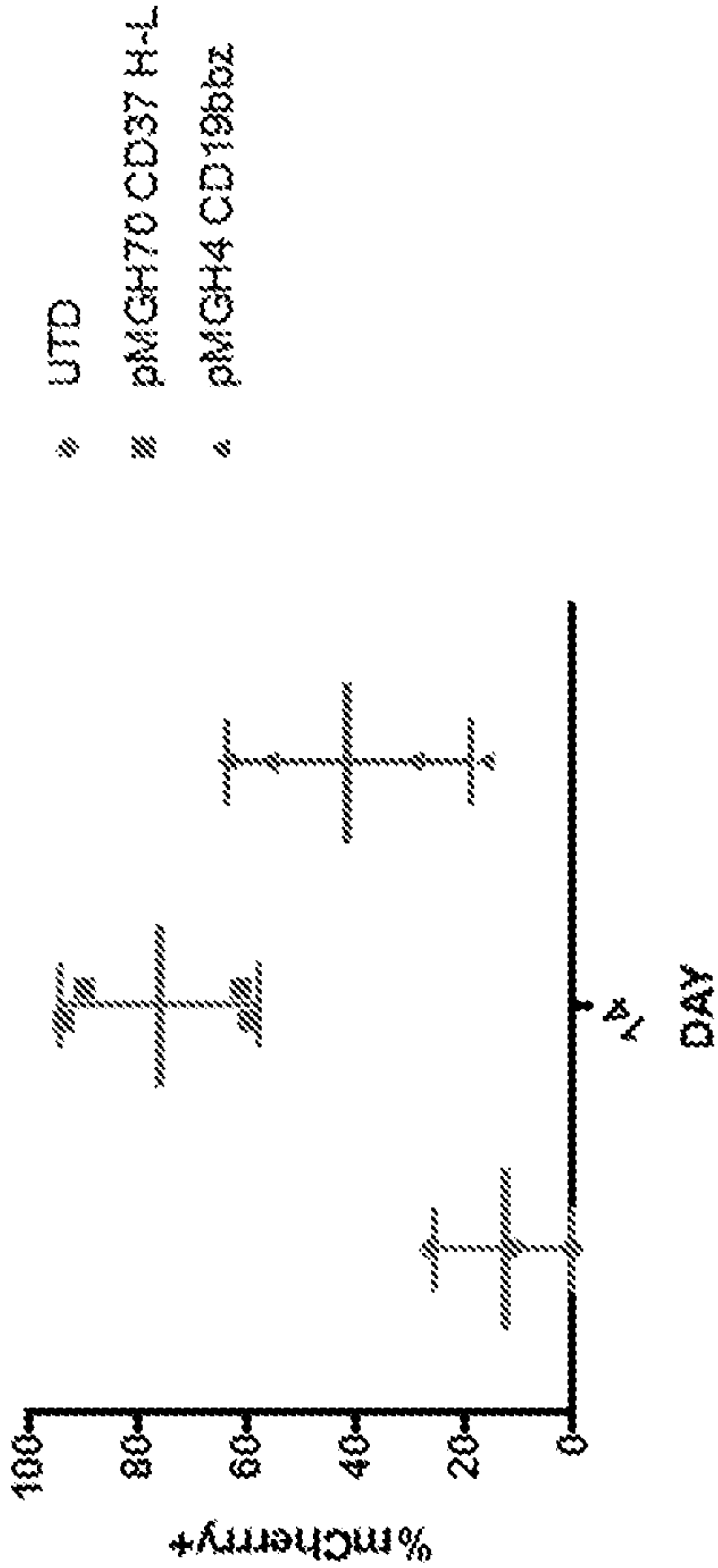
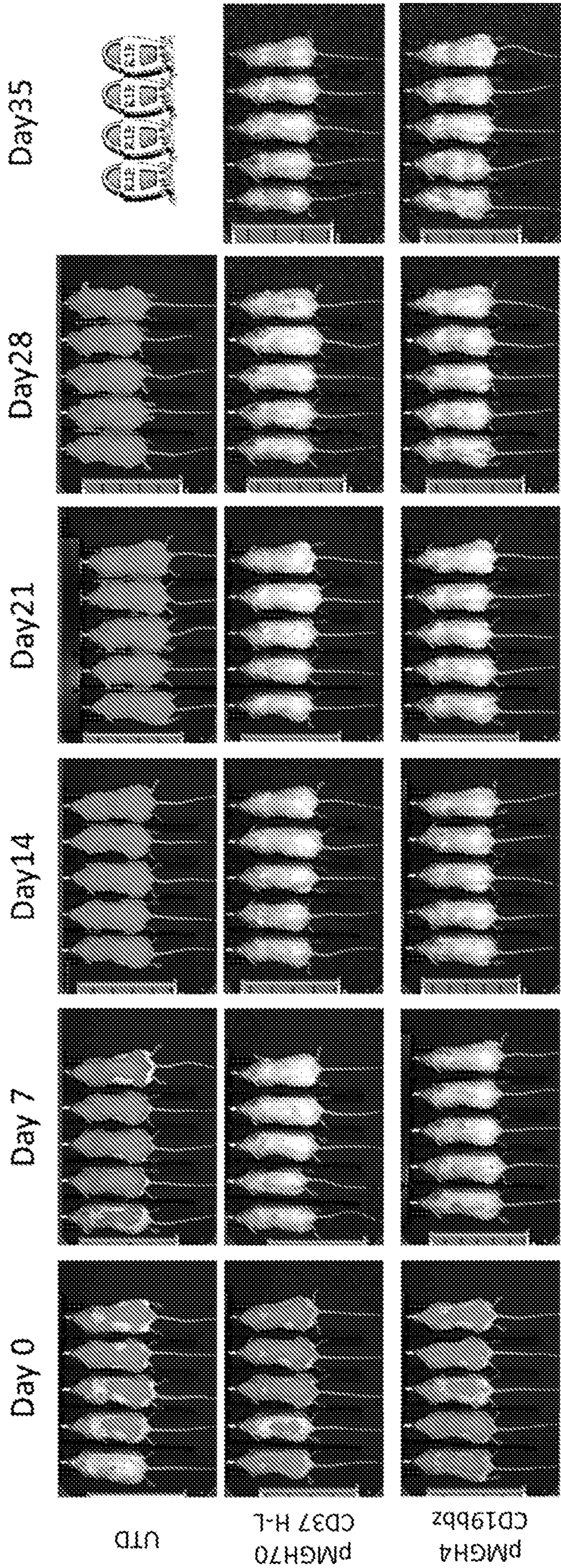


FIG. 17



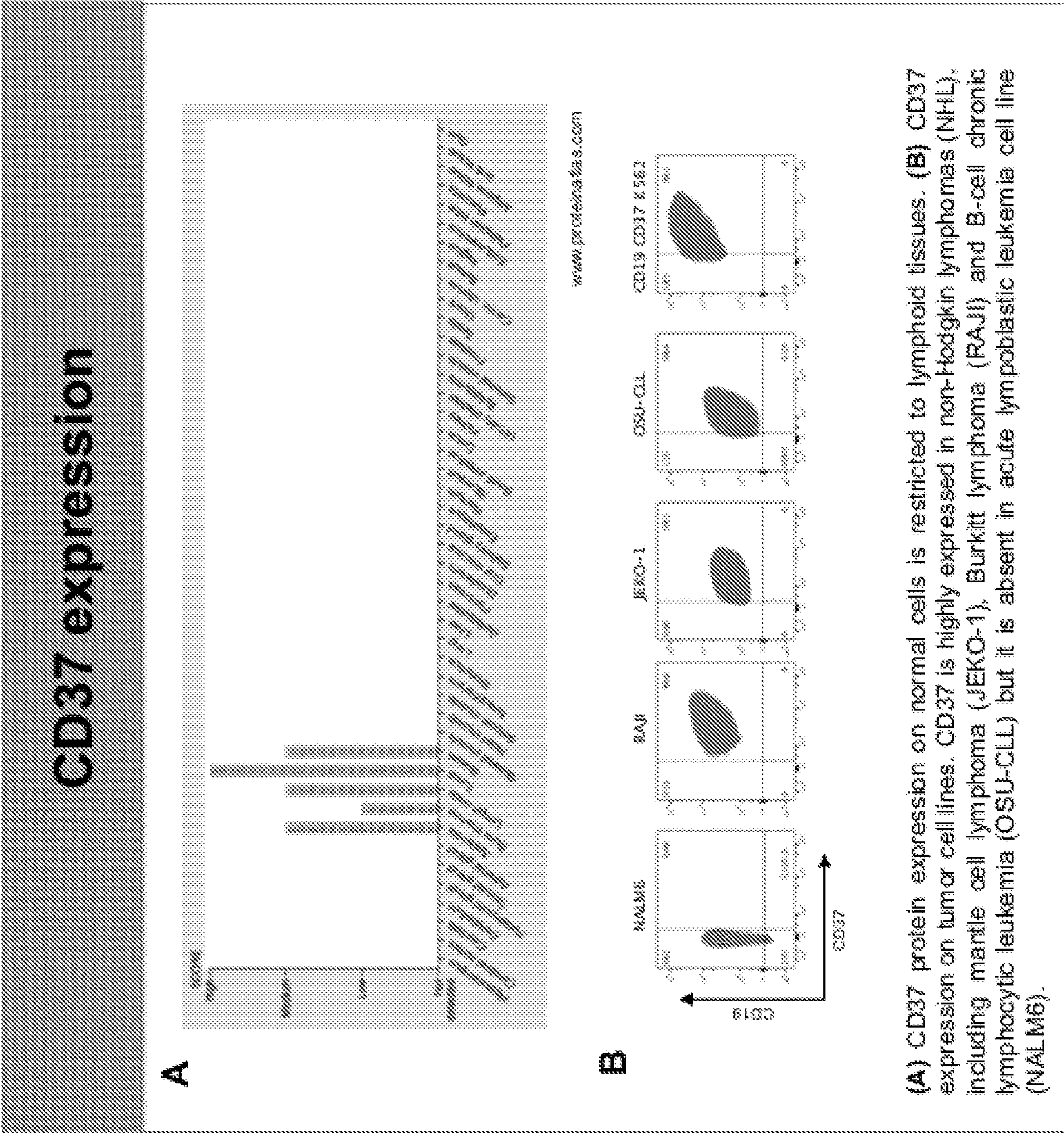


FIG. 18A-B

FIG. 19

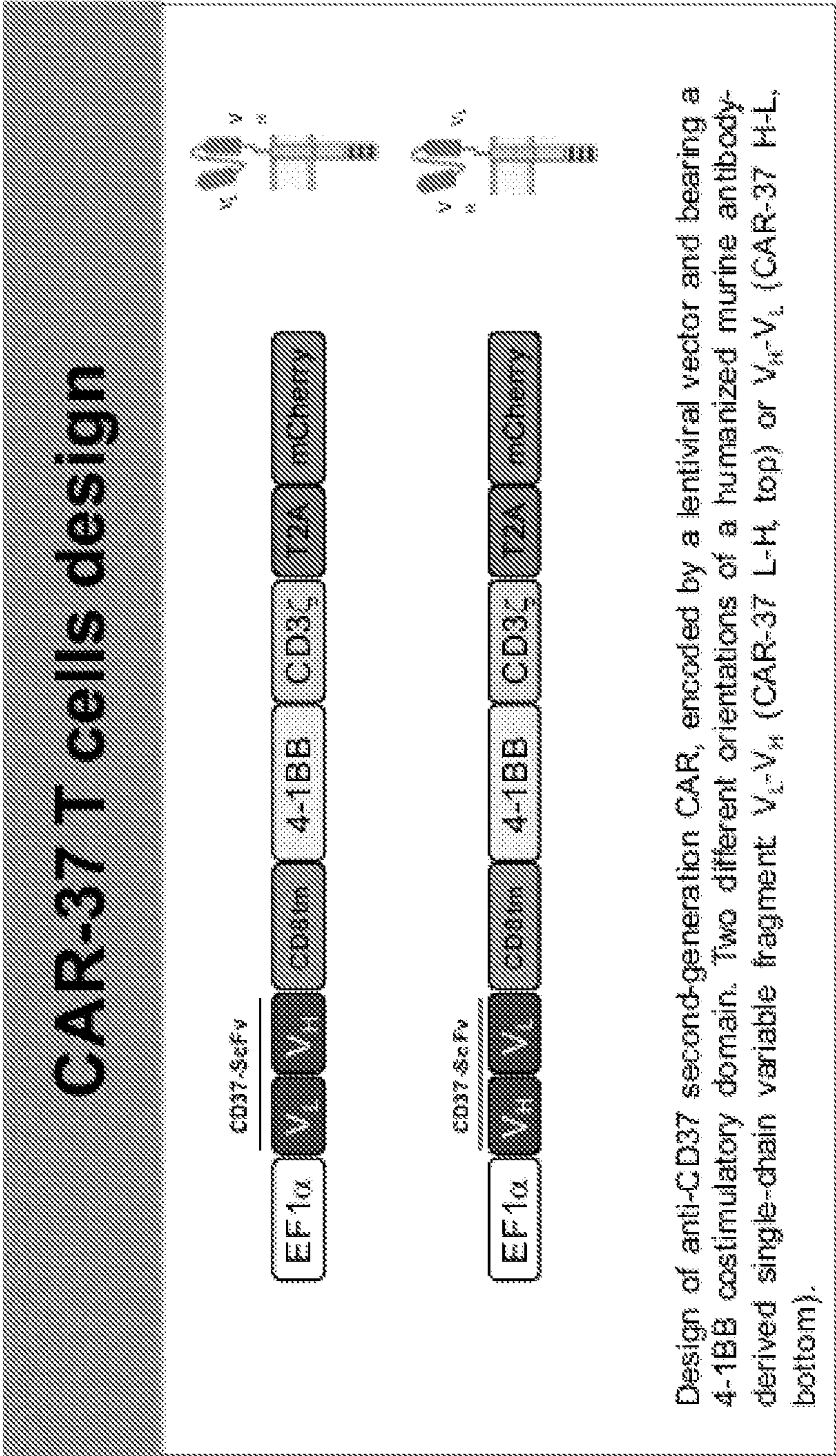


FIG. 20A-20D

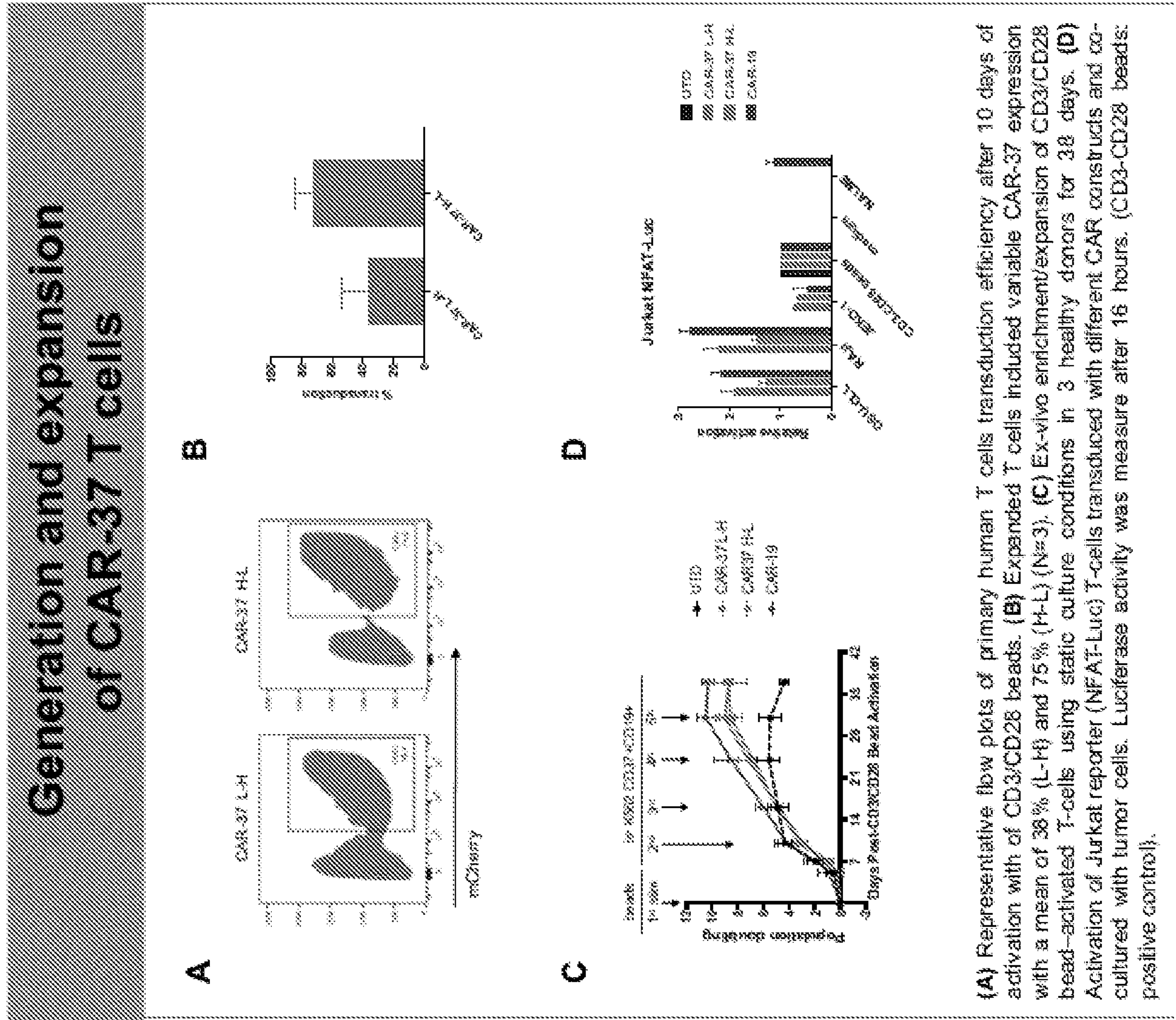
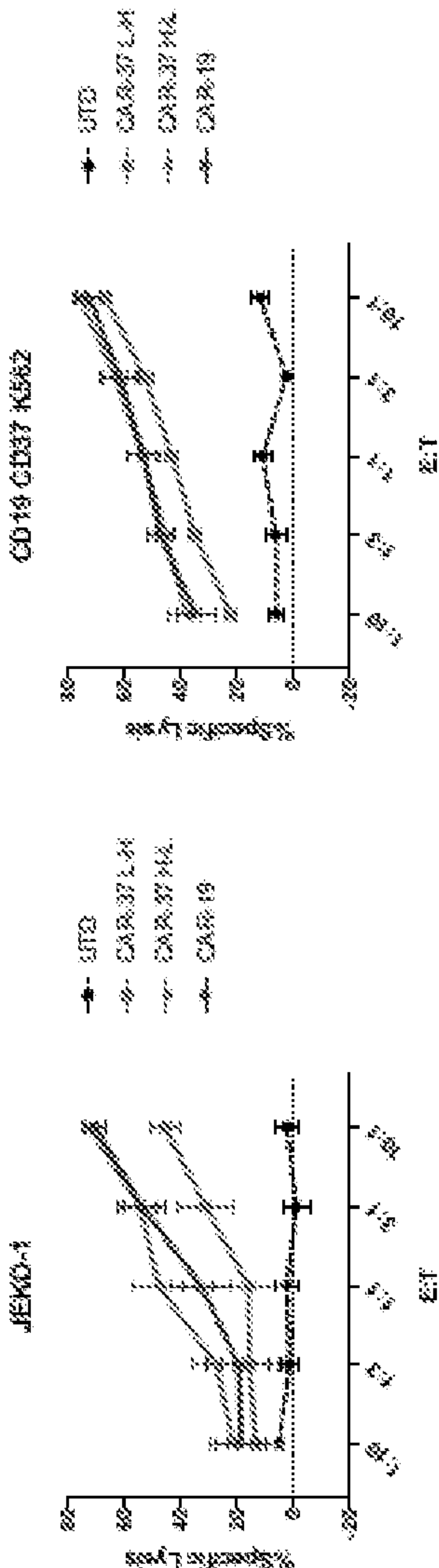


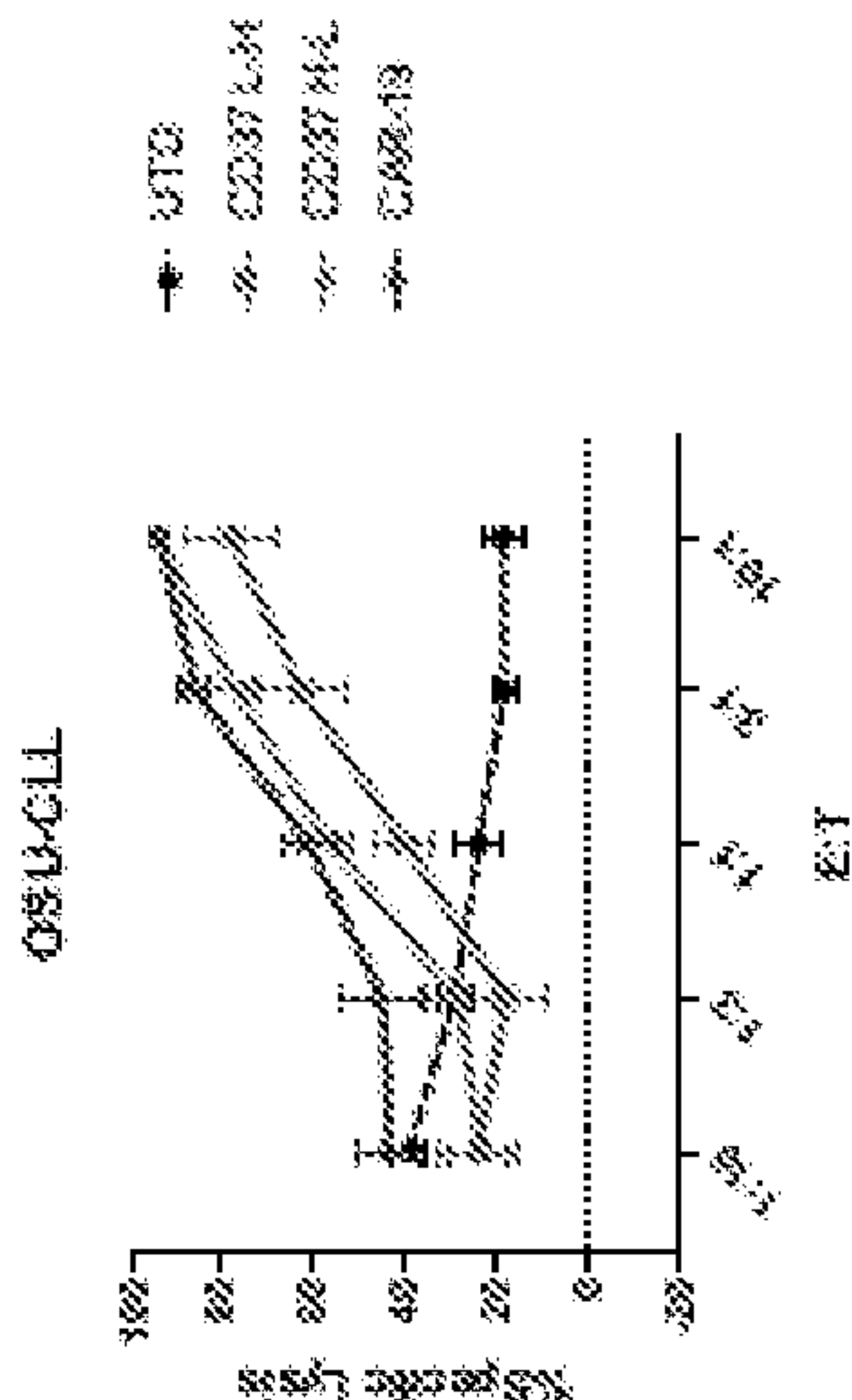
FIG. 21A- 21C

In vitro CAR-37 T cells cytotoxic activity

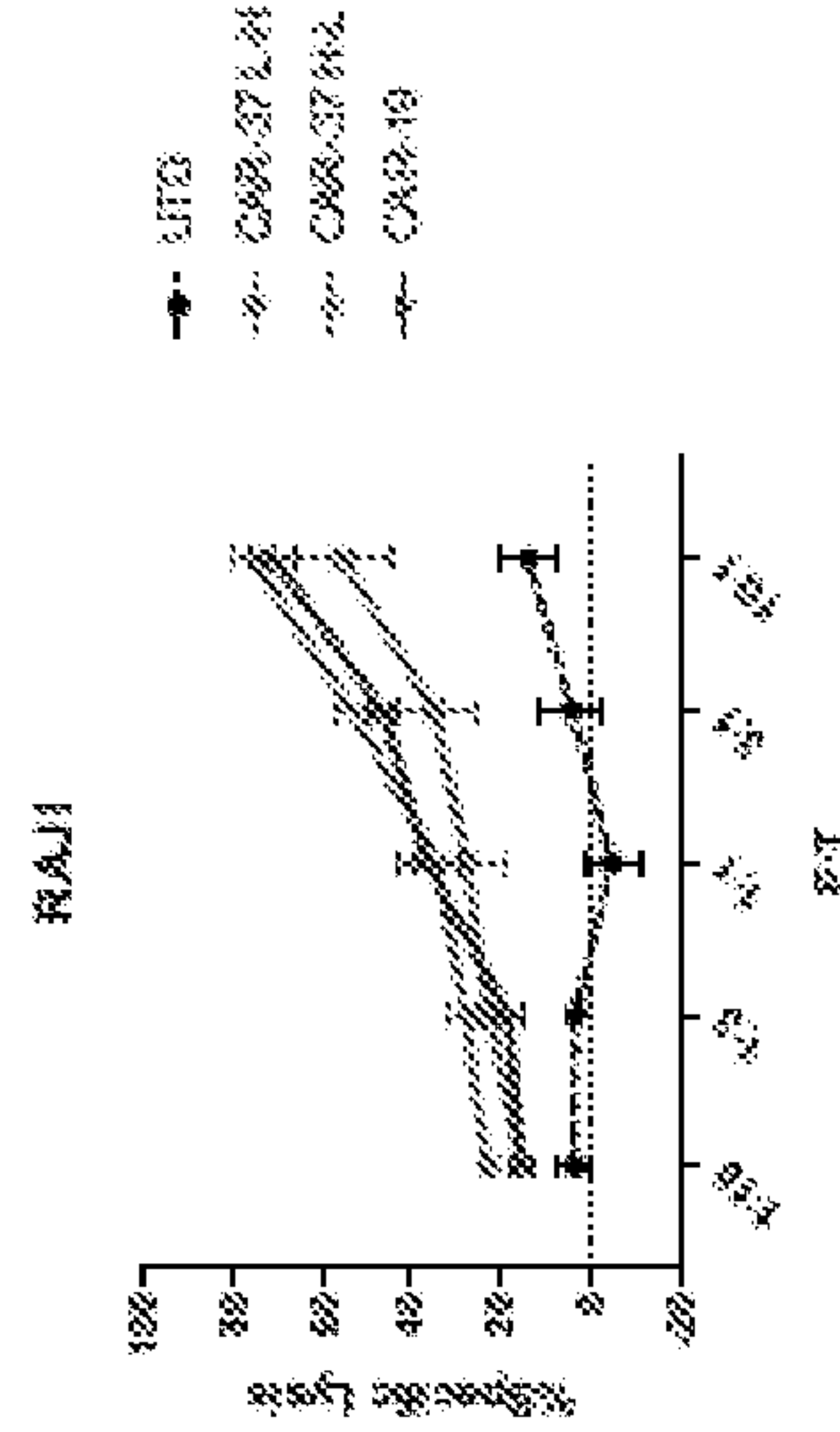
A



B



C



Cytotoxicity at 16 hours of CAR T cells co-cultured at indicated E:T ratios with Jeko-1, K562 expressing CD19 and CD37 (A), OSU-CLL (B) and RAJI (C). Increasing concentration of CAR-37 and CAR-19 T cells led to specific killing while no killing was observed in the control group (UTD).

FIG. 22A-22C

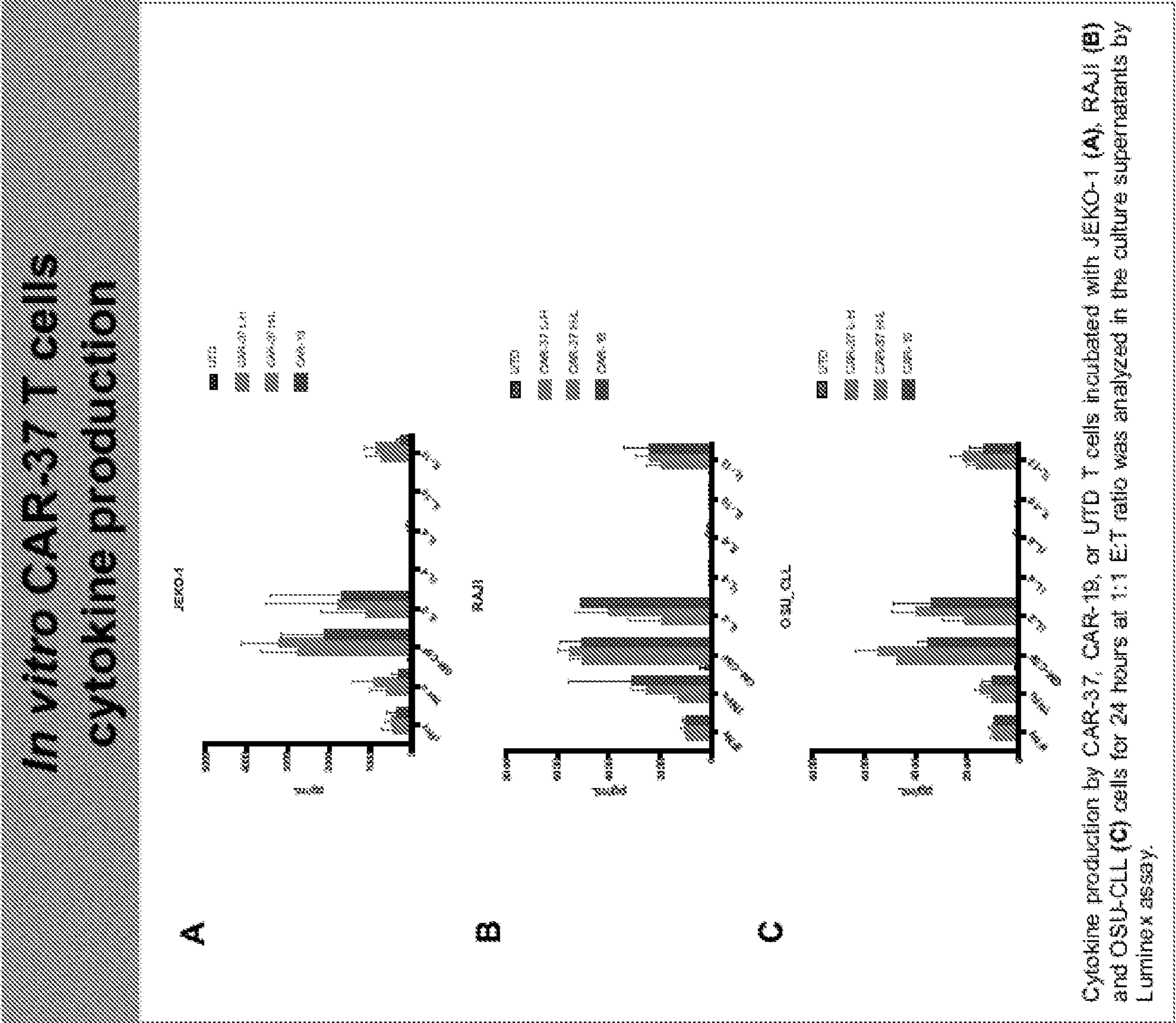


FIG. 23A-23C

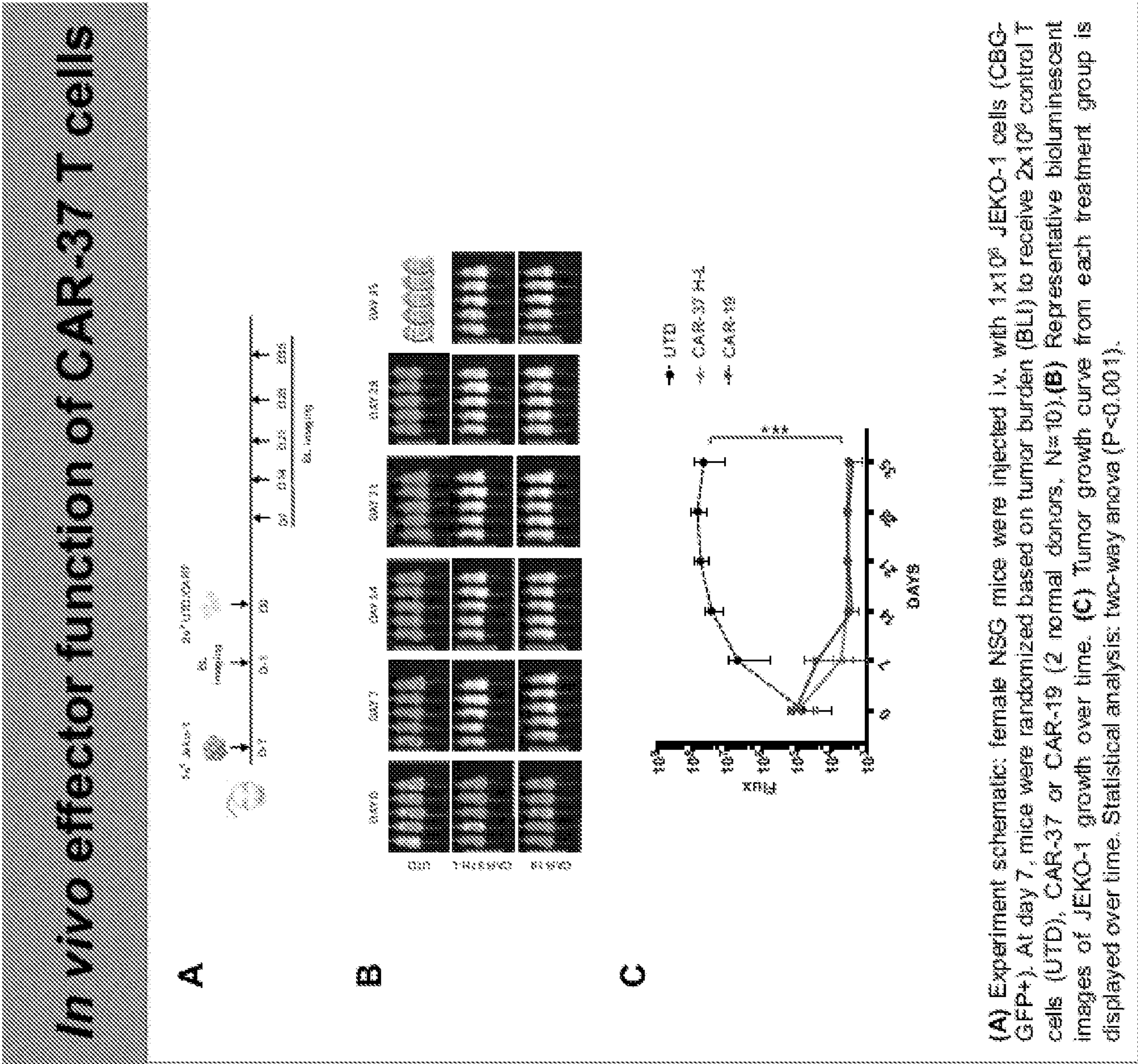


FIG. 24

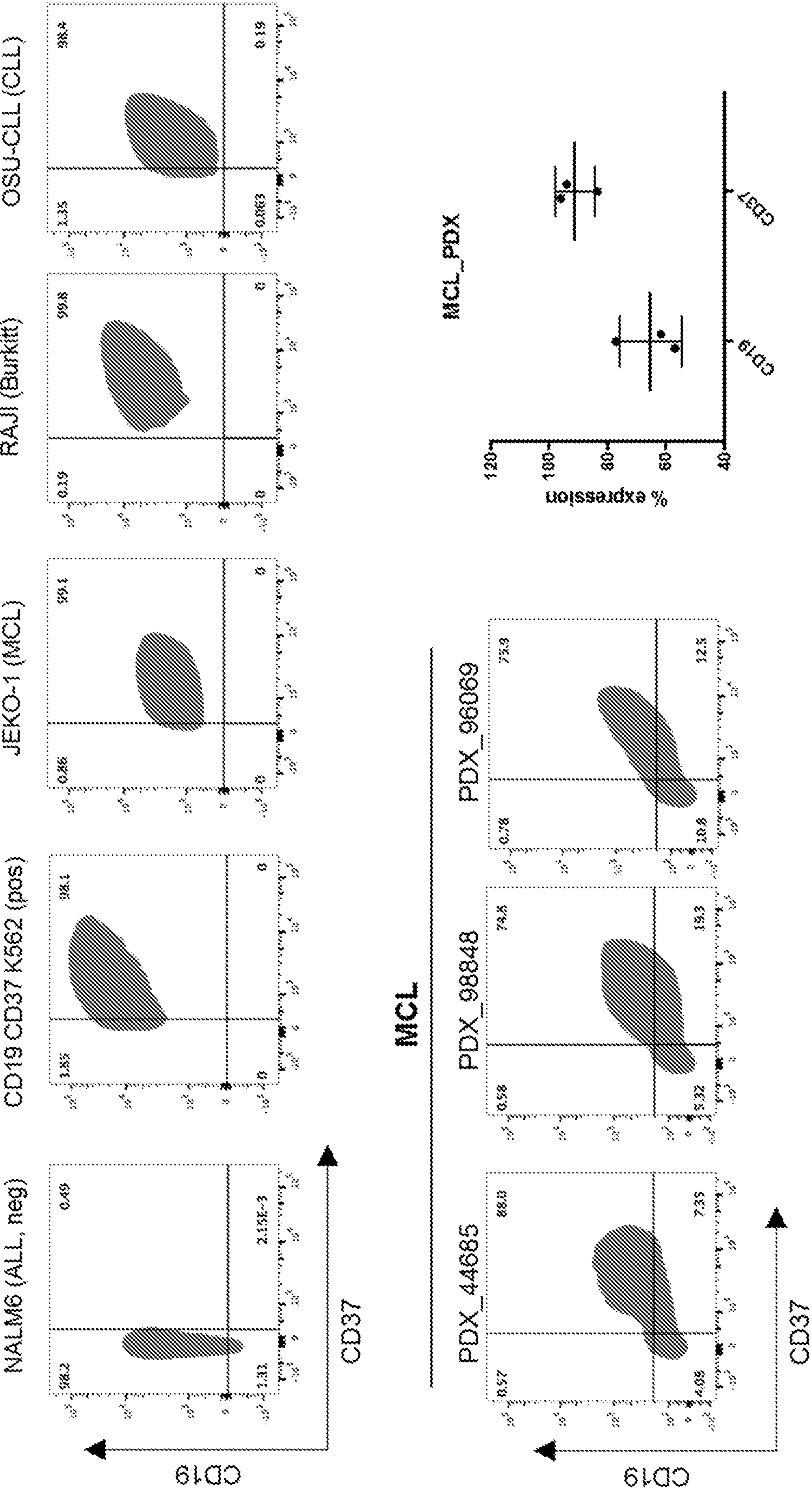
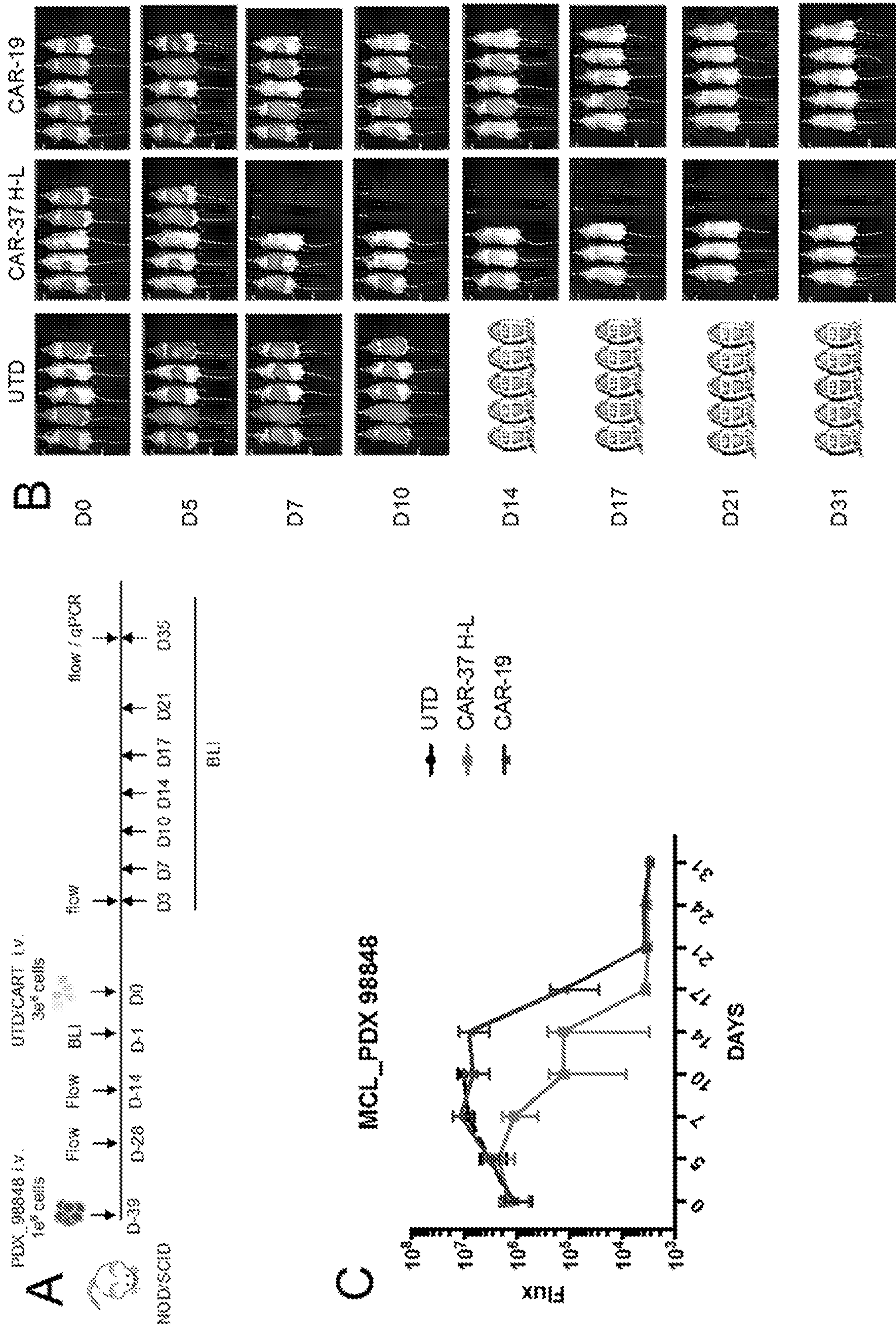


FIG. 25A-25C



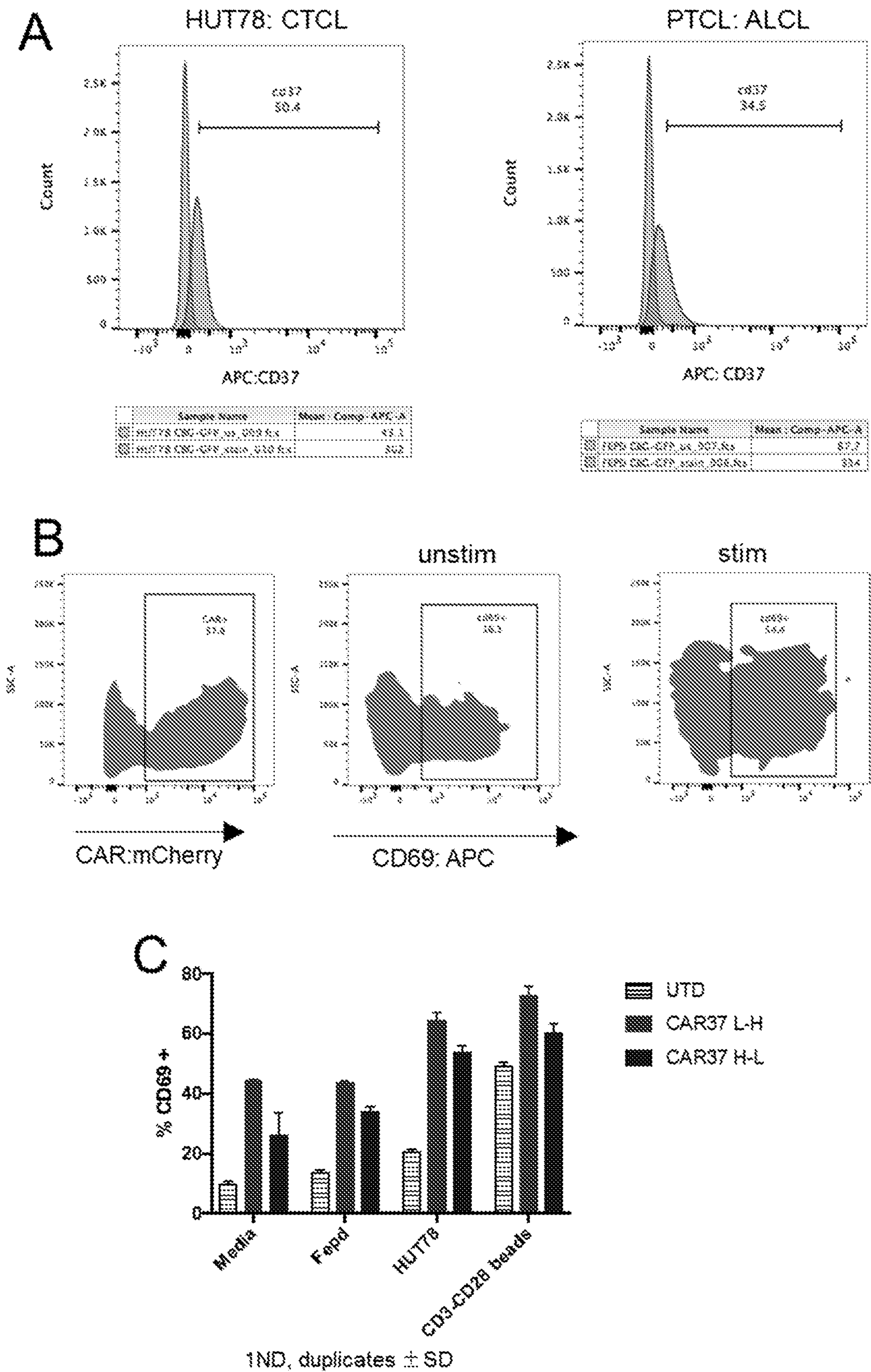


FIG. 26A-26C

FIG. 27

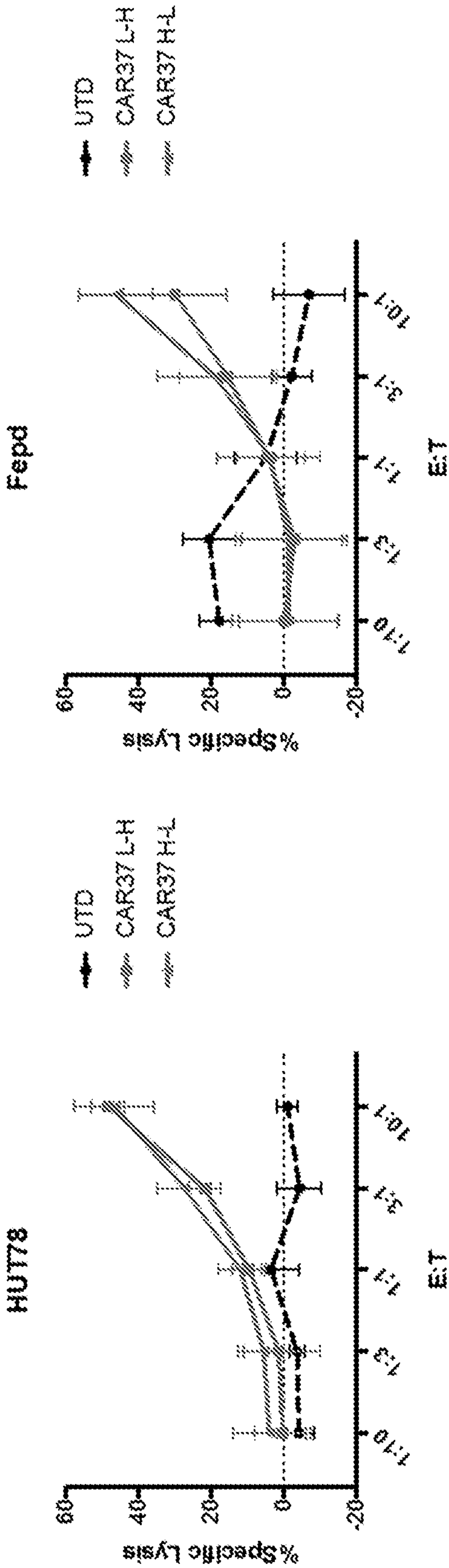


FIG. 28

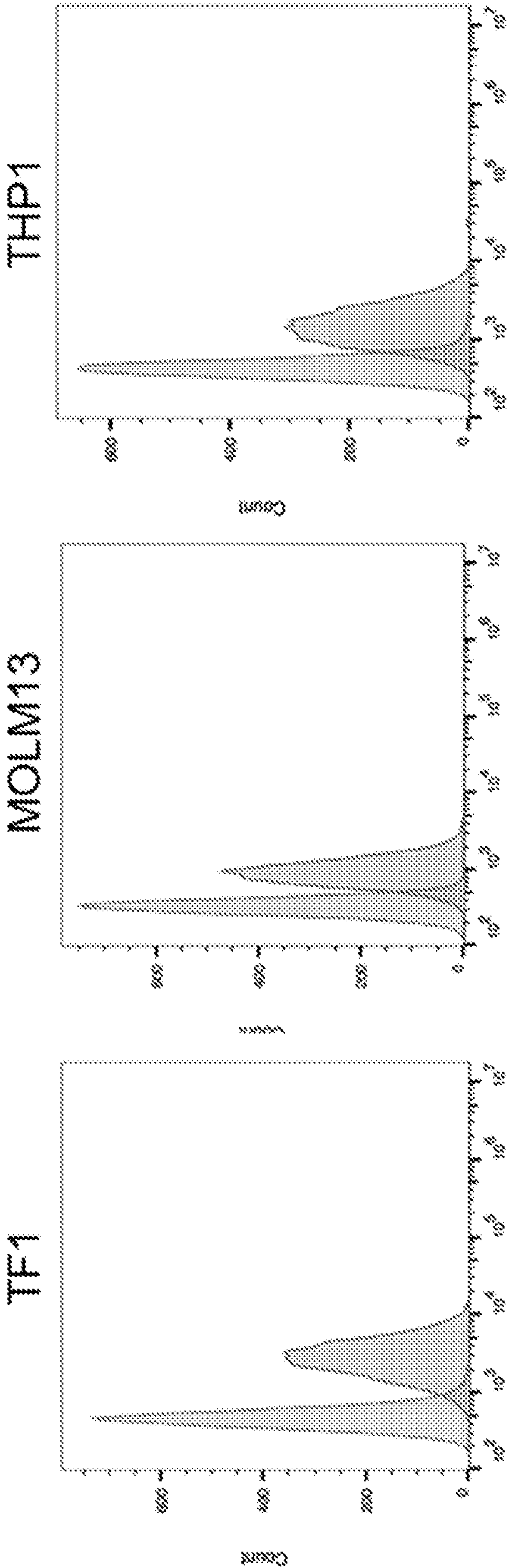
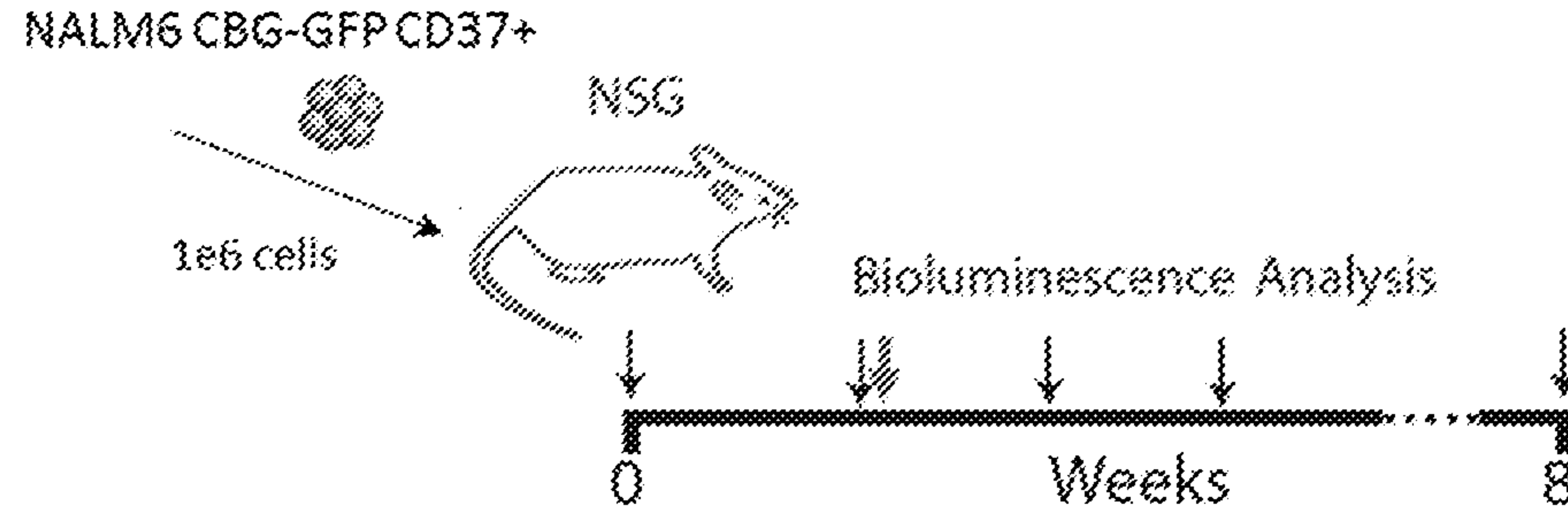


FIG. 8



5 mice injected iv per group

- UTD
- CD37 L-H CAR T cells
- CD37 H-L CAR T cells
- CD19 CAR T cells

▨ = CARTs: 1e6/mouse
@ Day 7