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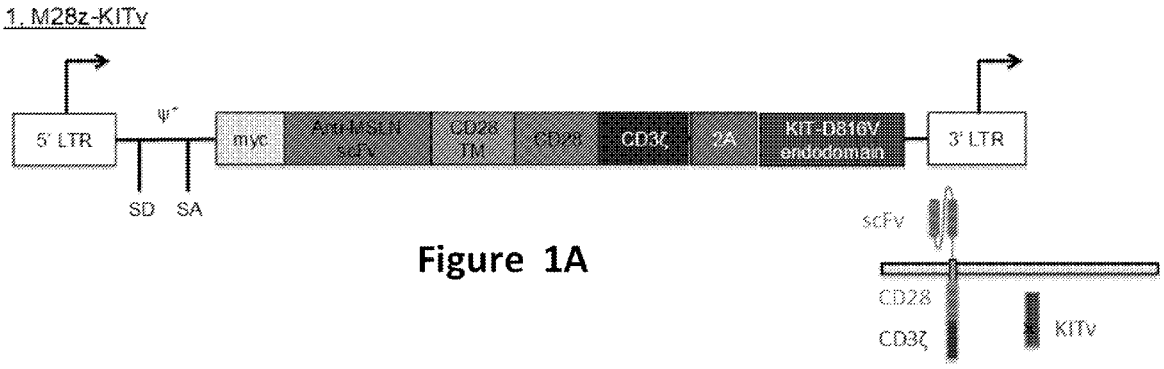


Figure 1A

(57) Abstract: The present disclosure provides methods and compositions for enhancing the immune response toward cancers and pathogens. The presently disclosed subject matter provides methods and compositions for enhancing the immune response toward cancers and pathogens. It relates to cells comprising a c-Kit mutant, e.g., a c-Kit mutant comprising an activating mutation. The cells can further comprise an antigen- recognizing receptor (*e.g.*, a chimeric antigen receptors (CAR) or a T cell receptors (TCR)). The presently disclosed subject matter relates to the use of cells for treatment, e.g., treating cancers.

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CELLS EXPRESSING C-KIT MUTATIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to United States Provisional Application No. 62/943,032 filed December 3, 2019, the content of which is incorporated by reference in its entirety herein, and to which priority is claimed.

SEQUENCE LISTING

The present application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 3, 2020, is named 0727341174_ST25 and is 57,790 bytes in size.

1. INTRODUCTION

The presently disclosed subject matter provides methods and compositions for enhancing the immune response toward cancers and pathogens. It relates to cells comprising a c-Kit mutant, e.g., a c-Kit mutant comprising an activating mutation. The cells can further comprise an antigen-recognizing receptor (*e.g.*, a chimeric antigen receptors (CAR) or a T cell receptors (TCR)). The presently disclosed subject matter relates to the use of cells for treatment, e.g., treating cancers.

2. BACKGROUND OF THE INVENTION

Cell-based immunotherapy is a therapy with curative potential for the treatment of cancer. T cells and other immune cells may be modified to target tumor antigens through the introduction of genetic material coding for natural or modified T cell receptors (TCR) or synthetic receptors for antigen, termed Chimeric Antigen Receptors (CARs), specific to selected antigens. Patient-engineered CAR T cells have demonstrated remarkable efficacy against a range of liquid and solid malignancies.

CARs that are in clinic and in preclinical development predominantly use co-stimulatory domains such as CD28 or 4-1BB. Persistence, especially functional persistence of these CARs has shown been to be associated with better comes. There is unmet need for improved CARs having enhanced proliferation and persistence without a co-stimulatory domain, and/or with improved efficiency and activities as compared to the existing CARs.

3. SUMMARY OF THE INVENTION

The presently disclosed subject matter provides cells comprising: (a) an antigen-recognizing receptor that binds to an antigen, and (b) a mutant of human c-Kit. In certain embodiments, the c-Kit mutant comprises an activating mutation.

In certain embodiments, the c-Kit is human c-Kit. In certain embodiments, the activating mutation is within the intracellular region of human c-Kit. In certain embodiments, the intracellular region comprises amino acids 544 to 977 of human c-Kit.

In certain embodiments, the activating mutation is within amino acids 816 to 826 of human c-Kit. In certain embodiments, the activating mutation is at amino acid position 816 or amino acid position 822.

In certain embodiments, the activating mutation is within amino acids 550 to 570 of human c-Kit. In certain embodiments, the activating mutation is at amino acid position 560 of human c-Kit.

In certain embodiments, the activating mutation is selected from D816V, D816Y, D816H, D816F, N822K, V560G, or a combination thereof. In certain embodiments, the activating mutation comprises or consists of D816V.

In certain embodiments, the human c-Kit comprises or consists of the amino acid sequence set forth in SEQ ID NO:1.

In certain embodiments, the mutant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2 or a portion thereof. In certain embodiments, the mutant consists of amino acids 543 to 976 of SEQ ID NO: 2

In certain embodiments, the c-Kit mutant is operably linked to an inducible promoter. In certain embodiments, the inducible promoter is selected from nuclear factor of activated T cells (NFAT), transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter.

In certain embodiments, the c-Kit mutant enhances cell persistence of the cell. In certain embodiments, the c-Kit mutant reduces apoptosis or anergy of the cell.

In certain embodiments, the antigen-recognizing receptor is exogenous or endogenous. In certain embodiments, the antigen-recognizing receptor is recombinantly expressed. In certain embodiments, the antigen-recognizing receptor is expressed from a vector. In certain embodiments, the c-Kit mutant is expressed from a vector.

In certain embodiments, the cell is an immunoresponsive cell. In certain embodiments, the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage. In certain embodiments, the cell of the lymphoid lineage is selected from T cells, B cells,

Natural Killer (NK) cells, dendritic cells. In certain embodiments, the cell is a T cell. In certain embodiments, the T cell is a cytotoxic T lymphocyte (CTL), a γδ T cell, a tumor-infiltrating lymphocyte (TIL), a regulatory T cell, or a Natural Killer T (NKT) cell.

In certain embodiments, the antigen is a tumor antigen or a pathogen antigen. In certain embodiments, the antigen is a tumor antigen. In certain embodiments, the tumor antigen is selected from the group consisting of mesothelin, CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-B2, Erb-B3, Erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-α, GD2, GD3, HER-2, hTERT, IL-13R-a2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, ERBB2, MAGEA3, CT83 (also known as KK-LC-1), p53, MART1,GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, and CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, HPV E6 oncoprotein, HPV E7 oncoprotein, and ERBB. In certain embodiments, the antigen is mesothelin.

In certain embodiments, the antigen-recognizing receptor is selected from a T cell receptor (TCR), a chimeric antigen receptor (CAR), and a TCR like fusion molecule. In certain embodiments, the antigen-recognizing receptor is a CAR. In certain embodiments, the CAR comprises an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain. In certain embodiments, the intracellular signaling domain of the CAR further comprises at least one co-stimulatory signaling region. In certain embodiments, the at least one co-stimulatory signaling region comprises a CD28 polypeptide. In certain embodiments, the CAR does not comprise a co-stimulatory signaling region.

Furthermore, the present disclosure provides methods for producing an antigen-specific cell. In certain embodiments, the method comprises introducing into a cell (a) a first nucleic acid sequence encoding an antigen-recognizing receptor that binds to an antigen; and (b) a second nucleic sequence encoding a c-Kit mutant comprising an activating mutation. In certain embodiments, the first nucleic acid sequence is operably linked to a first promoter. In certain embodiments, the second nucleic acid sequence is operably linked to a second promoter. In certain embodiments, one or both of the first and the second nucleic acid sequences are comprised in a vector. In certain embodiments, the vector is a retroviral vector.

Furthermore, the present disclosure provides compositions comprising: a) a mutant of human c-Kit comprising an activating mutation; and b) an antigen-recognizing receptor that binds to an antigen. In certain embodiments, the -Kit mutant is operably linked to a first promoter. In certain embodiments, the antigen-recognizing receptor is operably linked to a second promoter. In certain embodiments, one or both of the first and second promoters are inducible promoters. In certain embodiments, the inducible promoter is selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter. The present disclosure further provides cells comprising a composition disclosed herein.

Furthermore, the present disclosure provides nucleic acid compositions comprising (a) a first polynucleotide encoding an antigen-recognizing receptor that binds to an antigen; and (b) a second polynucleotide encoding a mutant of human c-Kit comprising an activating mutation. In certain embodiments, the nucleic acid composition further comprises a first promoter that is operably linked to the c-Kit mutant. In certain embodiments, the nucleic acid composition further comprises a second promoter that is operably linked to the antigen-recognizing receptor. In certain embodiments, one or both of the first and second promoters are inducible promoters. In certain embodiments, the inducible promoter is selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter. In certain embodiments, one or both of the first and second polynucleotides are comprised in a vector. In certain embodiments, the vector is a retroviral vector. The present disclosure further provides cells comprising a nucleic acid composition disclosed herein.

The present disclosure further provides vectors comprising the nucleic acid composition disclosed herein. The present disclosure further provides cells comprising a vector disclosed herein.

The present disclosure also provides pharmaceutical compositions comprising the cell disclosed herein and a pharmaceutically acceptable excipient. In certain embodiments, the composition further comprises an inhibitor of c-Kit. In certain embodiments, the inhibitor of c-Kit is selected from dasatinib (BMS-354825), midostaurin (PKC412), ponatinib, imatinib, and a combination thereof. In certain embodiments, the pharmaceutical composition is for treating and/or preventing a neoplasm, a pathogen infection, or an infection disease.

The present disclosure further provides methods of reducing tumor burden in a subject. The present disclosure also provides, the method comprises administering to the subject a cell disclosed herein or a pharmaceutical composition disclosed herein. In certain embodiments, the method reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.

The present disclosure further provides methods of treating and/or preventing a neoplasm. In certain embodiments, the method comprises administering to the subject a cell disclosed herein or a pharmaceutical composition disclosed herein.

The present disclosure further provides methods of lengthening survival of a subject having a neoplasia. In certain embodiments, the method comprises administering to the subject a cell disclosed herein or a pharmaceutical composition disclosed herein.

In certain embodiments, the tumor or neoplasm is a solid tumor. In certain embodiments, the solid tumor is selected from the group consisting of mesothelioma, lung cancer, pancreatic cancer, ovarian cancer, breast cancer, colon cancer, pleural tumor, glioblastoma, esophageal cancer, gastric cancer, synovial sarcoma, thymic carcinoma, endometrial carcinoma, stomach cancer, cholangiocarcinoma, and a combination thereof. In certain embodiments, the solid tumor is mesothelioma. In certain embodiments, the solid tumor is lung cancer.

In certain embodiments, the method further comprises administering an inhibitor of c-Kit. In certain embodiments, the inhibitor of c-Kit is selected from dasatinib (BMS-354825), midostaurin (PKC412), ponatinib, imatinib, and a combination thereof.

Furthermore, the present disclosure provides kits comprising a cell disclosed herein, a composition disclosed herein, a nucleic acid composition disclosed herein, or a vector disclosed herein. In certain embodiments, the kit further comprises written instructions for treating and/or preventing a neoplasm, a pathogen infection, or an infectious disease.

4. BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the presently disclosed subject matter to specific embodiments described, may be understood in conjunction with the accompanying drawings.

Figures 1A and 1B depict a composition in accordance with certain embodiments of the presently disclosed subject matter. The composition shown in Figure 1A, i.e., "M28z-KITv" (also referred to as M28z-KITm") comprises a c-Kit mutant D816V and a second generation CAR comprising an anti-mesothelin (MSLN) scFv, a CD28

transmembrane domain, a CD28 cytoplasmic signaling domain, a CD3zeta signaling domain. The composition shown in Figure 1B, i.e., "Mz-KITv" comprises a c-Kit mutant D816V and a first-generation CAR comprising an anti-mesothelin (MSLN) scFv, a CD28 transmembrane domain, and a CD3zeta signaling domain. LTR represents long terminal repeat.

Figure 2 depicts transduction efficiency of various constructs to T cells.

Figures 3A and 3B depict that M28z-KITv CAR-T constitutively exhibited activated pKIT signaling. Figure 3A depicts western blot results, which show that M28z-KITv CAR-T exhibited p-KIT activity without SCF, while M28z did not express KIT protein. Figure 3B depicts that M28z-KITv CAR-T exhibited higher p-STAT3 and p-STAT5 activity than M28z-KITwt control

Figure 4 depicts cumulative expansion of CAR-T cells during serial coculture. Arrows indicate time points of T-cell re-stimulation with A549GM tumor cells (E:T=3:1).

Figure 5 depicts enhanced proliferation of M28z CAR T cells and M28z-KITm CAR T cells. Far red cell trace 7 days first antigen stimulation (E:T=2:1). Target cells were A549GM cells.

Figure 6 depicts proliferation of M28z CAR T cells and M28z-KITm CAR T cells. Far red cell trace 7 days first antigen stimulation (E:T=2:1). Target cells were A549GM cells.

Figures 7A and 7B depict cytolytic activity of Mz CAR T cells, M28z CAR T cells, P28z CAR T cells, Mz-KITv CAR T cells, or M28z-KITv CAR T cells in Donor 1. Figure 7A shows the cytolytic activity at 4 hours. Figure 7B shows the cytolytic activity at 18 hours. Target cells were high MLSN A549M cells.

Figures 8A and 8B depict cytolytic activity of Mz CAR T cells, M28z CAR T cells, Mz-KITv CAR T cells, or M28z-KITv CAR T cells in Donor 2 after two stimulation with target cells (high MLSN A549GM cells) for every 4 day (E:T=3:1). Figure 8A shows the cytolytic activity at 4 hours post second antigen stimulation. Figure 8B shows the cytolytic activity at 18 hours post second antigen stimulation.

Figure 9 depicts cytolytic activity of Mz CAR T cells, M28z CAR T cells, Mz-KITv CAR T cells, or M28z-KITv CAR T cells. The target cells were low MSLN A549G cells.

Figures 10A and 10B depict PD1 expression of M28z CAR T cells, Mz-KITv CAR T cells, or M28z-KITv CAR T cells after antigen stimulation with A549GM cells. Figure 10A shows $CD4^+$ T cells. Figure 10B shows $CD8^+$ T cells.

Figures 11A and 11B depict KITv CAR T cells phenotype following antigen stimulation. Figure 11A depicts exhibition of stem cell-like memory T cells (T_{SCM}) cells of M28z CAR T cells, Mz-KITv CAR T cells, or M28z-KITv CAR T cells after stimulation with A546GM cells for every 4 days (E:T=3:1). Figure 11B depicts released IFN-γ, TNF-α and IL-2 assessed by Luminex assay, after 18 hours co-culture of CAR T cells with MSLN+ cells (E:T=3:1).

Figures 12A-12D depict *in vivo* efficacy of M28z CAR T cells, Mz-KITv CAR T cells, or M28z-KITv CAR T cells toward low MSLN lung tumor. Mice with established low MSLN A549G lung tumor were treated with a single dose of 1 x 10^5 T cells comprising M28z, Mz-KITv or M28z-KITv. UT represents untreated control. Figure 12A shows the results for UT. Figure 12B shows the results for M28z CAR T cells. Figure 12C shows the results for M28z-KITv CAR T cells. Figure 12D shows the results for Mz-KITv CAR T cells.

Figures 13A-13D depict *in vivo* efficacy of T cells comprising M28z, Mz-KITv or M28z-KITv toward high MSLN lung tumor. Mice with established high MSLN A549GM lung tumor were treated with a single dose of 1 x 10^5 T cells comprising M28z, Mz-KITv or M28z-KITv. UT represents untreated control. Figure 13A shows the results for UT. Figure 13B shows the results for M28z CAR T cells. Figure 13C shows the results for M28z-KITv CAR T cells. Figure 13D shows the results for Mz-KITv CAR T cells.

Figures 14A-14C depict Kaplan-Meier survival curves for in vivo treated mice depicted in Figures 12A-12D and 13A-13D. Figure 14A depicts the survival curve of mice with established low-antigen (mesothelin) expressing lung tumors. Figure 14 B depicts the survival curve of mice with established high-antigen (mesothelin) expressing lung tumors. Figure 14C depicts the FACS measurements of mesothelin expression levels in low mesothelin-expressing lung cancer (A549G) and high mesothelin-expressing lung cancer (A549GM).

Figure 15 depicts sensitivity of M28z CAR T cells and M28z-KITv CAR T cells to clinical tyrosine kinase inhibitors.

Figures 16A-16C depict the anti-tumor activity of M28z, Mz-KITv or M28z-KITv CAR T cells towards high MSLN-expressing lung tumor. NSG Mice with

established high MSLN A549GM lung tumor were treated with a single dose of 1×10^5 M28z, M28z-KITv or Mz-KITv CAR T cells. UT: untreated control. Figure 16A depicts the results of *in vivo* monitoring of tumor burden in mice using bioluminescent imaging (BLI). Figures 16B and 16C depict the Kaplan-Meier survival analysis of the mice, indicating the *in vivo* efficacy of i.v. administration of different CAR T cells. *, $P<0.05$. ***, $P<0.001$.

Figures 17A-17C depict the anti-tumor activity of M28z, Mz-KITv and M28z-KITv CAR T cells towards low MSLN-expressing lung tumor. NSG Mice with established low MSLN A549G lung tumor were treated with a single dose of 1×10^5 M28z, M28z-KITv or Mz-KITv CAR T cells. UT: untreated control. Figure 17A depicts the results of *in vivo* monitoring of tumor burden in mice using bioluminescent imaging (BLI). Figures 17B and 17C depict the Kaplan-Meier survival analysis of the mice, indicating the *in vivo* efficacy of i.v. administration of different CAR T cells. **, $P<0.01$.

Figures 18A and 18B depict Kaplan-Meier survival analysis that compared the *in vivo* efficacy of intrapleural administration of M28z, Mz-KITv or M28z-KITv CAR T cells in pleural mesothelioma tumor model. NSG Mice with established high MSLN MGM mesothelioma were treated with a single dose of 5×10^4 P28z, Mz, M28z, M28z-KITv and Mz-KITv CAR T cells. *, $P<0.05$. Figure 18A depicts the comparison among all groups. Figure 18B depicts the comparison between M28z and M28z-KITv, and between Mz and Mz-KITv groups.

Figures 19A and 19B depict the anti-tumor activity of M28z, Mz-KITv and M28z-KITv CAR T cells towards low MSLN-expressing mesothelioma. Figure 19A depicts the expression of low or high MSLN protein in MSTO cell to produce MG-LM and MGM cell respectively. Figure 19B depicts NSG Mice with established low MSLN mesothelioma (MG-LM) were treated with a single dose of 5×10^4 P28z, M28z, M28z-KITv or Mz-KITv CAR T cells. Kaplan-Meier survival analysis compared the *in vivo* efficacy of intrapleural administration of different CAR T cells. *, $P<0.05$. **, $P<0.01$.

Figure 20 depicts p-ERK signal of CAR T cells after antigen stimulation. After co-culture with MGM cell (E:T=1:2) for 5 min, p-ERK levels of CD4+ and CD8+ CAR T cell were measured by FACS. Both CD4 and CD8 of CAR T cells of M28z-KITv and Mz-KITv had stronger p-ERK activity than M28z.

Figures 21A and 21B depict that CAR T cells obtained from mice were exposed to high mesothelin-expressing mesothelioma cells and were analyzed for PD1 expression. Figure 21A depicts the quantification of PD1 expression at different E:T

ratios. Figure 21B depicts the flow cytometry graph measuring PD1 expression at different E:T ratios.

Figures 22A and 22B depict enriched gene sets related to phenotypic and functional T cell features in M28z-KITv CAR T cells. After co-culture with $MSLN^+$ tumor cells for 24 hours, CD8 CAR T cells of M28z and M28z-KITv were collected for analysis by nano string for CAR T panel genes, n=3 for each group. Figure 22A depicts that 87 out of 780 detected genes had significant fold change. Figure 22B depicts a heatmap of Pathway scores showing enriched gene sets related to phenotypic and functional T cell features in M28z-KITv CAR T cells. Figure 22C provides the gene pathways that were upregulated in KIT CAR T cells.

Figures 23A-23B depict significant upregulation of interferon signaling genes in M28z-KITv CAR T cells compared to M28z CAR T cells. After co-cultured with MSLN+ cancer cells for 24 hours, CD8 CAR T cells of M28z and M28z-KITv were collected for analysis by nano string for CAR T panel genes, n=3 for each group. The expression of Type I interferon signaling genes (Figure 23A) and Type II interferon signaling genes (Figure 23B) significantly increased in M28z-KITv CAR T cells.

5. DETAILED DESCRIPTION OF THE INVENTION

The presently disclosed subject matter provides cells comprising a c-Kit mutant, wherein the c-Kit mutant comprises an activating mutation. The cells can be genetically modified immunoresponsive cells (*e.g.*, T cells or NK cells), cells comprise an antigen-recognizing receptor (*e.g.*, a T-cell receptor (TCR) or a chimeric antigen receptor (CAR)). The presently disclosed subject matter also provides methods of using such cells for inducing and/or enhancing an immune response to a target antigen, and/or for treating and/or preventing a neoplasia, a pathogen infection, or other diseases/disorders (*e.g.*, a disease/disorder where an increase in an antigen-specific immune response is desired). The presently disclosed subject matter is based, at least in part, on the discovery that a c-Kit mutant (e.g., c-KitD816V) can enhance the cell proliferation of cells (e.g., T cells) comprising an antigen-recognizing receptor (e.g., a CAR). .

Non-limiting embodiments of the present disclosure are described by the present specification and Examples.

For purposes of clarity of disclosure and not by way of limitation, the detailed description is divided into the following subsections:

5.1. Definitions;

5.2. c-Kit Mutants;

5.3. Cells;
5.4. Antigen-Recognizing Receptors;
5.5. Dominant Negative Form of Programmed Death 1 (PD-1 DN)
5.6. Compositions and Vectors;
5.7. Polypeptides and Analogs;
5.8. Administration;
5.9. Formulations;
5.10. Methods of Treatment; and
5.11. Kits

5.1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art. The following references provide one of skill with a general definition of many of the terms used in the presently disclosed subject matter: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

As used herein, the term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, *e.g.*, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, *e.g.*, within 5-fold or within 2-fold, of a value.

By "immunoresponsive cell" is meant a cell that functions in an immune response or a progenitor, or progeny thereof.

By "activates an immunoresponsive cell" is meant induction of signal transduction or changes in protein expression in the cell resulting in initiation of an immune response. For example, when CD3 Chains cluster in response to ligand binding and immunoreceptor tyrosine-based inhibition motifs (ITAMs) a signal transduction cascade is produced. In certain embodiments, when an endogenous TCR or an

exogenous CAR binds to an antigen, a formation of an immunological synapse occurs that includes clustering of many molecules near the bound receptor (e.g. CD4 or CD8, CD3γ/δ/ε/ζ, etc.). This clustering of membrane bound signaling molecules allows for ITAM motifs contained within the CD3 chains to become phosphorylated. This phosphorylation in turn initiates a T cell activation pathway ultimately activating transcription factors, such as NF-κB and AP-1. These transcription factors induce global gene expression of the T cell to increase IL-2 production for proliferation and expression of master regulator T cell proteins in order to initiate a T cell mediated immune response.

By "stimulates an immunoresponsive cell" is meant a signal that results in a robust and sustained immune response. In various embodiments, this occurs after immune cell (*e.g.*, T-cell) activation or concomitantly mediated through receptors including, but not limited to, CD28, CD137 (4-lBB), OX40, CD40 and ICOS. Receiving multiple stimulatory signals can be important to mount a robust and long-term T cell mediated immune response. T cells can quickly become inhibited and unresponsive to antigen. While the effects of these co-stimulatory signals may vary, they generally result in increased gene expression in order to generate long lived, proliferative, and anti-apoptotic T cells that robustly respond to antigen for complete and sustained eradication.

The term "antigen-recognizing receptor" as used herein refers to a receptor that is capable of activating an immune or immunoresponsive cell (*e.g.*, a T-cell) in response to its binding to an antigen.

As used herein, the term "antibody" means not only intact antibody molecules, but also fragments of antibody molecules that retain immunogen-binding ability. Such fragments are also well known in the art and are regularly employed both *in vitro* and *in vivo*. Accordingly, as used herein, the term "antibody" means not only intact immunoglobulin molecules but also the well-known active fragments $F(ab')_2$, and Fab. $F(ab')_2$, and Fab fragments that lack the Fe fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J Nucl Med* (1983); 24:316-325). As used herein, antibodies include whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain variable fragments (scFvs), fusion polypeptides, and unconventional antibodies. In certain embodiments, an antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and

a heavy chain constant (C_H) region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant C_L region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further sub-divided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is 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. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

As used herein, "CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. *See*, *e.g.*, Kabat et al., Sequences of Proteins of Immunological Interest, 4th U. S. Department of Health and Human Services, National Institutes of Health (1987). Generally, antibodies comprise three heavy chain and three light chain CDRs or CDR regions in the variable region. CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. In certain embodiments, the CDRs regions are delineated using the Kabat system (Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services (1991); NIH Publication No. 91-3242).

As used herein, the term "single-chain variable fragment" or "scFv" is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of an immunoglobulin covalently linked to form a V_H::V_L heterodimer. The V_H and V_L are either joined directly or joined by a peptide-encoding linker (*e.g.*, 10, 15, 20, 25 amino acids), which connects the N-terminus of the V_H with the C-terminus of the V_L, or the C-terminus of the V_H with the N-terminus of the V_L. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. "Linker", as used herein, shall mean a functional group (e.g., chemical or polypeptide) that covalently attaches two or more polypeptides or nucleic acids so that they are connected to one another. As used herein, a "peptide linker" refers to one or more amino acids used to

couple two proteins together (e.g., to couple V_H and V_L domains). In certain embodiments, the linker comprises a sequence set forth in SEQ ID NO: 16, which is provided below:

GGGGSGGGGSGGGGS [SEQ ID NO: 16].

In certain embodiments, the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 16 is set forth in SEQ ID NO: 17, which is provided below:

GGAGGTGGAGGCTCAGGAGGAGGAGGCAGTGGAGGTGGTGGGTCA [SEQ ID NO: 17].

In certain embodiments, the nucleotide sequence encoding the amino acid sequence f SEQ ID NO: 16 is set forth in SEQ ID NO: 18, which is provided below.

GGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCA [SEQ ID NO: 18]

Despite removal of the constant regions and the introduction of a linker, scFv proteins retain the specificity of the original immunoglobulin. Single chain Fv polypeptide antibodies can be expressed from a nucleic acid including V_H - and V_L -encoding sequences as described by Huston et al., *Proc Nat Acad Sci USA* (1988);85:5879-5883,; U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754. Antagonistic scFvs having inhibitory activity have been described (Zhao et al., *Hyrbidoma (Larchmt)* 2008; 27(6):455-51; Peter et al., *J Cachexia Sarcopenia Muscle* (2013); 4(1):79-86; Shieh et al., *J Imunol* (2009);183(4):2277-85; Giomarelli et al., *Thromb Haemost* (2007);97(6):955-63; Fife et al., *J C I* (2006);116(8):2252-61; Brocks et al., *Immunotechnology* (1997); 3(3):173-84; Moosmayer et al., *Ther Immunol* (1995); 2(10):31-40). Agonistic scFvs having stimulatory activity have been described (Peter et al., *J Biol Chem* (2003); 25278(38):36740-7; Xie et al., *Nat Biotech* (1997); 15(8):768-71; Ledbetter et al., *Crit Rev Immunol* (1997);17(5-6):427-55; Ho et al., *BioChem Biophys Acta* (2003); 1638(3):257-66).

As used herein, the term "affinity" is meant a measure of binding strength. Affinity can depend on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and/or on the distribution of charged and hydrophobic groups. As used herein, the term "affinity" also includes "avidity", which refers to the strength of the antigen-antibody bond after formation of reversible complexes. Methods for calculating the affinity of an antibody for an antigen are known in the art, including, but not limited to, various antigen-binding experiments, *e.g.*, functional assays (*e.g.*, flow cytometry assay).

The term "chimeric antigen receptor" or "CAR" as used herein refers to a molecule comprising an extracellular antigen-binding domain that is fused to an intracellular signaling domain that is capable of activating or stimulating an immunoresponsive cell, and a transmembrane domain. In certain embodiments, the extracellular antigen-binding domain of a CAR comprises a scFv. The scFv can be derived from fusing the variable heavy and light regions of an antibody. Alternatively or additionally, the scFv may be derived from Fab's (instead of from an antibody, *e.g.*, obtained from Fab libraries). In certain embodiments, the scFv is fused to the transmembrane domain and then to the intracellular signaling domain. In certain embodiments, the CAR is selected to have high binding affinity or avidity for the antigen.

As used herein, the term "nucleic acid molecules" include any nucleic acid molecule that encodes a polypeptide of interest. Such nucleic acid molecules need not to be 100% homologous or identical with an endogenous nucleic acid sequence, but may exhibit substantial identity.

Polynucleotides having "substantial identity" or "substantial homology" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant a pair to form a double-stranded molecule between complementary polynucleotide sequences (*e.g.*, a gene described herein), or portions thereof, under various conditions of stringency. (Wahl et al., *Methods Enzymol* (1987); 152:399; Kimmel, *Methods Enzymol* (1987); 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, *e.g.*, less than about 500 mM NaCl and 50 mM trisodium citrate, or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, *e.g.*, at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, at least about 37° C, or at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In certain embodiments, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In certain embodiments, hybridization will occur at 37° C in 500

mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In certain embodiments, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps can be less than about 30 mM NaCl and 3 mM trisodium citrate, *e.g.*, less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, of at least about 42° C, or of at least about 68° C. In certain embodiments, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 42° C. in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described (Benton et al., *Science* (1977); 196:180; Grunstein et al., *Proc Natl Acad Sci, USA* (1975);72:3961,; Ausubel et al., *Current Protocols in Molecular Biology* (2001);Wiley Interscience, New York; ; Berger et al., *Guide to Molecular Cloning Techniques* (1987); Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, (1987); Cold Spring Harbor Laboratory Press, New York).

By "substantially identical" or "substantially homologous" is meant a polypeptide or nucleic acid molecule exhibiting at least about 50% homologous or identical to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In certain embodiments, such a sequence is 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 99%, or at least about 100% homologous or identical to the sequence of the amino acid or nucleic acid used for comparison.

Sequence identity can be measured by using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e-3 and e-100 indicating a closely related sequence.

By "analog" is meant a structurally related polypeptide or nucleic acid molecule having the function of a reference polypeptide or nucleic acid molecule.

The term "ligand" as used herein refers to a molecule that binds to a receptor. In certain embodiments, the ligand binds to a receptor on another cell, allowing for cell-to-cell recognition and/or interaction.

The term "constitutive expression" or "constitutively expressed" as used herein refers to expression or expressed under all physiological conditions.

By "disease" is meant any condition, disease or disorder that damages or interferes with the normal function of a cell, tissue, or organ, *e.g.*, neoplasia, and pathogen infection of cell.

By "effective amount" is meant an amount sufficient to have a therapeutic effect. In certain embodiments, an "effective amount" is an amount sufficient to arrest, ameliorate, or inhibit the continued proliferation, growth, or metastasis (*e.g.*, invasion, or migration) of a neoplasia.

By "enforcing tolerance" is meant preventing the activity of self-reactive cells or immunoresponsive cells that target transplanted organs or tissues.

By "endogenous" is meant a nucleic acid molecule or polypeptide that is normally expressed in a cell or tissue.

By "exogenous" is meant a nucleic acid molecule or polypeptide that is not endogenously present in a cell. The term "exogenous" would therefore encompass any recombinant nucleic acid molecule or polypeptide expressed in a cell, such as foreign, heterologous, and over-expressed nucleic acid molecules and polypeptides. By "exogenous" nucleic acid is meant a nucleic acid not present in a native wild-type cell;

for example, an exogenous nucleic acid may vary from an endogenous counterpart by sequence, by position/location, or both. For clarity, an exogenous nucleic acid may have the same or different sequence relative to its native endogenous counterpart; it may be introduced by genetic engineering into the cell itself or a progenitor thereof, and may optionally be linked to alternative control sequences, such as a non-native promoter or secretory sequence.

By a "heterologous nucleic acid molecule or polypeptide" is meant a nucleic acid molecule (*e.g.*, a cDNA, DNA or RNA molecule) or polypeptide that is not normally present in a cell or sample obtained from a cell. This nucleic acid may be from another organism, or it may be, for example, an mRNA molecule that is not normally expressed in a cell or sample.

By "modulate" is meant positively or negatively alter. Exemplary modulations include a about 1%, about 2%, about 5%, about 10%, about 25%, about 50%, about 75%, or about 100% change.

By "increase" is meant to alter positively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, about 100% or more.

By "reduce" is meant to alter negatively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, or even by about 100%.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high-performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation,

different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated cell" is meant a cell that is separated from the molecular and/or cellular components that naturally accompany the cell.

The term "antigen-binding domain" as used herein refers to a domain capable of specifically binding a particular antigenic determinant or set of antigenic determinants present on a cell.

By "neoplasm" is meant a disease characterized by the pathological proliferation of a cell or tissue and its subsequent migration to or invasion of other tissues or organs. Neoplastic growth is typically uncontrolled and progressive, and occurs under conditions that would not elicit, or would cause cessation of, multiplication of normal cells. Neoplasm can affect a variety of cell types, tissues, or organs, including but not limited to an organ selected from the group consisting of bladder, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and vagina, or a tissue or cell type thereof. Neoplasia include cancers, such as sarcomas, carcinomas, or plasmacytomas (malignant tumor of the plasma cells). In certain embodiments, the neoplasm is a cancer. In certain embodiments, the neoplasm is a solid tumor.

By "receptor" is meant a polypeptide, or portion thereof, present on a cell membrane that selectively binds one or more ligand.

By "recognize" is meant selectively binds to a target. A T cell that recognizes a tumor can expresses a receptor (*e.g.*, a TCR or CAR) that binds to a tumor antigen.

By "reference" or "control" is meant a standard of comparison. For example, the level of scFv-antigen binding by a cell expressing a CAR and an scFv may be compared to the level of scFv-antigen binding in a corresponding cell expressing CAR alone.

By "secreted" is meant a polypeptide that is released from a cell via the secretory pathway through the endoplasmic reticulum, Golgi apparatus, and as a vesicle that transiently fuses at the cell plasma membrane, releasing the proteins outside of the cell.

By "signal sequence" or "leader sequence" is meant a peptide sequence (*e.g.*, 5, 10, 15, 20, 25 or 30 amino acids) present at the N-terminus of newly synthesized proteins that directs their entry to the secretory pathway. Exemplary leader sequences include, but is not limited to, the IL-2 signal sequence: MYRMQLLSCIALSLALVTNS [SEQ ID

NO: 43] (human), MYSMQLASCVTLTLVLLVNS [SEQ ID NO: 44] (mouse); the kappa leader sequence: METPAQLLFLLLLWLPDTTG [SEQ ID NO: 45] (human), METDTLLLWVLLLWVPGSTG [SEQ ID NO: 46] (mouse); the CD8 leader sequence: MALPVTALLLPLALLLHAARP [SEQ ID NO: 47] (human); the truncated human CD8 signal peptide: MALPVTALLLPLALLLHA [SEQ ID NO: 48] (human); the albumin signal sequence: MKWVTFISLLFSSAYS [SEQ ID NO: 49] (human); and the prolactin signal sequence: MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS [SEQ ID NO: 20] (human). By "soluble" is meant a polypeptide that is freely diffusible in an aqueous environment (*e.g.*, not membrane bound).

By "specifically binds" is meant a polypeptide or fragment thereof that recognizes and binds to a biological molecule of interest (*e.g.*, a polypeptide), but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a presently disclosed polypeptide.

The terms "comprises", "comprising", and are intended to have the broad meaning ascribed to them in U.S. Patent Law and can mean "includes", "including" and the like.

As used herein, "treatment" refers to clinical intervention in an attempt to alter the disease course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Therapeutic effects of treatment include, without limitation, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By preventing progression of a disease or disorder, a treatment can prevent deterioration due to a disorder in an affected or diagnosed subject or a subject suspected of having the disorder, but also a treatment may prevent the onset of the disorder or a symptom of the disorder in a subject at risk for the disorder or suspected of having the disorder.

An "individual" or "subject" herein is a vertebrate, such as a human or non-human animal, for example, a mammal. Mammals include, but are not limited to, humans, primates, farm animals, sport animals, rodents and pets. Non-limiting examples of non-human animal subjects include rodents such as mice, rats, hamsters, and guinea pigs; rabbits; dogs; cats; sheep; pigs; goats; cattle; horses; and non-human primates such as apes and monkeys. The term "immunocompromised" as used herein refers to a subject who has an immunodeficiency. The subject is very vulnerable to opportunistic infections,

infections caused by organisms that usually do not cause disease in a person with a healthy immune system but can affect people with a poorly functioning or suppressed immune system.

Other aspects of the presently disclosed subject matter are described in the following disclosure and are within the ambit of the presently disclosed subject matter.

5.2. c-Kit Mutants

Proto-oncogene KIT is a receptor tyrosine kinase protein and also known as CD117; KIT; PBT; stem cell growth factor receptor (SCFR); MASTC. GenBank ID: 3815 (human), 16590(mouse). The protein product of KIT includes, but is not limited to, NCBI Reference Sequences NP_000213 (human isoform 1), NP 001122733 (mouse isoform 1).

c-Kit, known as CD117, is a cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. Signaling through c-Kit plays a role in cell survival, proliferation and differentiation (Ceredig et al., *Nat Rev Immunol* (2002); 2(11):888-97).

c-Kit binds to stem cell factor (SCF). Upon binding, c-Kit and SCF form a dimer that activates its intrinsic tyrosine kinase activity and in turn phosphorylates and activates signal transduction molecules that propagate the signal in the cell. A presently disclosed c-Kit activating mutation (e.g., D816V mutation) results in constitutive activation absent SCF, e.g., without forming the cKit/SCF dimers (Hirota et al., *Science* (1998);279(5350):577-80; Kitamura et al., *Mut Res* (2001):165-71).

In certain embodiments, a c-Kit mutant is a human c-Kit mutant. In certain embodiments, a human c-Kit protein comprises or consists of the sequence having a NCBI Reference No. NP_000213 (SEQ ID NO: 1). SEQ ID NO: 1 is provided below.

```
1 MRGARGAWDF LCVLLLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD
61 PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV
121 DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH
181 RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
241 SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
301 VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
361 DYPKSENESN IRYVSELHLT RLKGTEGGTY TFLVSNSDVN AAIAFNVYVN TKPEILTYDR
421 LVNGMLQCVA AGFPEPTIDW YFCPGTEQRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS
481 SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
541 MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
601 GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
661 TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
721 YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
781 AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES
841 IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY
901 DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV
961 GSTASSSQPL LVHDDV [SEQ ID NO: 1]
```

The cells of the presently disclosed subject matter comprise a c-Kit mutant. In certain embodiments, the c-Kit mutant comprises an activating mutation.

In certain embodiments, the activating mutation is a gain-of-function mutation. In certain embodiments, the activating mutation is a mutation whose gene product consists of an enhanced effect as compared to a gene product without such mutation (e.g., the wild-type protein).

In certain embodiments, the activating mutation (e.g., D816V mutation) is present in early lineage of hemopoietic cells, and is lost during maturation. In certain embodiments, the activating mutation of c-Kit leads to c-Kit activation independent of the interaction of the c-Kit and its ligand (e.g., SCF). In certain embodiments, the activating mutation of c-Kit leads to c-Kit activation absent a c-Kit ligand, e.g., SCF. In certain embodiments, the activating mutation of c-Kit leads to constitutive activation of c-Kit. In certain embodiments, the activating mutation of c-Kit leads to c-Kit activation independent of the inhibition of Tyrosine phosphatase-1 (SHP-1) and/or Tyrosine phosphatase-2 (SHP-2). In certain embodiments, the activating mutation of c-Kit leads to c-Kit activation in the presence of inhibition of SHP-1 and/or SHP-2.

The activating mutation of c-Kit promotes proliferation and anti-apoptosis of cells comprising the c-Kit mutant. The activating mutation of c-Kit makes cells comprising the c-Kit mutant resistant to PD-L1/2-PD-1 inhibition.

In certain embodiments, the activating mutation is within the intracellular region of human cKIT, e.g., a human cKIT consisting of the amino acid sequence set forth in SEQ ID NO: 1. In certain embodiments, the intracellular region of human cKIT comprises amino acids 544 to 977 of SEQ ID NO: 1. In certain embodiments, the activating mutation is within amino acids 816 to 826 of human cKIT, e.g., a human cKIT consisting of the amino acid sequence set forth in SEQ ID NO: 1. In certain embodiments, the activating mutation is at amino acid position 816, or amino acid position 822. In certain embodiments, the activating mutation is within amino acids 550 to 570 of human cKIT, e.g., a human cKIT consisting of the amino acid sequence set forth in SEQ ID NO: 1. In certain embodiments, the activating mutation is at amino acid position 560. Non-limiting examples of c-Kit activating mutation include D816V, D816Y, D816H, D816F, N822K, V560G, or a combination thereof. In certain embodiments, the activating mutation is D816V.

The c-Kit mutant can be operably linked to a promoter. The promoters can be endogenous or exogenous. Non-limiting examples of exogenous promoters include an

elongation factor (EF)-1 promoter, a cytomegalovirus immediate-early promoter (CMV) promoter, a simian virus 40 early promoter (SV40) promoter, a phosphoglycerate kinase (PGK) promoter, and a metallothionein promoter. In certain embodiments, the promoter is an inducible promoter. Non-limiting examples of inducible promoter are selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter. The inducible promoter can control the activation of c-Kit, e.g., with the control of the inducible promoter, c-Kit is activated only upon the activation of the cells comprising the c-Kit mutant (e.g., T cells or CAR-T cells).

In certain embodiments, the c-Kit mutant comprises or consists of an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, at least about 100% identical to the amino acid sequence set forth in SEQ ID NO: 2 or a portion thereof. In certain embodiments, the c-Kit mutant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2 or a portion thereof. In certain embodiments, the c-Kit mutant comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 2, which is at least 50, or at least 100, or at least 150, or at least 200, or at least 250, or at least 300, or at least 350, or at least 400, or at least 450, or at least 500, or at least 550, or at least 600, or at least 650, or at least 700, or at least 750, or at least 800, or at least 850, or at least 900, or at least 950, and up to 976 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the c-Kit mutant comprises or consists of an amino acid sequence of amino acids 1 to 976, 1 to 200, 400 to 976, 500 to 976, or 543 to 976 of SEQ ID NO: 2. In certain embodiments, the c-Kit mutant comprises or consists of amino acids 543 to 976 of SEQ ID NO: 2.

SEQ ID NO: 2 is provided below.

```
1 MRGARGAWDF LCVLLLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD
61 PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV
121 DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH
181 RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
241 SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
301 VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
361 DYPKSENESN IRYVSELHLT RLKGTEGGTY TFLVSNSDVN AAIAFNVYVN TKPEILTYDR
421 LVNGMLQCVA AGFPEPTIDW YFCPGTEQRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS
481 SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
541 MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
601 GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
661 TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
721 YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
781 AFLASKNCIH RDLAARNILL THGRITKICD FGLARVIKND SNYVVKGNAR LPVKWMAPES
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841 IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY
901 DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV
961 GSTASSSQPL LVHDDV [SEQ ID NO: 2]

5.3. Cells

The presently disclosed subject matter provides cells comprising a c-Kit mutant disclosed herein (e.g., one disclosed in Section 5.2). In certain embodiments, the c-Kit mutant is an exogenous c-Kit mutant.

In certain embodiments, the cell is selected from cells of lymphoid lineage and cells of myeloid lineage. In certain embodiments, the cell is an immunoresponsive cell. In certain embodiments, the immunoresponsive cell is a cell of lymphoid lineage.

In certain embodiments, the cell is a cell of the lymphoid lineage. Cells of the lymphoid lineage can provide production of antibodies, regulation of cellular immune system, detection of foreign agents in the blood, detection of cells foreign to the host, and the like. Non-limiting examples of cells of the lymphoid lineage include T cells, Natural Killer (NK) cells, B cells, dendritic cells, stem cells from which lymphoid cells may be differentiated. In certain embodiments, the stem cell is a pluripotent stem cell (e.g., embryonic stem cell).

In certain embodiments, the cell is a T cell. T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. The T cells of the presently disclosed subject matter can be any type of T cells, including, but not limited to, helper T cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: *e.g.*, TEM cells and TEMRA cells, Regulatory T cells (also known as suppressor T cells), tumor-infiltrating lymphocyte (TIL), Natural killer T cells, Mucosal associated invariant T cells, and γδ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells may be genetically modified to target specific antigens through the introduction of an antigen-recognizing receptor, *e.g.*, a CAR or a TCR. In certain embodiments, the immunoresponsive cell is a T cell. The T cell can be a $CD4^+$ T cell or a $CD8^+$ T cell. In certain embodiments, the T cell is a $CD4^+$ T cell. In certain embodiments, the T cell is a $CD8^+$ T cell.

In certain embodiments, the cell is a NK cell. Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune

response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

Types of human lymphocytes of the presently disclosed subject matter include, without limitation, peripheral donor lymphocytes. *e.g.*, those disclosed in Sadelain et al., *Nat Rev Cancer* (2003); 3:35-45 (disclosing peripheral donor lymphocytes genetically modified to express CARs), in Morgan, R.A., *et al*. 2006 *Science* 314:126-129 (disclosing peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the α and β heterodimer), in Panelli et al., *J Immunol* (2000);164:495-504; Panelli et al., *J Immunol* (2000);164:4382-4392 (disclosing lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies), and in Dupont et al., Cancer Res (2005);65:5417-5427; Papanicolaou et al., *Blood* (2003);102:2498-2505 (disclosing selectively *in vitro*-expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells).

The cells (*e.g.*, T cells) can be autologous, non-autologous (*e.g.*, allogeneic), or derived *in vitro* from engineered progenitor or stem cells. In certain embodiments, the cell is allogeneic.

The cells of the presently disclosed subject matter can be cells of the myeloid lineage. Non-limiting examples of cells of the myeloid lineage include monocytes, macrophages, basophils, neutrophils, eosinophils, megakaryocytes, mast cell, erythrocyte, thrombocytes, and stem cells from which myeloid cells may be differentiated.

In certain embodiments, the stem cell is a pluripotent stem cell (e.g., an embryonic stem cell or an induced pluripotent stem cell).

In certain embodiments, the presently disclosed cells are capable of modulating the tumor microenvironment. Tumors have a microenvironment that is hostile to the host immune response involving a series of mechanisms by malignant cells to protect themselves from immune recognition and elimination. This "hostile tumor microenvironment" comprises a variety of immune suppressive factors including infiltrating regulatory $CD4^+$ T cells (Tregs), myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs), immune suppressive cytokines including TGF-β, and expression of ligands targeted to immune suppressive receptors expressed by activated T cells (CTLA-4 and PD-1). These mechanisms of immune suppression play a

role in the maintenance of tolerance and suppressing inappropriate immune responses, however within the tumor microenvironment these mechanisms prevent an effective anti-tumor immune response. Collectively these immune suppressive factors can induce either marked anergy or apoptosis of adoptively transferred CAR modified T cells upon encounter with targeted tumor cells.

In certain embodiments, the presently disclosed cells have increased cell proliferation, and/or cell persistence. In certain embodiments, the presently disclosed immunoresponsive cells have decreased apoptosis and/or anergy.

In certain embodiments, the cell further comprises an antigen-recognizing receptor (*e.g.*, a CAR or a TCR) that binds to an antigen. The cells can be transduced with the antigen-recognizing receptor and the c-Kit mutant exogenous activating such that the cells co-express the antigen-recognizing receptor and the c-Kit mutant.

The c-Kit mutant can be operably linked to a first promoter. The antigen-recognizing receptor can be operably linked to a second promoter. The first promoter can be the same as the second promoter. Alternatively, the first promoter is different from the second promoter. The first and the second promoters can be endogenous or exogenous. Non-limiting examples of exogenous promoters include an elongation factor (EF)-1 promoter, a cytomegalovirus immediate-early promoter (CMV) promoter, a simian virus 40 early promoter (SV40) promoter, a phosphoglycerate kinase (PGK) promoter, and a metallothionein promoter. In certain embodiments, one or both of the first and second promoters are inducible promoters. Non-limiting examples of inducible promoter are selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter.

5.4. Antigen-Recognizing Receptors

In certain embodiments, a presently disclosed cell further comprises an antigen-recognizing receptor. In certain embodiments, the antigen-recognizing receptors binds to an antigen. In certain embodiments, the antigen-recognizing receptor is a chimeric antigen receptor (CAR). In certain embodiments, the antigen-recognizing receptor is a T-cell receptor (TCR). In certain embodiments, the antigen-recognizing receptor is a TCR like fusion molecule.

5.4.1. Antigens

The antigen-recognizing receptor can bind to a tumor antigen or a pathogen antigen.

In certain embodiments, the antigen-recognizing receptor binds to a tumor antigen. Any tumor antigen (antigenic peptide) can be used in the tumor-related embodiments described herein. Sources of antigen include, but are not limited to, cancer proteins. The antigen can be expressed as a peptide or as an intact protein or portion thereof. The intact protein or a portion thereof can be native or mutagenized. In certain embodiments, the antigen is expressed in a tumor tissue. Non-limiting examples of tumor antigens include Mesothelin, CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-B2, Erb-B3, Erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-α, GD2, GD3, HER-2, hTERT, IL-13R-a2, κ-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, ERBB2, MAGEA3, CT83 (also known as KK-LC-1), p53, MART1,GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, HPV E6 oncoprotein, HPV E7 oncoprotein, and ERBB. In certain embodiments, the tumor antigen is mesothelin.

In certain embodiments, the antigen-recognizing receptor binds to mesothelin. In certain embodiments, the antigen-recognizing receptor binds to human mesothelin consisting of the sequence with a NCBI Reference No: AAV87530.1 (SEQ ID NO: 3), or fragments thereof. SEQ ID NO: 3 is provided below:

MALPTARPLL GSCGTPALGS LLFLLFSLGW VQPSRTLAGE TGQEAAPLDG VLANPPNISS LSPRQLLGFP
CAEVSGLSTE RVRELAVALA QKNVKLSTEQ LRCLAHRLSE PPEDLDALPL DLLLFLNPDA FSGPQACTHF
FSRITKANVD LLPRGAPERQ RLLPAALACW GVRGSLLSEA DVRALGGLAC DLPGRFVAES AEVLLPRLVS
CPGPLDQDQQ EAARAALQGG GPPYGPPSTW SVSTMDALRG LLPVLGQPII RSIPQGIVAA WRQRSSRDPS
WRQPERTILR PRFRREVEKT ACPSGKKARE IDESLIFYKK WELEACVDAA LLATQMDRVN AIPFTYEQLD
VLKHKLDELY PQGYPESVIQ HLGYLFLKMS PEDIRKWNVT SLETLKALLE VNKGHEMSPQ VATLIDRFVK
GRGQLDKDTL DTLTAFYPGY LCSLSPEELS SVPPSSIWAV RPQDLDTCDP RQLDVLYPKA RLAFQNMNGS
EYFVKIQSFL GGAPTEDLKA LSQQNVSMDL ATFMKLRTDA VLPLTVAEVQ KLLGPHVEGL KAEERHRPVR
DWILRQRQDD LDTLGLGLQG GIPNGYLVLD LSVQEALSGT PCLLGPGPVL TVLALLLAST LA [SEQ ID NO:3]

In certain embodiments, the antigen-recognizing receptor binds to a pathogen antigen, *e.g.*, for use in treating and/or preventing a pathogen infection or other infectious disease, for example, in an immunocompromised subject. Non-limiting examples of pathogens include a virus, bacteria, fungi, parasite and protozoa capable of causing disease.

Non-limiting examples of viruses include, *Retroviridae* (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HDTV-III, LAVE or

HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; *Picornaviridae* (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); *Calciviridae* (e.g. strains that cause gastroenteritis); *Togaviridae* (e.g. equine encephalitis viruses, rubella viruses); *Flaviridae* (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); *Coronoviridae* (e.g. coronaviruses); *Rhabdoviridae* (*e.g.*,vesicular stomatitis viruses, rabies viruses); *Filoviridae* (e.g., Ebola viruses); *Paramyxoviridae* (*e.g.*, parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); *Orthomyxoviridae* (e.g., influenza viruses); *Bungaviridae* (e.g. Hantaan viruses, bunga viruses, phleboviruses and Naira viruses); *Arena viridae* (hemorrhagic fever viruses); *Reoviridae* (e.g. reoviruses, orbiviurses and rotaviruses); *Birnaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvovirida* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses); *Adenoviridae* (most adenoviruses); *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); and *Iridoviridae* (*e.g.*, African swine fever virus); and unclassified viruses (e.g. the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 = internally transmitted; class 2 = parenterally transmitted (*i.e.*, Hepatitis C); Norwalk and related viruses, and astroviruses), human papilloma virus (*i.e.* HPV), JC virus, Epstein Bar Virus, Merkel cell polyoma virus .

Non-limiting examples of bacteria include *Pasteurella*, *Staphylococci*, *Streptococcus*, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria include but are not limited to, *Helicobacter pyloris*, *Borelia burgdorferi*, *Legionella pneumophilia*, *Mycobacteria sps* (e.g. *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansaii*, *M. gordonae*), *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A Streptococcus), *Streptococcus agalactiae* (Group B Streptococcus), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic sps.), *Streptococcus pneumoniae*, pathogenic *Campylobacter sp.*, *Enterococcus sp.*, *Haemophilus influenzae*, *Bacillus antracis*, *corynebacterium diphtheriae*, *corynebacterium sp.*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringers*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasturella multocida*, *Bacteroides sp.*, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidium*, *Treponema pertenue*, *Leptospira*, *Rickettsia*, clostridium difficile, and *Actinomyces israelli*.

In certain embodiments, the pathogen antigen is a viral antigen present in Cytomegalovirus (CMV), a viral antigen present in Epstein Barr Virus (EBV), a viral antigen present in Human Immunodeficiency Virus (HIV), or a viral antigen present in influenza virus.

5.4.2. T-Cell Receptor (TCR)

In certain embodiments, the antigen-recognizing receptor is a TCR. A TCR is a disulfide-linked heterodimeric protein consisting of two variable chains expressed as part of a complex with the invariant CD3 chain molecules. A TCR found on the surface of T cells is responsible for recognizing antigens as peptides bound to major histocompatibility complex (MHC) molecules. In certain embodiments, a TCR comprises an alpha chain and a beta chain (encoded by TRA and TRB, respectively). In certain embodiments, a TCR comprises a gamma chain and a delta chain (encoded by TRG and TRD, respectively).

Each chain of a TCR is composed of two extracellular domains: Variable (V) region and a Constant (C) region. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail. The Variable region binds to the peptide/MHC complex. The variable domain of both chains each consists of three complementarity determining regions (CDRs).

In certain embodiments, a TCR can form a receptor complex with three dimeric signaling modules CD3δ/ε, CD3γ/ε and CD247 ζ/ζ or ζ/η. When a TCR complex engages with its antigen and MHC (peptide/MHC), the T cell expressing the TCR complex is activated.

In certain embodiments, the TCR is an endogenous TCR. In certain embodiments, the antigen-recognizing receptor is naturally occurring TCR.

In certain embodiments, the antigen-recognizing receptor is an exogenous TCR. In certain embodiments, the antigen-recognizing receptor is a recombinant TCR. In certain embodiments, the antigen-recognizing receptor is a non-naturally occurring TCR. In certain embodiments, the non-naturally occurring TCR differs from any naturally occurring TCR by at least one amino acid residue. In certain embodiments, the non-naturally occurring TCR differs from any naturally occurring TCR by at least about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100 or more amino acid residues. In certain embodiments, the non-naturally occurring TCR is modified from a naturally occurring

TCR by at least one amino acid residue. In certain embodiments, the non-naturally occurring TCR is modified from a naturally occurring TCR by at least about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 20, about 25, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100 or more amino acid residues.

5.4.3. Chimeric Antigen Receptor (CAR)

In certain embodiments, the antigen-recognizing receptor is a CAR. CARs are engineered receptors, which graft or confer a specificity of interest onto an immune effector cell. CARs can be used to graft the specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by retroviral vectors.

There are three generations of CARs. "First-generation" CARs are typically composed of an extracellular antigen-binding domain (*e.g.*, a scFv), which is fused to a transmembrane domain, which is fused to cytoplasmic/intracellular signaling domain. "First generation" CARs can provide *de novo* antigen recognition and cause activation of both $CD4^+$ and $CD8^+$ T cells through their CD3ζ chain signaling domain in a single fusion molecule, independent of HLA-mediated antigen presentation. "Second generation" CARs add intracellular signaling domains from various co-stimulatory molecules (*e.g.*, CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. "Second generation" CARs comprise those that provide both co-stimulation (*e.g.*, CD28 or 4-1BB) and activation (CD3ζ). "Third generation" CARs comprise those that provide multiple co-stimulation (*e.g.*, CD28 and 4-1BB) and activation (CD3ζ). In certain embodiments, the antigen-recognizing receptor is a first-generation CAR. In certain embodiments, the antigen-recognizing receptor is a CAR that does not comprise an intracellular signaling domain of a co-stimulatory molecule. In certain embodiments, the antigen-recognizing receptor is a second-generation CAR.

In certain embodiments, the CAR can comprise an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain specifically binds to an antigen, which can be a tumor antigen or a pathogen antigen.

5.4.3.1. Extracellular Antigen-Binding Domain of A CAR

In certain embodiments, the extracellular antigen-binding domain specifically binds to an antigen. In certain embodiments, the antigen is mesothelin. In certain embodiments, the extracellular antigen-binding domain is an scFv. In certain

embodiments, the scFv is a human scFv. In certain embodiments, the scFv is a humanized scFv. In certain embodiments, the scFv is a murine scFv. In certain embodiments, the extracellular antigen-binding domain is a Fab, which is optionally crosslinked. In certain embodiments, the extracellular antigen-binding domain is a $F(ab)_2$. In certain embodiments, any of the foregoing molecules may be comprised in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain. In certain embodiments, the scFv is identified by screening scFv phage library with an antigen-Fc fusion protein.

In certain non-limiting embodiments, the extracellular antigen-binding domain of the CAR (embodied, for example, an scFv or an analog thereof) binds to an antigen with a dissociation constant (K_d) of about 2×10^{-7} M or less. In certain embodiments, the K_d is about 2×10^{-7} M or less, about 1×10^{-7} M or less, about 9×10^{-8} M or less, about 1×10^{-8} M or less, about 9×10^{-9} M or less, about 5×10^{-9} M or less, about 4×10^{-9} M or less, about 3×10^{-9} or less, about 2×10^{-9} M or less, or about 1×10^{-9} M or less. In certain non-limiting embodiments, the K_d is about 3×10^{-9} M or less. In certain non-limiting embodiments, the K_d is from about 1×10^{-9} M to about 3×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5×10^{-9} M to about 3×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5×10^{-9} M to about 2.7×10^{-7} M.

In certain non-limiting embodiments, the extracellular antigen-binding domain of the CAR has a high binding specificity as well as high binding affinity to human mesothelin. For example, in such embodiments, the extracellular antigen-binding domain of the CAR (embodied, for example, in an scFv) binds to human mesothelin with an EC50 value of from about 1 nM to about 25 nM as measured by enzyme-linked immunosorbent assay (ELISA). In certain embodiments, the extracellular antigen-binding domain of the CAR has an EC50 value of about 20 nM as measured by ELISA. In certain embodiments, the extracellular antigen-binding domain of the CAR comprises an anti-mesothelin antibody or an antigen-binding portion thereof described in U.S. Patent No. 8,357,783, which is herein incorporated by reference in its entirety. In certain embodiments, the extracellular antigen-binding domain of the CAR is derived from a heavy chain variable region and a light chain variable region of an antibody that binds to human mesothelin, *e.g.*, antibody m912 as disclosed in Feng *et al.*, *Mol. Cancer Therapy* (2009);8(5):1113-1118, which is herein incorporated by reference in its entirety. Antibody m912 was isolated from a human Fab library by panning against

recombinant mesothelin. In certain embodiments, the extracellular antigen-binding domain of the CAR is derived from Fab's (e.g., from human or mouse Fab libraries).

Binding of the extracellular antigen-binding domain (embodiment, for example, in an scFv) of the CAR can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detect the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody, or an scFv) specific for the complex of interest. For example, the scFv can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain embodiments, the mesothelin targeted extracellular antigen-binding domain is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (e.g., EBFP, EBFP2, Azurite, and mKalama1), cyan fluorescent protein (e.g., ECFP, Cerulean, and CyPet), and yellow fluorescent protein (e.g., YFP, Citrine, Venus, and YPet). In one embodiment, the mesothelin-targeted human scFv is labeled with GFP.

In certain non-limiting embodiments, the extracellular antigen-binding domain of the CAR recognizes or binds to human mesothelin with a mesothelin level of about 1,000 or more mesothelin binding sites/cell. In certain embodiments, the extracellular antigen-binding domain of the CAR recognizes or binds to human mesothelin with a mesothelin level of from about 1,000 to about 50,000 mesothelin binding sites/cell. In some embodiments, the extracellular antigen-binding domain of the CAR does not recognize or bind to human mesothelin with a mesothelin expression level of less than 1,000 mesothelin binding sites/cell, *e.g.*, the human mesothelin expressed in normal tissues, e.g., normal pleura, pericardium, and peritoneum tissues. In certain embodiments, the extracellular antigen-binding domain of the CAR does not recognize or bind to human mesothelin with a mesothelin expression level of more than 50,000 mesothelin binding sites/cell. In certain embodiments, a human scFv comprised in the CAR recognizes or binds to human mesothelin with a mesothelin expression level of from about 1,000 to about 50,000 mesothelin binding sites/cell. In certain embodiments, a human scFv comprised in the CAR does not recognize or bind to human mesothelin with a

mesothelin expression level of more than 50,000 or less than 1,000 mesothelin binding sites/cell.

In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 4, or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5 or a conservative modification thereof, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6, a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 4, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., a scFv) comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., a scFv) comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9.

In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 4 or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5 or a conservative modification thereof, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6, a conservative modification thereof, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 4, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6, a V_L CDR1

comprising the amino acid sequence set forth in SEQ ID NO: 7, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8 and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the CDRs are identified according to the Kabat numbering system.

In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a heavy chain variable region (V_H) comprising the amino acid sequence set forth in SEQ ID NO: 10. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a light chain variable region (V_L) comprising the amino acid sequence set forth in SEQ ID NO: 11. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., a scFv) comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 10, and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 11, optionally with (iii) a linker sequence, for example a linker peptide, between the V_H and the V_L. In certain embodiments, the linker comprises amino acids consisting of the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 10. For example, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H comprising an amino acid sequence that is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to SEQ ID NO: 10. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino sequence set forth in SEQ ID NO: 10. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 11. For example, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_L comprising an amino acid sequence that is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to SEQ ID NO: 11. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_L

comprising the amino acid sequence set forth in SEQ ID NO: 11. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 10, and a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 11. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 10 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 11.

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 10 is set forth in SEQ ID NO: 12.

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 11 is set forth in SEQ ID NO: 13.

In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises an amino acid sequence that is at least about 80%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% (*e.g.*, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%) homologous or identical to the amino acid sequence set forth in SEQ ID NO: 14. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) comprises or consists of the amino acid sequence set forth in SEQ ID NO: 14. In certain embodiments, the extracellular antigen-binding domain of the CAR (e.g., an scFv) specifically binds to a human mesothelin polypeptide (e.g., a human mesothelin polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 3).

In certain embodiments, an exemplary nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 14 is set forth in SEQ ID NO: 15.

In certain embodiments, the scFv is a human scFv.

SEQ ID Nos: 4-15 are provided below:

GGSVSSGSYY [SEQ ID NO: 4]

IYYSGST [SEQ ID NO: 5]

AREGKNGAFDIW [SEQ ID NO: 6]

QSISSY [SEQ ID NO: 7]

AASS [SEQ ID NO: 8]

QQSYSTPLTF [SEQ ID NO: 9]

QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTI
SVDTSKNQFSLKLSSVTAADTAVYYCAREGKNGAFDIWGQGTMVTVSS [SEQ ID NO: 10]

RHQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKRT [SEQ ID NO: 11]

CAGGTTCAGCTTCAGGAGAGTGGCCCAGGCCTGGTGAAGCCAAGTGAGACTCTCAGCTTGACTTGCACAGT
TTCTGGAGGCAGTGTCTCCTCAGGCAGCTATTATTGGTCCTGGATTCGGCAGCCCCCTGGGAAAGGCCTGG
AGTGGATTGGGTACATATATTACAGTGGCAGCACAAATTACAATCCATCCCTGAAGTCTCGAGTAACTATC
AGTGTGGACACAAGCAAGAATCAGTTTTCACTCAAACTGTCTTCTGTGACTGCTGCTGACACTGCTGTTTA
TTATTGTGCCAGGGAGGGGAAAAATGGGGCATTTGATATTTGGGGTCAGGGCACAATGGTGACAGTCAGCT
CT [SEQ ID NO: 12]

CGCCATCAGATGACTCAGTCCCCCTCCAGTCTTTCTGCCTCAGTTGGGGATAGAGTGACCATCACATGCAG
AGCAAGTCAGAGCATATCATCCTATCTGAACTGGTACCAGCAGAAGCCAGGGAAAGCCCCCAAATTGCTGA
TTTATGCAGCCTCAAGTCTCCAGAGTGGGGTGCCAAGCAGGTTCTCAGGCAGTGGCAGTGGGACAGATTTC
ACATTGACAATCAGCTCCCTCCAACCTGAAGATTTTGCCACCTACTATTGCCAGCAATCCTACAGCACGCC
CCTGACTTTTGGAGGTGGCACAAAGGTAGAGATCAAGAGGACT [SEQ ID NO: 13]

QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTI
SVDTSKNQFSLKLSSVTAADTAVYYCAREGKNGAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSRHQMTQSP
SSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ
PEDFATYYCQQSYSTPLTFGGGTKVEIKRT [SEQ ID NO: 14]

CAGGTTCAGCTTCAGGAGAGTGGCCCAGGCCTGGTGAAGCCAAGTGAGACTCTCAGCTTGACTTGCACAGT
TTCTGGAGGCAGTGTCTCCTCAGGCAGCTATTATTGGTCCTGGATTCGGCAGCCCCCTGGGAAAGGCCTGG
AGTGGATTGGGTACATATATTACAGTGGCAGCACAAATTACAATCCATCCCTGAAGTCTCGAGTAACTATC
AGTGTGGACACAAGCAAGAATCAGTTTTCACTCAAACTGTCTTCTGTGACTGCTGCTGACACTGCTGTTTA
TTATTGTGCCAGGGAGGGGAAAAATGGGGCATTTGATATTTGGGGTCAGGGCACAATGGTGACAGTCAGCT
CTGGAGGTGGAGGCTCAGGAGGAGGAGGCAGTGGAGGTGGTGGGTCACGCCATCAGATGACTCAGTCCCCC
TCCAGTCTTTCTGCCTCAGTTGGGGATAGAGTGACCATCACATGCAGAGCAAGTCAGAGCATATCATCCTA
TCTGAACTGGTACCAGCAGAAGCCAGGGAAAGCCCCCAAATTGCTGATTTATGCAGCCTCAAGTCTCCAGA
GTGGGGTGCCAAGCAGGTTCTCAGGCAGTGGCAGTGGGACAGATTTCACATTGACAATCAGCTCCCTCCAA
CCTGAAGATTTTGCCACCTACTATTGCCAGCAATCCTACAGCACGCCCCTGACTTTTGGAGGTGGCACAAA
GGTAGAGATCAAGAGGACT [SEQ ID NO: 15]

As used herein, the term "a conservative sequence modification" refers to an amino acid modification that does not significantly affect or alter the binding characteristics of the presently disclosed mesothelin-targeted CAR (*e.g.*, the extracellular antigen-binding domain of the CAR) comprising the amino acid sequence. Conservative modifications can include amino acid substitutions, additions and deletions. Modifications can be introduced into the extracellular antigen-binding domain of the presently disclosed CAR by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Amino acids can be classified into groups

according to their physicochemical properties such as charge and polarity. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid within the same group. For example, amino acids can be classified by charge: positively-charged amino acids include lysine, arginine, histidine, negatively-charged amino acids include aspartic acid, glutamic acid, neutral charge amino acids include alanine, asparagine, cysteine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In addition, amino acids can be classified by polarity: polar amino acids include arginine (basic polar), asparagine, aspartic acid (acidic polar), glutamic acid (acidic polar), glutamine, histidine (basic polar), lysine (basic polar), serine, threonine, and tyrosine; non-polar amino acids include alanine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and valine. Thus, one or more amino acid residues within a CDR region can be replaced with other amino acid residues from the same group and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein. In certain embodiments, no more than one, no more than two, no more than three, no more than four, no more than five residues within a specified sequence or a CDR region are altered.

The V_H and/or V_L amino acid sequences having at least about 80%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% (*e.g.*, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%) homology or identity to a specific sequence (*e.g.*, SEQ ID NO: 10 or SEQ ID NO: 11) may contain substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the specified sequence(s), but retain the ability to bind to a target antigen (e.g., mesothelin). In certain embodiments, a total of 1 to 10 amino acids are substituted, inserted and/or deleted in a specific sequence (*e.g.*, SEQ ID NO: 10 or SEQ ID NO: 11). In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (*e.g.*, in the FRs) of the extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 10 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 11, including post-translational modifications of these sequences (SEQ ID NO: 10 and SEQ ID NO: 11).

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

The percent homology between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent homology between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the amino acids sequences of the presently disclosed subject matter can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the specified sequences (*e.g.*, heavy and light chain variable region sequences of scFv m903, m904, m905, m906, and m900) disclosed herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

5.4.3.2. Transmembrane Domain of a CAR

In certain non-limiting embodiments, the transmembrane domain of the CAR comprises a hydrophobic alpha helix that spans at least a portion of the membrane. Different transmembrane domains result in different receptor stability. After antigen recognition, receptors cluster and a signal are transmitted to the cell. In accordance with

the presently disclosed subject matter, the transmembrane domain of the CAR can comprise a native or modified transmembrane domain of CD8, CD28, CD3ζ, CD4, 4-1BB, OX40, ICOS, CD84, CD166, CD8a, CD8b, ICAM-1, CTLA-4, CD27, CD40, NKGD2, a synthetic peptide (not based on a protein associated with the immune response), or a combination thereof.

In certain embodiments, the transmembrane domain of the CAR comprises a CD8 polypeptide (e.g., a transmembrane domain of CD8).

In certain embodiments, the CD8 polypeptide comprises or consists of an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to the sequence having a NCBI Reference No: NP_001139345.1 (SEQ ID NO: 19) (homology herein may be determined using standard software such as BLAST or FASTA) as provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain embodiments, the CD8 polypeptide comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 19, which is at least 20, or at least 30, or at least 40, or at least 50, and up to 235 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD8 polypeptide comprises or consists of an amino acid sequence of amino acids 1 to 235, 1 to 50, 50 to 100, 100 to 150, 150 to 200, 137 to 209 or 200 to 235 of SEQ ID NO: 19. In certain embodiments, the transmembrane domain of the CAR comprises a CD8 polypeptide comprising or having amino acids 137 to 209 of SEQ ID NO: 19.

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLL
YLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPAKPTTTPA
PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRR
VCKCPRPVVKSGDKPSLSARYV [SEQ ID NO: 19]

In certain embodiments, the CD8 polypeptide comprises or consists of an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to the sequence having a NCBI Reference No: AAA92533.1 (SEQ ID NO: 20) (homology herein may be determined using standard software such as BLAST or FASTA) as provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain embodiments, the CD8 polypeptide comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 20, which is at least about 20, or at least about 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 100, or at least about 200, and up to 247 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD8 polypeptide comprises or consists of an amino acid sequence of amino acids 1 to 247, 1 to 50, 50 to 100, 100 to 150, 150 to 200, 151 to 219, or 200 to 247 of SEQ ID NO: 20. In certain embodiments, the transmembrane domain of the CAR comprises a CD8 polypeptide comprising or having amino acids 151 to 219 of SEQ ID NO: 20.

```
1   MASPLTRFLS LNLLLMGESI ILGSGEAKPQ APELRIFPKK MDAELGQKVD LVCEVLGSVS
61  QGCSWLFQNS SSKLPQPTFV VYMASSHNKI TWDEKLNSSK LFSAVRDTNN KYVLTLNKFS
121 KENEGYYFCS VISNSVMYFS SVVPVLQKVN STTTKPVLRT PSPVHPTGTS QPQRPEDCRP
181 RGSVKGTGLD FACDIYIWAP LAGICVAPLL SLIITLICYH RSRKRVCKCP RPLVRQEGKP
241 RPSEKIV [SEQ ID NO: 20]
```

In certain embodiments, the transmembrane domain of the CAR comprises a CD8 polypeptide comprising or having the amino acid sequence set forth in SEQ ID NO: 21, which is provided below:

```
STTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVALLLSLIITLICY
[SEQ ID NO: 21]
```

In accordance with the presently disclosed subject matter, a "CD8 nucleic acid molecule" refers to a polynucleotide encoding a CD8 polypeptide.

In certain embodiments, an exemplary CD8 nucleic acid molecule encoding the CD8 polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 21 is set forth in SEQ ID NO: 22, which is provided below.

TCTACTACTACCAAGCCAGTGCTGCGAACTCCCTCACCTGTGCACCCTACCGGGACATCTCAGCCCCAGAG ACCAGAAGATTGTCGGCCCCGTGGCTCAGTGAAGGGGACCGGATTGGACTTCGCCTGTGATATTTACATCT GGGCACCCTTGGCCGGAATCTGCGTGGCCCTTCTGCTGTCCTTGATCATCACTCTCATCTGCTAC [SEQ ID NO: 22]

In certain embodiments, the transmembrane domain of a presently disclosed CAR comprises a CD28 polypeptide (e.g., a transmembrane domain of CD28).

The CD28 polypeptide can consist of an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous or identical to the sequence having a NCBI Reference No: P10747 or NP_006130 (SEQ ID No: 2), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 23 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 220 amino acids in length. In non-limiting various embodiments, the CD28 polypeptide comprises or consists of an amino acid

sequence of amino acids 1 to 220, 1 to 50, 50 to 100, 100 to 150, 150 to 200, 153 to 179. or 200 to 220 of SEQ ID NO: 23. In certain embodiments, the transmembrane domain of the CAR comprises a CD28 polypeptide comprising or consisting of amino acids 153 to 179 of SEQ ID NO: 23. SEQ ID NO: 23 is provided below:

```
1   MLRLLLALNL FPSIQVTGNK ILVKQSPMLV AYDNAVNLSC KYSYNLFSRE FRASLHKGLD
61  SAVEVCVVYG NYSQQLQVYS KTGFNCDGKL GNESVTFYLQ NLYVNQTDIY FCKIEVMYPP
121 PYLDNEKSNG TIIHVKGKHL CPSPLFPGPS KPFWVLVVVG GVLACYSLLV TVAFIIFWVR
181 SKRSRLLHSD YMNMTPRRPG PTRKHYQPYA PPRDFAAYRS [SEQ ID NO: 23]
```

In accordance with the presently disclosed subject matter, a "CD28 nucleic acid molecule" refers to a polynucleotide encoding a CD28 polypeptide.

In certain embodiments, an exemplarCD28 nucleic acid molecule encoding the CD28 polypeptide consisting of amino acids 153 to 179 of SEQ ID NO: 23 is set forth in SEQ ID NO: 24, which is provided below.

```
ttttgggtgctggtggtggttggtggagtcctggcttgctatagcttgctagtaacagtggcctttattat
tttctgggtg [SEQ ID NO: 24]
```

In certain embodiments, the transmembrane domain of the CAR comprises a CD28 polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 25. SEQ ID NO: 25 is provided below:

```
FWVLVVVGGV LACYSLLVTV AFIIFWV [SEQ ID NO: 25]
```

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 25 is set forth in SEQ ID NO: 26, which is provided below.

```
TTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTAT
TTTCTGGGTG [SEQ ID NO: 26]
```

In certain non-limiting embodiments, a CAR further comprises a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region can be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition.

In certain non-limiting embodiments, the hinge/spacer region of the CAR comprises a native or modified hinge region of CD8, CD28, CD3ζ, CD40, 4-1BB, OX40, CD84, CD166, CD8a, CD8b, ICOS, ICAM-1, CTLA-4, CD27, CD40, NKGD2, a synthetic polypeptide (not based on a protein associated with the immune response), or a combination thereof. The hinge/spacer region can be the hinge region from IgG1, or the CH_2CH_3 region of immunoglobulin and portions of CD3, a portion of a CD28 polypeptide (e.g., a portion of SEQ ID NO: 23), a portion of a CD8 polypeptide (e.g., a portion of SEQ ID NO: 19, or a portion of SEQ ID NO: 20), a variation of any of the foregoing which is at least about 80%, at least about 85%, at least about 90%, at least

about 95%, or at least about 100% homologous or identical thereto, or a synthetic spacer sequence.

5.4.3.3. Intracellular Signaling Domain of a CAR

In certain non-limiting embodiments, the CAR comprises an intracellular signaling domain. In certain non-limiting embodiments, the intracellular signaling domain of the CAR comprises a CD3ζ polypeptide. CD3ζ can activate or stimulate a cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell). Wild type ("native") CD3ζ comprises three functional immunoreceptor tyrosine-based activation motifs (ITAMs), three functional basic-rich stretch (BRS) regions (BRS1, BRS2 and BRS3). CD3ζ transmits an activation signal to the cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell) after antigen is bound. The intracellular signaling domain of the CD3ζ-chain is the primary transmitter of signals from endogenous TCRs.

In certain embodiments, the intracellular signaling domain of the CAR comprises a native CD3ζ. In certain embodiments, the CD3ζ polypeptide comprises or consists of an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to the sequence having a NCBI Reference No: NP_932170 (SEQ ID NO: 27), or a fragment thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain non-limiting embodiments, the CD3ζ polypeptide comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 27, which is at least 20, or at least 30, or at least 40, or at least 50, and up to 164 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD3ζ polypeptide comprises or consists of an amino acid sequence of amino acids 1 to 164, 1 to 50, 50 to 100, 52 to 164, 100 to 150, or 150 to 164 of SEQ ID NO: 27. In certain embodiments, the intracellular signaling domain of the CAR comprises a CD3ζ polypeptide comprising or having amino acids 52 to 164 of SEQ ID NO: 27. SEQ ID NO: 27 is provided below:

```
1   MKWKALFTAA ILQAQLPITE AQSFGLLDPK LCYLLDGILF IYGVILTALF LRVKFSRSAD
61  APAYQQGQNQ LYNELNLGRR EEYDVLDKRR GRDPEMGGKP QRRKNPQEGL YNELQKDKMA
121 EAYSEIGMKG ERRRGKGHDG LYQGLSTATK DTYDALHMQA LPPR [SEQ ID NO: 27]
```

In certain embodiments, the intracellular signaling domain of the CAR comprises a modified CD3ζ polypeptide. In certain embodiments, the modified CD3ζ polypeptide is one disclosed in International Patent Publication No. WO2019/133969, which is incorporated hereby in its entirety.

In certain embodiments, the modified CD3ζ polypeptide comprises or consists of an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, at least about 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO: 28 or a fragment thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 28 is provided below:

RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLFN ELQKDKMAEA FSEIGMKGER RRGKGHDGLF QGLSTATKDT FDALHMQALP PR [SEQ ID NO: 28]

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 28 is set forth in SEQ ID NO: 29, which is provided below.

agagtgaagttcagcaggagcgcagacgcccccgcgtaccagcagggccagaaccagctctataacgagct caatctaggacgaagagaggagtacgatgttttggacaagagacgtggccgggaccctgagatgggggggaa agccgagaaggaagaaccctcaggaaggcctgtTcaatgaactgcagaaagataagatggcggaggcctTc agtgagattgggatgaaaggcgagcgccggaggggcaaggggcacgatggcctttTccaggggctcagtac agccaccaaggacacctTcgacgcccttcacatgcaggccctgcccccctcgc [SEQ ID NO: 29]

In certain non-limiting embodiments, the intracellular signaling domain of the CAR further comprises at least a co-stimulatory signaling region. In certain embodiments, the co-stimulatory region comprises at least one co-stimulatory molecule or a portion thereof. In certain embodiments, the co-stimulatory signaling region comprises an intracellular domain of at least one co-stimulatory molecule or a portion thereof.

As used herein, a "co-stimulatory molecule" refers to a cell surface molecule other than antigen receptor or its ligand that can provide an efficient response of lymphocytes to an antigen. In certain embodiments, a co-stimulatory molecule can provide optimal lymphocyte activation. Non-limiting examples of co-stimulatory molecules includeCD28, 4-1BB, OX40, ICOS, DAP-10, CD27, CD40, NKGD2, CD2, and combinations thereof. The co-stimulatory molecule can bind to a co-stimulatory ligand, which is a protein expressed on cell surface that upon binding to its receptor produces a co-stimulatory response, *i.e.*, an intracellular response that effects the stimulation provided when an antigen-recognizing receptor (e.g., a chimeric antigen receptor (CAR)) binds to its target antigen. As one example, a 4-1BB ligand (*i.e.*, 4-1BBL) may bind to 4-1BB for providing an intracellular signal that in combination with a CAR signal induces an effector cell function of the CAR^+ T cell.

In certain embodiments, the intracellular signaling domain of the CAR comprises a co-stimulatory signaling region that comprises a CD28 polypeptide, e.g., an intracellular domain of CD28 or a portion thereof. In certain embodiments, the CD28 polypeptide comprises or consists of an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, at least about 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO: 23), or a fragment thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 23 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 220 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD28 polypeptide comprises or consists of an amino acid sequence of amino acids 1 to 220, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, 180 to 220, or 200 to 220 of SEQ ID NO: 23. In certain embodiments, the intracellular signaling domain of the CAR comprises a co-stimulatory signaling region that comprises a CD28 polypeptide comprising or consisting of an amino acid sequence of amino acids 180 to 220 of SEQ ID NO: 23.

An exemplary nucleic acid sequence encoding amino acids 180 to 220 of SEQ ID NO: 23 is set forth in SEQ ID NO: 30, which is provided below.

AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCC CACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC [SEQ ID NO: 30]

In certain embodiments, the CD28 polypeptide comprises or consists of an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, at least about 100% homologous or identical to the sequence having a NCBI Reference No: NP_031668.3 (SEQ ID NO: 31), or a fragment thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or consists of an amino acid sequence that is a consecutive portion of SEQ ID NO: 31 which is at least about 20, or at least about 30, or at least about 40, or at least about 50, and up to 218 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD28 polypeptide comprises or consists of an amino acid

sequence of amino acids 1 to 218, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, 178 to 218, or 200 to 220 of SEQ ID NO: 31. In certain embodiments, the co-stimulatory signaling region of a presently disclosed CAR comprises a CD28 polypeptide that comprises or consists of the amino acids 178 to 218 of SEQ ID NO: 31. SEQ ID NO: 31 is provided below:

1 MTLRLLFLAL NFFSVQVTEN KILVKQSPLL VVDSNEVSLS CRYSYNLLAK EFRASLYKGV
61 NSDVEVCVGN GNFTYQPQFR SNAEFNCDGD FDNETVTFRL WNLHVNHTDI YFCKIEFMYP
121 PPYLDNERSN GTIIHIKEKH LCHTQSSPKL FWALVVVAGV LFCYGLLVTV ALCVIWTNSR
181 RNRLLQSDYM NMTPRRPGLT RKPYQPYAPA RDFAAYRP [SEQ ID NO: 31]

An exemplary nucleotide sequence encoding amino acids 178 to 218 of SEQ ID NO: 31 is set forth in SEQ ID NO: 32, which is provided below.

aat agtagaagga acagactcct tcaaagtgac tacatgaaca tgactccccg gaggcctggg ctcactcgaa agccttacca gccctacgcc cctgccagag actttgcagc gtaccgcccc [SEQ ID NO: 32]

In certain embodiments, the intracellular signaling domain of the CAR comprises a co-stimulatory signaling region that comprises two co-stimulatory molecules or portions thereof (e.g., intracellular domains of the co-stimulatory molecules), e.g., intracellular domains of CD28 and 4-1BB, or intracellular domains of CD28 and OX40.

In certain non-limiting embodiments, the intracellular signaling domain of the CAR does not comprise a co-stimulatory signaling region, *i.e.*, the CAR is a first-generation CAR. For example, the intracellular signaling domain of the CAR does not comprise an intracellular signaling domain of a co-stimulatory molecule, e.g., 4-1BB, CD28, etc. The co-stimulatory signaling domain comprised in the CAR may result in uncontrolled proliferation. The activation of c-Kit comprised in the cells can be controlled. For example, as disclosed in Section 5.2, the c-Kit mutant can be operably linked to a promoter, e.g., an inducible promoter, which can control the activation of c-Kit, e.g., with the control of the inducible promoter, c-Kit is activated only upon the activation of the cells comprising the c-Kit mutant (e.g., T cells or CAR-T cells).

5.4.3.4. Exemplified CARs

In certain embodiments, the CAR is a mesothelin-targeted CAR. In certain embodiments, the CAR comprises (a) an extracellular antigen-binding domain comprising a V_H CDR1 having the amino acid sequence set forth in SEQ ID NO: 4, a V_H CDR2 having the amino acid sequence set forth in SEQ ID NO: 5, a V_H CDR3 having the amino acid sequence set forth in SEQ ID NO: 6, a V_L CDR1 having the amino acid sequence set forth in SEQ ID NO: 7, a V_L CDR2 having the amino acid sequence set

forth in SEQ ID NO: 8, and a V_L CDR3 having the amino acid sequence set forth in SEQ ID NO: 9; (b) a transmembrane domain comprising a transmembrane domain of CD28, and (c) an intracellular signaling domain comprising (i) a CD3ζ polypeptide, and (ii) a co-stimulatory signaling region comprising a CD28 polypeptide (e.g., an intracellular domain of CD28).

In certain embodiments, the CAR is a mesothelin-targeted CAR. In certain embodiments, the CAR comprises (a) an extracellular antigen-binding domain comprising a V_H CDR1 consisting of the amino acid sequence set forth in SEQ ID NO: 4, a V_H CDR2 consisting of the amino acid sequence set forth in SEQ ID NO: 5, a V_H CDR3 consisting of the amino acid sequence set forth in SEQ ID NO: 6, a V_L CDR1 consisting of the amino acid sequence set forth in SEQ ID NO: 7, a V_L CDR2 consisting of the amino acid sequence set forth in SEQ ID NO: 8, and a V_L CDR3 consisting of the amino acid sequence set forth in SEQ ID NO: 9; (b) a transmembrane domain comprising a transmembrane domain of CD8, and (c) an intracellular signaling domain comprising a CD3ζ polypeptide, and does not comprise a co-stimulatory signaling region.

In certain embodiments, a presently disclosed CAR further comprises an inducible promoter, for expressing nucleic acid sequences in human cells. Promoters for use in expressing CAR genes can be a constitutive promoter, such as ubiquitin C (UbiC) promoter.

5.4.4. TCR like Fusion Molecules

In certain embodiments, the antigen-recognizing receptor is a TCR like fusion molecule. Non-limiting examples of TCR fusion molecules include HLA-Independent TCR-based Chimeric Antigen Receptor (also known as "HIT-CAR", e.g., those disclosed in International Patent Application No. PCT/US19/017525, which is incorporated by reference in its entirety), and T cell receptor fusion constructs (TRuCs) (e.g., those disclosed in Baeuerle et al., "Synthetic TRuC receptors engaging the complete T cell receptor for potent anti-tumor response," *Nature Communications* volume 10, Article number: 2087 (2019), which is incorporated by reference in its entirety).

The presently disclosed subject matter provides polypeptide compositions comprising a mesothelin-targeted chimeric antigen receptor (CAR) and a dominant negative form of programmed death 1 (PD-1 DN).

5.5. Dominant Negative Form of Programmed Death 1 (PD-1 DN)

In certain embodiments, the cells of the presently disclosed subject matter further comprise a dominant negative form of programmed death 1 (referred to as "PD-1 DN").

The PD-1 DN can enhance the therapeutic efficacy of an immunoresponsive cell comprising a CAR. In certain embodiments, the PD-1 DN comprises (a) at least a portion of an extracellular domain of programmed death 1 (PD-1) comprising a ligand binding region, and (b) a transmembrane domain.

In certain embodiments, a cell, such as a T cell, is engineered to express a dominant negative form (DN form) of PD-1.

Malignant cells adapt to generate an immunosuppressive microenvironment that protects the cells from immune recognition and elimination (Sharpe et al., Dis. Model Mech. 8:337-350 (2015)). The immunosuppressive microenvironment puts limitations on immunotherapy methods. Details of DN forms of inhibitors of a cell-mediated immune response are disclosed in WO2017/040945 and WO2017/100428, the contents of each of which are incorporated herein in their entireties.

Programmed cell death protein 1 (PD-1) is a negative immune regulator of activated T cells upon engagement with its corresponding ligands, PD-L1 and PD-L2, expressed on endogenous macrophages and dendritic cells. PD-1 is a type I membrane protein of 268 amino acids. PD-1 consists of two ligands, PD-L1 and PD-L2, which are members of the B7 family. The protein's structure comprises an extracellular IgV domain followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif. PD-1 negatively regulates TCR signals. SHP-1 and SHP-2 phosphatases bind to the cytoplasmic tail of PD-1 upon ligand binding. Upregulation of PD-L1 is one mechanism tumor cells use to evade the host immune system. In pre-clinical and clinical trials, PD-1 blockade by antagonistic antibodies induced anti-tumor responses mediated through the host endogenous immune system.

In certain embodiments, a PD-1 polypeptide consists of the amino acid with a GenBank No. NP_005009.2 (SEQ ID NO: 33), or fragments thereof. In certain embodiments, amino acids 1 to 20 of SEQ ID NO: 33 is the signal peptide (or peptide

signal) of PD-1. In certain embodiments, amino acids 21 to 170 of SEQ ID NO: 33 is the extracellular domain of PD-1. In certain embodiments, amino acids 171 to 191 of SEQ ID NO: 33 is the transmembrane domain of PD-1. In certain embodiments, amino acids 192 to 288 of SEQ ID NO: 33 is the intracellular domain of PD-1. SEQ ID NO: 33 is provided below:

MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS
LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP
CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL [SEQ ID NO: 33]

In certain embodiments, the extracellular domain of PD-1 comprises a ligand binding domain (referred to as "extracellular ligand binding domain"). In certain embodiments, the extracellular ligand binding domain of PD-1 is fused to one or more heterologous polypeptide sequences, that is, the PD-1 DN is a chimeric sequence. For example, the extracellular ligand binding domain of PD-1 can be fused at its N-terminus to a signal peptide that is optionally a heterologous signal peptide, including various signal peptides described herein. In addition, the PD-1 DN can comprise a transmembrane domain that is optionally a heterologous transmembrane domain, including any of various transmembrane domains described herein.

In certain embodiments, the PD-1 DN comprises the extracellular domain of a PD-1 polypeptide (e.g., amino acids 21 to 170 of SEQ ID NO: 33) or a ligand binding portion thereof (e.g., amino acids 21 to 165 of SEQ ID NO: 33). A cell expressing such a PD-1 DN may lack the ability or have reduced ability to signal in a PD-1 immune checkpoint pathway. In certain embodiments, the PD-1 DN is a deletion mutant having a deletion of the intracellular domain (e.g., the PD-1 DN lacks amino acids 192 to 288 of SEQ ID NO: 33) or a portion thereof. A PD-1 having a deletion of the intracellular domain may have reduced or inhibited immune checkpoint pathway mediated by PD-1.

In certain embodiments, the PD-1 DN comprises the extracellular ligand binding domain of PD-1. In certain embodiments, the PD-1 DN comprises the extracellular ligand binding domain of a PD-1 polypeptide, and the transmembrane domain of a PD-1 polypeptide. In certain embodiments, the PD-1 DN comprises or consists of amino acids 21 to 165 of SEQ ID NO: 33.

An exemplary nucleotide sequence encoding amino acids 21 to 165 of SEQ ID NO: 33 is set forth in SEQ ID NO: 34, which is provided below.

CCAGGATGGTTCTTAGACTCCCCAGACAGGCCCTGGAACCCCCCCACCTTCTCCCCAGCCCTGCTCGTGGT
GACCGAAGGGGACAACGCCACCTTCACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGT
ACCGCATGAGCCCCAGCAACCAGACGGACAAGCTGGCCGCTTTCCCCGAGGACCGCAGCCAGCCCGGCCAG

GACTGCCGCTTCCGTGTCACACAACTGCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGCG
CAATGACAGCGGCACCTACCTCTGTGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGC
GGGCAGAGCTCAGGGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGCCA
GCCGGCCAG [SEQ ID NO: 34]

In certain embodiments, the PD-1 DN further comprises a signal peptide, e.g., the PD-1 DN comprises the extracellular ligand binding domain of a PD-1 polypeptide, the transmembrane domain of a PD-1 polypeptide, and the signal peptide of a PD-1 polypeptide. In certain embodiments, the signal peptide comprises or consists of amino acids 1-20 of SEQ ID NO: 33. An exemplary nucleotide sequence encoding amino acids 1-20 of SEQ ID NO: 33 is set forth in SEQ ID NO: 35, which is provided below.

ATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTACAACTGGGCTGGCGG [SEQ ID NO: 35]

In certain embodiments, the PD-1 DN comprises or consists of amino acids 1 to 165 of SEQ ID NO: 33.

An exemplary nucleotide sequence encoding amino acids 1-165 of SEQ ID NO: 33 is set forth in SEQ ID NO: 36, which is provided below.

ATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTACAACTGGGCTGGCGGCCAGGATGGTT
CTTAGACTCCCCAGACAGGCCCTGGAACCCCCCCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGG
ACAACGCCACCTTCACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGAGC
CCCAGCAACCAGACGGACAAGCTGGCCGCTTTCCCCGAGGACCGCAGCCAGCCCGGCCAGGACTGCCGCTT
CCGTGTCACACAACTGCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCG
GCACCTACCTCTGTGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTC
AGGGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGCCAGCCGGCCAG
[SEQ ID NO: 36]

In certain embodiments, the PD-1 DN comprises or consists of amino acids 21 to 151 of SEQ ID NO: 33. In certain embodiments, a PD-1 DN comprises or consists of amino acids 1 to 151 of SEQ ID NO: 33. In certain embodiments, a PD-1 DN comprises or consists of amino acids 21 to 151 of SEQ ID NO: 33. In certain embodiments, thePD-1 DN comprises or consists of an amino acid sequence starting at amino acid 21 of SEQ ID NO: 33 through an amino acid between amino acids 151 to 165 of SEQ ID NO: 33.

In certain embodiments, the PD-1 DN further comprises a CD8 polypeptide. In certain embodiments, the PD-1 DN comprises the extracellular domain of PD-1 or a portion thereof (e.g., the extracellular ligand binding domain) fused to the transmembrane domain and/or the hinge domain of CD8. In certain embodiments, the PD-1 DN comprises the transmembrane domain of CD8 (e.g., amino acids 183 to 203 of SEQ ID NO: 19). Such embodiments are representative of a chimeric DN form comprising a transmembrane domain from a different (heterologous) polypeptide. As described above, a PD-1 DN comprising a heterologous domain such as a transmembrane domain can optionally include additional sequence from the heterologous

polypeptide. In certain embodiments, the PD-1 DN comprises an additional sequence from the heterologous polypeptide N-terminal of the transmembrane domain. In certain embodiments, the PD-1 DN comprises the hinge domain of CD8. In certain embodiments, the heterologous sequence comprises an additional N-terminal sequence of a CD8 polypeptide (e.g., amino acids 137 to 182 (or optionally starting at amino acids 138 or 139) of SEQ ID NO: 19). In certain embodiments, the PD-1 DN comprises an additional sequence from the heterologous polypeptide C-terminal of the transmembrane domain of CD8. In certain embodiments, the additional C-terminal sequence is amino acids 204 to 209 of SEQ ID NO: 19.

In certain embodiments, the PD-1 DN comprises the transmembrane domain of a CD8 polypeptide (e.g., amino acids 183 to 203 of SEQ ID NO: 19), a hinge domain of a CD8 polypeptide (e.g., amino acids 137 to 182 of SEQ ID NO: 19), and an additional C-terminal sequence of a CD8 polypeptide (e.g., amino acids 204 to 207 of SEQ ID NO: 19. In certain embodiments, the PD-1 DN comprises a CD8 polypeptide consisting of amino acids 137 to 207 of SEQ ID NO: 19.

An exemplary nucleotide sequence encoding amino acids 137-207 of SEQ ID NO: 19 is set forth in SEQ ID NO: 37, which is provided below:

CCCACCACGACGCCAGCGCCGCGACCACCAACCCCGGCGCCCACGATCGCGTCGCAGCCCCTGTCCCTGCG
CCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCT
ACATCTGGGCGCCCCTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAC
[SEQ ID NO: 37]

In certain embodiments, the PD-1 DN comprises the transmembrane domain of a CD8 polypeptide (e.g., amino acids 183 to 203 of SEQ ID NO: 19), a hinge domain of a CD8 polypeptide (e.g., amino acids 137 to 182 of SEQ ID NO: 19), and an additional C-terminal sequence of a CD8 polypeptide (e.g., amino acids 204 to 209 of SEQ ID NO: 19. In certain embodiments, the PD-1 DN comprises a CD8 polypeptide having amino acids 137 to 209 of SEQ ID NO: 19.

An exemplary nucleotide sequence encoding amino acids 137-209 of SEQ ID NO: 19 is set forth in SEQ ID NO: 38, which is provided below:

CCCACCACGACGCCAGCGCCGCGACCACCAACCCCGGCGCCCACGATCGCGTCGCAGCCCCTGTCCCTGCG
CCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCT
ACATCTGGGCGCCCCTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAC
CACAGG [SEQ ID NO: 38]

In certain embodiments, the PD-1 DN comprises the amino acid sequence set forth in SEQ ID NO: 39, which is provided below.

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNA
TFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQL
PNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAE

VPTAHPSPSPRPAGQAAA*PTTTPAPRPPTPAPTIASQPLSLRPEACRPAA*
*GGAVHTRGLDFACDIYIWAPLAGT*CGVLLLSLVITLYCN [SEQ ID NO: 39]

An exemplary nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 39 is set forth in SEQ ID NO: 40, which is provided below:

ATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTACAACTGGGCTGGCGGCCAGGATGGTT
CTTAGACTCCCCAGACAGGCCCTGGAACCCCCCCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGG
ACAACGCCACCTTCACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGAGC
CCCAGCAACCAGACGGACAAGCTGGCCGCTTTCCCCGAGGACCGCAGCCAGCCCGGCCAGGACTGCCGCTT
CCGTGTCACACAACTGCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCG
GCACCTACCTCTGTGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTC
AGGGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGCCAGCCGGCCAGGC
GGCCGCACCCACCACGACGCCAGCGCCGCGACCACCAACCCCGGCGCCCACGATCGCGTCGCAGCCCCTGT
CCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGT
GATATCTACATCTGGGCGCCCCTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTA
CTGCAAC [SEQ ID NO: 40]

In certain embodiments, the PD-1 DN comprises the amino acid sequence set forth in SEQ ID NO: 41, which is provided below.

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNA
TFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQL
PNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAE
VPTAHPSPSPRPAGQAAA*PTTTPAPRPPTPAPTIASQPLSLRPEACRPAA*
*GGAVHTRGLDFACDIYIWAPLAGT*CGVLLLSLVITLYCNHR [SEQ ID NO: 41]

An exemplary nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 41 is set forth in SEQ ID NO: 42, which is provided below:

ATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTACAACTGGGCTGGCGGCCAGGATGGTT
CTTAGACTCCCCAGACAGGCCCTGGAACCCCCCCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGG
ACAACGCCACCTTCACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGAGC
CCCAGCAACCAGACGGACAAGCTGGCCGCTTTCCCCGAGGACCGCAGCCAGCCCGGCCAGGACTGCCGCTT
CCGTGTCACACAACTGCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCG
GCACCTACCTCTGTGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTC
AGGGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGCCAGCCGGCCAGGC
GGCCGCACCCACCACGACGCCAGCGCCGCGACCACCAACCCCGGCGCCCACGATCGCGTCGCAGCCCCTGT
CCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGT
GATATCTACATCTGGGCGCCCCTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTA
CTGCAACCACAGG [SEQ ID NO: 42]

In certain non-limiting embodiments, the transmembrane domain of the PD-1 DN comprises a hydrophobic alpha helix that spans at least a portion of the membrane. Different transmembrane domains result in different receptor stability. In accordance with the presently disclosed subject matter, the transmembrane domain of the PD-1 DN can comprise a native or modified transmembrane domain of any polypeptide disclose herein, e.g., any transmembrane domain that can be comprised in a chimeric antigen receptor. In certain embodiments, the transmembrane domain is a CD8 polypeptide, a CD28 polypeptide, a CD3ζ polypeptide, a CD40 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD84 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1 polypeptide, a CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, a synthetic

polypeptide (not based on a protein associated with the immune response), or a combination thereof. In certain embodiments, the transmembrane domain is a CD8 polypeptide. Detail of these transmembrane domains are described in Section 5.4.

5.6. Compositions and Vectors

The presently disclosed subject matter provides compositions comprising (a) a c-Kit mutant disclosed herein (e.g., disclosed in Section 5.2) and an antigen-recognizing receptor disclosed herein (e.g., disclosed in Section 5.4). Also provided are cells comprising such compositions.

In certain embodiments, the c-Kit mutant is operably linked to a first promoter. In certain embodiments, the antigen-recognizing receptor is operably linked to a second promoter.

Furthermore, the present discloses subject matter provides nuclei acid compositions comprising a first polynucleotide encoding a c-Kit mutant disclosed herein (e.g., disclosed in Section 5.2) and a second polynucleotide encoding an antigen-recognizing receptor disclosed herein (e.g., disclosed in Section 5.4). Also provided are cells comprising such nucleic acid compositions.

In certain embodiments, the nucleic acid composition further comprises a first promoter that is operably linked to the c-Kit mutant. In certain embodiments, the nucleic acid composition further comprises a second promoter that is operably linked to the antigen-recognizing receptor.

In certain embodiments, one or both of the first and second promoters are endogenous or exogenous. In certain embodiments, the exogenous promoter is selected from an elongation factor (EF)-1 promoter, a CMV promoter, a SV40 promoter, a PGK promoter, and a metallothionein promoter. In certain embodiments, one or both of the first and second promoters are inducible promoters. In certain embodiment, the inducible promoter is selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter.

The compositions and nucleic acid compositions can be administered to subjects or and/delivered into cells by art-known methods or as described herein. Genetic modification of a cell (*e.g.*, a T cell or a NK cell) can be accomplished by transducing a substantially homogeneous cell composition with a recombinant DNA construct. In certain embodiments, a retroviral vector (e.g., gamma-retroviral vector or lentiviral vector) is employed for the introduction of the DNA construct into the cell. For example, a polynucleotide encoding an antigen-recognizing receptor can be cloned into a retroviral

vector and expression can be driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest. Non-viral vectors may be used as well.

For initial genetic modification of a cell to include an antigen-recognizing receptor (*e.g.*, a CAR or a TCR), a retroviral vector is generally employed for transduction, however any other suitable viral vector or non-viral delivery system can be used. The antigen-recognizing receptor and the c-Kit mutant can be constructed in a single, multicistronic expression cassette, in multiple expression cassettes of a single vector, or in multiple vectors. Examples of elements that create polycistronic expression cassette include, but is not limited to, various viral and non-viral Internal Ribosome Entry Sites (IRES, *e.g.*, FGF-1 IRES, FGF-2 IRES, VEGF IRES, IGF-II IRES, NF-κB IRES, RUNX1 IRES, p53 IRES, hepatitis A IRES, hepatitis C IRES, pestivirus IRES, aphthovirus IRES, picornavirus IRES, poliovirus IRES and encephalomyocarditis virus IRES) and cleavable linkers (*e.g.,* 2A peptides , *e.g.*, P2A, T2A, E2A and F2A peptides). Combinations of retroviral vector and an appropriate packaging line are also suitable, where the capsid proteins will be functional for infecting human cells. Various amphotropic virus-producing cell lines are known, including, but not limited to, PA12 (Miller *et al.*, (1985) *Mol Cell Biol* (1985);5:431-437); PA317 (Miller., *et al.*, *Mol Cell Biol* (1986); 6:2895-2902); and CRIP (Danos *et al.*, *Proc Natl Acad Sci* USA (1988);85:6460-6464). Non-amphotropic particles are suitable too, *e.g.*, particles pseudotyped with VSVG, RD114 or GALV envelope and any other known in the art.

Possible methods of transduction also include direct co-culture of the cells with producer cells (Bregni *et al.*, *Blood* (1992);80:1418-1422), or culturing with viral supernatant alone or concentrated vector stocks with or without appropriate growth factors and polycations(Xu *et al.*, *Exp Hemat* (1994); 22:223-230; and Hughes *et al. J Clin Invest* (1992); 89:1817).

Other transducing viral vectors can be used to modify an immunoresponsive cell. In certain embodiments, the chosen vector exhibits high efficiency of infection and stable integration and expression (*see, e.g.*, Cayouette et al., Human Gene Therapy 8:423-430, 1997; Kido et al., Current Eye Research 15:833-844, 1996; Bloomer et al., Journal of Virology 71:6641-6649, 1997; Naldini et al., Science 272:263-267, 1996; and Miyoshi et al., Proc. Natl. Acad. Sci. U.S.A. 94:10319, 1997). Other viral vectors that can be used include, for example, adenoviral, lentiviral, and adena-associated viral vectors, vaccinia virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see,

for example, the vectors of Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis et al., BioTechniques 6:608-614, 1988; Tolstoshev et al., Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechnology 7:980-990, 1989; LeGal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S- 83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Pat. No. 5,399,346).

Non-viral approaches can also be employed for genetic modification of an immunoresponsive cell. For example, a nucleic acid molecule can be introduced into an immunoresponsive cell by administering the nucleic acid in the presence of lipofection (Feigner et al., Proc. Natl. Acad. Sci. U.S.A. 84:7413, 1987; Ono et al., Neuroscience Letters 17:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Methods in Enzymology 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu et al., Journal of Biological Chemistry 263:14621, 1988; Wu et al., Journal of Biological Chemistry 264:16985, 1989), or by micro-injection under surgical conditions (Wolff et al., Science 247:1465, 1990). Other non-viral means for gene transfer include transfection *in vitro* using calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be potentially beneficial for delivery of DNA into a cell. Transplantation of normal genes into the affected tissues of a subject can also be accomplished by transferring a normal nucleic acid into a cultivatable cell type *ex vivo* (e.g., an autologous or heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue or are injected systemically. Recombinant receptors can also be derived or obtained using transposases or targeted nucleases (e.g. Zinc finger nucleases, meganucleases, or TALE nucleases, CRISPR). Transient expression may be obtained by RNA electroporation.

Any targeted genome editing methods can also be used to deliver the c-Kit mutant and/or the antigen-recognizing receptor disclosed herein to a cell or a subject. In certain embodiments, a CRISPR system is used to deliver the c-Kit mutant and/or the antigen-recognizing receptor disclosed herein. In certain embodiments, zinc-finger nucleases are used to deliver the c-Kit mutant and/or the antigen-recognizing receptor disclosed herein. In certain embodiments, a TALEN system is used to deliver the c-Kit mutant and/or the antigen-recognizing receptor disclosed herein.

Clustered regularly-interspaced short palindromic repeats (CRISPR) system is a genome editing tool discovered in prokaryotic cells. When utilized for genome editing, the system includes Cas9 (a protein able to modify DNA utilizing crRNA as its guide), CRISPR RNA (crRNA, contains the RNA used by Cas9 to guide it to the correct section of host DNA along with a region that binds to tracrRNA (generally in a hairpin loop form) forming an active complex with Cas9), trans-activating crRNA (tracrRNA, binds to crRNA and forms an active complex with Cas9), and an optional section of DNA repair template (DNA that guides the cellular repair process allowing insertion of a specific DNA sequence). CRISPR/Cas9 often employs a plasmid to transfect the target cells. The crRNA needs to be designed for each application as this is the sequence that Cas9 uses to identify and directly bind to the target DNA in a cell. The repair template carrying CAR expression cassette need also be designed for each application, as it must overlap with the sequences on either side of the cut and code for the insertion sequence. Multiple crRNA's and the tracrRNA can be packaged together to form a single-guide RNA (sgRNA). This sgRNA can be joined together with the Cas9 gene and made into a plasmid in order to be transfected into cells.

A zinc-finger nuclease (ZFN) is an artificial restriction enzyme, which is generated by combining a zinc finger DNA-binding domain with a DNA-cleavage domain. A zinc finger domain can be engineered to target specific DNA sequences which allows a zinc-finger nuclease to target desired sequences within genomes. The DNA-binding domains of individual ZFNs typically contain a plurality of individual zinc finger repeats and can each recognize a plurality of base pairs. The most common method to generate new zinc-finger domain is to combine smaller zinc-finger "modules" of known specificity. The most common cleavage domain in ZFNs is the non-specific cleavage domain from the type IIs restriction endonuclease FokI. Using the endogenous homologous recombination (HR) machinery and a homologous DNA template carrying CAR expression cassette, ZFNs can be used to insert the CAR expression cassette into genome. When the targeted sequence is cleaved by ZFNs, the HR machinery searches for homology between the damaged chromosome and the homologous DNA template, and then copies the sequence of the template between the two broken ends of the chromosome, whereby the homologous DNA template is integrated into the genome.

Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. TALEN system operates on almost the same principle as ZFNs. They are generated by combining a transcription

activator-like effectors DNA-binding domain with a DNA cleavage domain. Transcription activator-like effectors (TALEs) are composed of 33-34 amino acid repeating motifs with two variable positions that have a strong recognition for specific nucleotides. By assembling arrays of these TALEs, the TALE DNA-binding domain can be engineered to bind desired DNA sequence, and thereby guide the nuclease to cut at specific locations in genome. cDNA expression for use in polynucleotide therapy methods can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element or intron (e.g. the elongation factor 1a enhancer/promoter/intron structure). For example, if desired, enhancers known to preferentially direct gene expression in specific cell types can be used to direct the expression of a nucleic acid. The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a genomic clone is used as a therapeutic construct, regulation can be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Methods for delivering the genome editing agents/systems can vary depending on the need. In certain embodiments, the components of a selected genome editing method are delivered as DNA constructs in one or more plasmids. In certain embodiments, the components are delivered via viral vectors. Common delivery methods include but is not limited to, electroporation, microinjection, gene gun, impalefection, hydrostatic pressure, continuous infusion, sonication, magnetofection, adeno-associated viruses, envelope protein pseudotyping of viral vectors, replication-competent vectors cis and trans-acting elements, herpes simplex virus, and chemical vehicles (e.g., oligonucleotides, lipoplexes, polymersomes, polyplexes, dendrimers, inorganic Nanoparticles, and cell-penetrating peptides).

5.7. Polypeptides and Analogs

Also included in the presently disclosed subject matter are a mesothelin, CD28, CD8, CD3ζ, and c-Kit polypeptides or fragments thereof that are modified in ways that enhance their anti-neoplastic activity when expressed in an immunoresponsive cell. The presently disclosed subject matter provides methods for optimizing an amino acid sequence or nucleic acid sequence by producing an alteration in the sequence. Such alterations may include certain mutations, deletions, insertions, or post-translational

modifications. The presently disclosed subject matter further includes analogs of any naturally-occurring polypeptide disclosed herein (including, but not limited to, mesothelin, CD8, CD28, CD3ζ, and c-Kit). Analogs can differ from a naturally-occurring polypeptide disclosed herein by amino acid sequence differences, by post-translational modifications, or by both. Analogs can exhibit at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more homologous to all or part of a naturally-occurring amino, acid sequence of the presently disclosed subject matter. The length of sequence comparison is at least 5, 10, 15 or 20 amino acid residues, *e.g.*, at least 25, 50, or 75 amino acid residues, or more than 100 amino acid residues. Again, in an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, *e.g.*, acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring polypeptides by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, *e.g.*, D-amino acids or non-naturally occurring or synthetic amino acids, *e.g.*, β or γ amino acids.

In addition to full-length polypeptides, the presently disclosed subject matter also provides fragments of any one of the polypeptides or peptide domains disclosed herein. As used herein, the term "a fragment" means at least 5, 10, 13, or 15 amino acids. In certain embodiments, a fragment comprises at least 20 contiguous amino acids, at least 30 contiguous amino acids, or at least 50 contiguous amino acids. In certain embodiments, a fragment comprises at least 60 to 80, 100, 200, 300 or more contiguous amino acids. Fragments can be generated by methods known to those skilled in the art or may result from normal protein processing (*e.g.*, removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Non-protein analogs have a chemical structure designed to mimic the functional activity of a protein disclosed herein (*e.g.*, the c-Kit mutant). Such analogs may exceed the physiological activity of the original polypeptide. Methods of analog design are well known in the art, and synthesis of analogs can be carried out according to such methods by modifying the chemical structures such that the resultant analogs increase the anti-neoplastic activity of the original polypeptide when expressed in an immunoresponsive cell. These chemical modifications include, but are not limited to, substituting alternative R groups and varying the degree of saturation at specific carbon atoms of a reference polypeptide. In certain embodiments, the protein analogs are relatively resistant to *in vivo* degradation, resulting in a more prolonged therapeutic effect upon administration. Assays for measuring functional activity include, but are not limited to, those described in the Examples below.

5.8. Administration

The presently disclosed subject matter also provides compositions comprising the presently disclosed cells.

Compositions comprising the presently disclosed cells can be provided systemically or directly to a subject for inducing and/or enhancing an immune response to an antigen and/or treating and/or preventing a neoplasm, a pathogen infection, or an infectious disease. In certain embodiments, the presently disclosed cells or compositions comprising thereof are directly injected into an organ of interest (*e.g.*, an organ affected by a neoplasia). Alternatively, the presently disclosed cells or compositions comprising thereof are provided indirectly to the organ of interest, for example, by administration into the circulatory system (*e.g.*, the tumor vasculature). Expansion and differentiation agents can be provided prior to, during or after administration of the cells or compositions to increase production of T cells or NK cells *in vitro* or *in vivo*.

The presently disclosed cells can be administered in any physiologically acceptable vehicle, normally intravascularly, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (*e.g.*, thymus). Usually, at least about 1×10^5 cells will be administered, eventually reaching about 1×10^{10} or more. The presently disclosed cells can comprise a purified population of cells. Those skilled in the art can readily determine the percentage of the presently disclosed cells in a population using various well-known methods, such as fluorescence activated cell sorting (FACS). Suitable ranges of purity in populations comprising the presently disclosed immunoresponsive

cells are about 50% to about 55%, about 5% to about 60%, and about 65% to about 70%. In certain embodiments, the purity is about 70% to about 75%, about 75% to about 80%, or about 80% to about 85%. In certain embodiments, the purity is about 85% to about 90%, about 90% to about 95%, and about 95% to about 100%. Dosages can be readily adjusted by those skilled in the art (*e.g.*, a decrease in purity may require an increase in dosage). The cells can be introduced by injection, catheter, or the like.

The presently disclosed compositions can be pharmaceutical compositions comprising the presently disclosed cells and a pharmaceutically acceptable carrier. Administration can be autologous or heterologous. For example, cells can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived cells or their progeny (*e.g.*, *in vivo*, *ex vivo* or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a presently disclosed composition (*e.g.*, a pharmaceutical composition comprising presently disclosed cells), it can be formulated in a unit dosage injectable form (solution, suspension, emulsion).

In certain embodiments, the composition further comprises an inhibitor of c-Kit (referred to as "c-Kit inhibitor"). The c-Kit inhibitor can inhibit the activity of c-Kit (e.g., kinase activity). In certain embodiments, the c-Kit inhibit specifically inhibits the c-Kit mutant, e.g., c-Kit D816V. In certain embodiments, the c-Kit inhibit is a multi-tyrosine kinase inhibitor. Non-liming examples of c-Kit inhibitors include Dasatinib, Midostaurin, ponatinib, imatinib.

5.9. Formulations

Compositions comprising the presently disclosed cells can be conveniently provided as sterile liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof.

Sterile injectable solutions can be prepared by incorporating the genetically modified cells in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (*e.g.*, methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the presently disclosed subject matter, however, any vehicle, diluent, or additive used would have to be compatible with the genetically modified cells.

The compositions can be isotonic, *i.e.*, they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride can be particularly for buffers containing sodium ions.

Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. For example, methylcellulose is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The concentration of the thickener can depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, *e.g.*, liquid

dosage form (*e.g.*, whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

The quantity of cells to be administered will vary for the subject being treated. In a one embodiment, between about 10^4 and about 10^{10}, between about 10^4 and about 10^6, between about 10^5 and about 10^9, or between about 10^6 and about 10^8 of the presently disclosed cells are administered to a human subject. More effective cells may be administered in even smaller numbers. In certain embodiments, at least about 1×10^5, about 2×10^5, about 3×10^5, about 4×10^5, or about 5×10^5 of the presently disclosed cells are administered to a human subject. The precise determination of what would be considered an effective dose may be based on factors individual to each subject, including their size, age, sex, weight, and condition of the particular subject. Dosages can be readily ascertained by those skilled in the art from this disclosure and the knowledge in the art.

The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions and to be administered in methods. Typically, any additives (in addition to the active cell(s) and/or agent(s)) are present in an amount of 0.001 to 50% (weight) solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %, about 0.0001 to about 1 wt %, about 0.0001 to about 0.05 wt% or about 0.001 to about 20 wt %, about 0.01 to about 10 wt %, or about 0.05 to about 5 wt %. For any composition to be administered to an animal or human, the followings can be determined: toxicity such as by determining the lethal dose (LD) and LD50 in a suitable animal model *e.g.*, rodent such as mouse; the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

5.10. Methods of Treatment

The cells and compositions comprising thereof of the presently disclosed subject matter can be used for the treatment and/or prevention of a neoplasia, pathogen infection, infectious disease, inflammatory disease, or graft rejection. Such cells can be administered to a subject (e.g., a human subject) in need thereof for the treatment or prevention of a solid tumor (e.g. mesothelioma, lung cancer, pancreatic cancer, ovarian cancer, breast cancer, colon cancer, pleural tumor, glioblastoma, esophageal cancer,

gastric cancer, synovial sarcoma, thymic carcinoma, endometrial carcinoma, stomach cancer, and/or cholangiocarcinoma). In certain embodiments, the cell is a T cell. The T cell can be a $CD4^+$ T cell or a $CD8^+$ T cell. In certain embodiments, the T cell is a $CD4^+$ T cell.

In certain embodiments, a cell of the neoplasm or tumor has a high expression level of mesothelin (referred to as "a high-MSLN expressing cell"). In certain embodiments, a high-MSLN expressing cell is a cell expressing mesothelin at an expression level of about 50 folds or more, about 50 folds or more, about 60 folds or more, about 70 folds or more, about 80 folds or more, about 90 folds or more, about 100 folds or more, about 150 folds or more, about 200 folds or more, about 300 folds or more, about 400 folds or more, or about 500 folds or more as compared to the mesothelin expression level of a normal cell.

In certain embodiments, a cell of the neoplasm or tumor has a low expression level of mesothelin (referred to as "a low-MSLN expressing cell"). In certain embodiments, a low-MSLN expressing cell is a cell expressing mesothelin at an expression level of about 50 folds or less, about 40 folds or less, about 30 folds or less, about 20 folds or less, about 10 folds or less, about 5 folds or less, about 4 folds or less, about 3 folds or less, or about 2 folds or less as compared to the mesothelin expression level of a normal cell.

In certain embodiments, the solid tumor is a lung cancer.

In certain embodiments, the solid tumor is a mesothelioma. In certain embodiments, the mesothelioma cell is a high-MSLN expressing cell. In certain embodiments, the cells used in treating a high-MSLN expressing mesothelioma cell comprise a CAR that does not comprise a co-stimulatory signaling region (e.g., a first-generation CAR).

The presently disclosed subject matter provides methods for inducing and/or increasing an immune response in a subject in need thereof. The presently disclosed cells and compositions comprising thereof can be used in a therapy or medicament. The presently disclosed cells and compositions comprising thereof can be used for treating and/or preventing a neoplasia in a subject. The presently disclosed cells and compositions comprising thereof can be used for prolonging the survival of a subject suffering from a neoplasia. The presently disclosed cells and compositions comprising thereof can also be used for treating and/or preventing a pathogen infection or other infectious disease in a subject, such as an immunocompromised human subject. Such

methods comprise administering the presently disclosed cells or a composition (*e.g.*, a pharmaceutical composition) comprising thereof to achieve the desired effect, be it palliation of an existing condition or prevention of recurrence. For treatment, the amount administered is an amount effective in producing the desired effect. An effective amount can be provided in one or a series of administrations. An effective amount can be provided in a bolus or by continuous perfusion.

An "effective amount" (or, "therapeutically effective amount") is an amount sufficient to affect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In terms of treatment, an effective amount is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the disease, or otherwise reduce the pathological consequences of the disease. The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art. Several factors are typically taken into account when determining an appropriate dosage to achieve an effective amount. These factors include age, sex and weight of the subject, the condition being treated, the severity of the condition and the form and effective concentration of the cells administered.

For adoptive immunotherapy using antigen-specific T cells, cell doses in the range of about 10^6-10^{10} (*e.g.*, about 10^9) are typically infused. Upon administration of the presently disclosed cells into the host and subsequent differentiation, T cells are induced that are specifically directed against the specific antigen. The modified cells can be administered by any method known in the art including, but not limited to, pleural administration, intravenous administration, subcutaneous administration, intranodal administration, intratumoral administration, intrathecal administration, intrapleural administration, intraperitoneal administration, and direct administration to the thymus. In certain embodiments, the immunoresponsive cells and the compositions comprising thereof are pleurally administered to the subject in need. The presently disclosed subject matter provides various methods of using the cells (e.g., T cells) or compositions comprising thereof. For example, the presently disclosed subject matter provides methods of reducing tumor burden in a subject. In certain embodiments, the method of reducing tumor burden comprises administering the presently disclosed cells or a composition comprising thereof to the subject. The presently disclosed cell can reduce the number of tumor cells, reduce tumor size, and/or eradicate the tumor in the subject. The tumor can be a solid tumor. Non-limiting examples of solid tumor include

mesothelioma, lung cancer, pancreatic cancer, ovarian cancer, breast cancer, colon cancer, pleural tumor, glioblastoma, esophageal cancer, gastric cancer, synovial sarcoma, thymic carcinoma, endometrial carcinoma, stomach cancer, and cholangiocarcinoma.

The presently disclosed subject matter also provides methods of increasing or lengthening survival of a subject having a neoplasm. In certain embodiments, the method of increasing or lengthening survival of a subject having neoplasm comprises administering an effective amount of the presently disclosed immunoresponsive cells or a composition comprising thereof to the subject. The method can reduce or eradicate tumor burden in the subject. Additionally, the presently disclosed subject matter provides methods for increasing an immune response in a subject, comprising administering the presently disclosed cell or a composition comprising thereof to the subject. The presently disclosed subject matter further provides methods for treating and/or preventing a neoplasia in a subject, comprising administering the presently disclosed cells or a composition comprising thereof to the subject.

As used herein, the term "neoplasm" refers to a disease characterized by the pathological proliferation of a cell or tissue and its subsequent migration to or invasion of other tissues or organs. Neoplastic growth is typically uncontrolled and progressive, and occurs under conditions that would not elicit, or would cause cessation of, multiplication of normal cells. Neoplasms can affect a variety of cell types, tissues, or organs, including but not limited to an organ selected from the group consisting of bladder, colon, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pleura, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and vagina, or a tissue or cell type thereof. Neoplasms include cancers, such as sarcomas, carcinomas, or plasmacytomas (malignant tumor of the plasma cells). In one embodiment, the neoplasm is a solid tumor. The neoplasm can a primary tumor or primary cancer. In addition, the neoplasm can be in metastatic status.

Cancers whose growth may be inhibited using the immunoresponsive cells of the presently disclosed subject matter comprise cancers typically responsive to immunotherapy. Non-limiting examples of cancers for treatment include mesothelioma, lung cancer (*e.g.* non-small cell lung cancer), pancreatic cancer, ovarian cancer, breast cancer (e.g., metastatic breast cancer, metastatic triple-negative breast cancer), colon cancer, pleural tumor, glioblastoma, esophageal cancer, gastric cancer, synovial sarcoma,

thymic carcinoma, endometrial carcinoma, stomach cancer, cholangiocarcinoma, cervical cancer, and salivary gland cancer. Additionally, the presently disclosed subject matter comprises refractory or recurrent malignancies whose growth may be inhibited using the immunoresponsive cells of the presently disclosed subject matter.

Examples of other neoplasms or cancers that may be treated using the methods of the presently disclosed subject matter include bone cancer, intestinal cancer, liver cancer, skin cancer, cancer of the head or neck, melanoma (cutaneous or intraocular malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), throat cancer, prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), blood cancers (e.g. leukemias, lymphomas, and myelomas), uterine cancer, rectal cancer, cancer of the anal region, bladder cancer, brain cancer, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, include Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, salivary gland cancer, uterine

cancer, testicular cancer, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

The subjects can have an advanced form of disease, in which case the treatment objective can include mitigation or reversal of disease progression, and/or amelioration of side effects. The subjects can have a history of the condition, for which they have already been treated, in which case the therapeutic objective will typically include a decrease or delay in the risk of recurrence.

Suitable human subjects for therapy typically comprise two treatment groups that can be distinguished by clinical criteria. Subjects with "advanced disease" or "high tumor burden" are those who bear a clinically measurable tumor. A clinically measurable tumor is one that can be detected on the basis of tumor mass (*e.g.*, by palpation, CAT scan, sonogram, mammogram or X-ray; positive biochemical or histopathologic markers on their own are insufficient to identify this population). A pharmaceutical composition is administered to these subjects to elicit an anti-tumor response, with the objective of palliating their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical improvement constitutes a benefit. Clinical improvement includes decreased risk or rate of progression or reduction in pathological consequences of the tumor.

A second group of suitable subjects is known in the art as the "adjuvant group." These are individuals who have had a history of a neoplasm, but have been responsive to another mode of therapy. The prior therapy can have included, but is not restricted to, surgical resection, radiotherapy, and traditional chemotherapy. As a result, these individuals have no clinically measurable tumor. However, they are suspected of being at risk for progression of the disease, either near the original tumor site, or by metastases. This group can be further subdivided into high-risk and low-risk individuals. The subdivision is made on the basis of features observed before or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different neoplasia. Features typical of high-risk subgroups are those in which the tumor has invaded neighboring tissues, or who show involvement of lymph nodes.

Another group have a genetic predisposition to neoplasia but have not yet evidenced clinical signs of neoplasia. For instance, women testing positive for a genetic mutation associated with breast cancer, but still of childbearing age, can wish to receive one or more of the immunoresponsive cells described herein in treatment

prophylactically to prevent the occurrence of neoplasia until it is suitable to perform preventive surgery.

As a consequence of surface expression of an antigen-recognizing receptor that binds to a tumor antigen and a c-Kit mutant that enhances the anti-tumor effect of the immunoresponsive cell, adoptively transferred T or NK cells are endowed with augmented and selective cytolytic activity at the tumor site. Furthermore, subsequent to their localization to tumor or viral infection and their proliferation, the T cells turn the tumor or viral infection site into a highly conductive environment for a wide range of immune cells involved in the physiological anti-tumor or antiviral response (tumor infiltrating lymphocytes, NK-, NKT- cells, dendritic cells, and macrophages).

Additionally, the presently disclosed subject matter provides methods for treating and/or preventing a pathogen infection (*e.g.*, viral infection, bacterial infection, fungal infection, parasite infection, or protozoal infection) in a subject, *e.g.*, in an immunocompromised subject. The method can comprise administering an effective amount of the presently disclosed cells or a composition comprising thereof to a subject having a pathogen infection. Exemplary viral infections susceptible to treatment include, but are not limited to, Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus infections.

Further modification can be introduced to the presently disclosed immunoresponsive cells (*e.g.*, T cells) to avert or minimize the risks of immunological complications (known as "malignant T-cell transformation"), *e.g.*, graft versus-host disease (GvHD), or when healthy tissues express the same target antigens as the tumor cells, leading to outcomes similar to GvHD. A potential solution to this problem is engineering a suicide gene into the presently disclosed immunoresponsive cells. Suitable suicide genes include, but are not limited to, Herpes simplex virus thymidine kinase (hsv-tk), inducible Caspase 9 Suicide gene (iCasp-9), and a truncated human epidermal growth factor receptor (EGFRt) polypeptide. In certain embodiments, the suicide gene is an EGFRt polypeptide. The EGFRt polypeptide can enable T cell elimination by administering anti-EGFR monoclonal antibody (*e.g.*, cetuximab). EGFRt can be covalently joined to the upstream of the antigen-recognizing receptor of a presently disclosed CAR. The suicide gene can be included within the vector comprising nucleic acids encoding a presently disclosed CAR. In this way, administration of a prodrug designed to activate the suicide gene (*e.g.*, a prodrug (*e.g.*, AP1903 that can activate iCasp-9) during malignant T-cell transformation (*e.g.*, GVHD) triggers apoptosis in the

suicide gene-activated CAR-expressing T cells. The incorporation of a suicide gene into the a presently disclosed CAR gives an added level of safety with the ability to eliminate the majority of CAR T cells within a very short time period. A presently disclosed immunoresponsive cell (*e.g.*, a T cell) incorporated with a suicide gene can be pre-emptively eliminated at a given timepoint post CAR T cell infusion, or eradicated at the earliest signs of toxicity.

In addition, the presently disclosed subject matter provides methods of preventing and/or treating an inflammatory disease in a subject. In certain embodiments, the method comprises administering the presently disclosed cells or a composition comprising thereof to the subject. In certain embodiments, the cell is an immunoinhibitory cell. In certain embodiments, the immunoinhibitory cell is a regulatory T cell. In one embodiment, the inflammatory disease is pancreatitis. In certain embodiments, the subject is a human. In certain embodiments, the subject is a recipient of an organ transplant, e.g., a recipient of a pancreas transplant.

Furthermore, the presently disclosed subject matter provides methods of preventing graft rejection in a subject who is a recipient of an organ transplant. In certain embodiments, the method comprises administering the presently disclosed cells or a composition comprising thereof to the subject. In certain embodiments, the cell is an immunoinhibitory cell. In certain embodiments, the immunoinhibitory cell is a regulatory T cell. In certain embodiments the subject is a human. In a further embodiment, the subject is a recipient of a pancreas transplant.

In certain embodiments, the method disclosed herein further comprises administering to the subject an inhibitor of c-Kit (e.g., one disclosed in Section 5.8). The c-Kit inhibitor can inhibit the activity of c-Kit (e.g., kinase activity). In certain embodiments, the c-Kit inhibit specifically inhibits the c-Kit mutant, e.g., c-Kit D816V. In certain embodiments, the c-Kit inhibit is a multi-tyrosine kinase inhibitor. Non-liming examples of c-Kit inhibitors include Dasatinib, Midostaurin, ponatinib, imatinib.

5.11. Kits

The presently disclosed subject matter provides kits for inducing and/or enhancing an immune response and/or treating and/or preventing a neoplasia or a pathogen infection, or an immune disorder in a subject. In certain embodiments, the kit comprises the presently disclosed cells or a composition comprising thereof. In certain embodiments, the kit comprises a sterile container; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container

forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. In certain non-limiting embodiments, the kit includes an isolated nucleic acid molecule encoding an antigen-recognizing receptor (*e.g.*, a CAR or a TCR) directed toward an antigen of interest and an isolated nucleic acid molecule encoding a c-Kit mutant in expressible form, which may optionally be comprised in the same or different vectors.

If desired, the cells and/or nucleic acid molecules are provided together with instructions for administering the cells or nucleic acid molecules to a subject having or at risk of developing a neoplasia or pathogen or immune disorder. The instructions generally include information about the use of the composition for the treatment and/or prevention of neoplasia or a pathogen infection. In certain embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of a neoplasia, pathogen infection, or immune disorder or symptoms thereof; precautions; warnings; indications; counter-indications; over-dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

6. EXAMPLES

The practice of the present disclosure employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides disclosed herein, and, as such, may be considered in making and practicing the presently disclosed subject matter. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the presently disclosed cells and compositions, and are not intended to limit the scope of what the inventors regard as their invention.

Example 1 – Generation of Constructs

Two constructs of the presently disclose subject matter were generated. One construct is designated as "M28z-KITv" (also referred to as "M28z-KITm"). The structure of M28z-KITv is shown in Figure 1A. As shown in Figure 1A, M28z-KITv comprises a c-Kit mutant (e.g., c-Kit D816V) and a second generation CAR comprising an anti-MSKN scFv, a transmembrane domain comprising a CD28 polypeptide (e.g., a CD28 transmembrane domain or a portion thereof), an intracellular domain that comprises a CD3ζ and a CD28 polypeptide (e.g., an intracellular domain of CD28). Another construct is designed as "Mz-KITv". The structure of Mz-KITv is shown in Figure 1B. As shown in Figure 1B, Mz-KITv comprises a c-Kit mutant (e.g., c-Kit D816V) and a first generation CAR comprising an anti-MSKN scFv, a transmembrane domain comprising a CD28 polypeptide (e.g., a CD28 transmembrane domain or a portion thereof), an intracellular domain that comprises a CD3ζ.

Example 2 – Transduction, Activation of pKIT signaling, Expansion, and Proliferation of the Constructs

The transduction, activation of pKIT signaling, expansion, and proliferation of M28z-KITv and Mz-KITv were tested. M28z comprises the same CAR construct as M28z-KITv but does not comprise a Kit mutant. The transduction results are shown in Figure 2. As shown in Figure 2, both M28z-KITv and Mz-KITv had good transduction. MFI value for T cells comprising M28z was higher than T cells comprising M28z-KITv (referred to as "M28z-KITv CAR T cells") or T cells comprising Mz-KITv (referred to as "Mz-KITv CAR T cells").

The results of the constructs for the ability to activate pKIT signaling are shown in Figures 3A and 3B. As shown in Figures 3A and 3B, M28z-KITv CAR T cells constitutively exhibited activated pKIT signaling. As shown in Figure 3A, M28z-KITv CAR T cells exhibited pKIT activity without SCF, while M28z CAR T cells did not express KIT protein. As shown in Figure 3B, M28z-KITv CAR T cells exhibited higher p-STAT3 and p-STAT5 activity than M28z-KITwt CAR T cells control.

The CAR T cells expansion results are shown in Figure 4. Figure 4 shows cumulative expansion of CAR T cells during serial co-culture. T-cell were re-stimulated

with A549GM tumor cells (E:T=3:1). As shown in Figure 4, M28z-KITv CAR T cells and M28z CAR T cells exhibited similar expansion level, while Mz-KITv CAR T cells exhibited less expansion. A549GM was non-small cell lung cancer cell line with overexpression of GFP-luciferase and mesothelin.

The proliferation results are shown in Figures 5 and 6. Far red cell trace staining of CAR T cells is measured after 7 days first antigen stimulation (E:T=2:1). The target cell was A549GM. As shown in Figures 5 and 6, M28z-KITm CAR T cells exhibited higher proliferation as compared to M28z CAR T cells.

Thus, the cKIT costimulatory constructs demonstrated successful transduction, and antigen-specific activation, and proliferation.

Example 3 – In Vitro Cytolytic Activity Of the Presently Disclosed Constructs

The *in vitro* cytolytic activity of M28z-KITv CAR T cells and Mz-KITv CAR T cells towards high MSLN expressing cells and low MSLN expressing cells were assessed. The results are shown in Figures 7A-7B, 8A-8B, and 9. As shown in Figures 7A-7B and 8A-8B, M28z-KITv CAR T cells and Mz-KITv CAR T cells killed high MSLN tumor cells faster than M28z CAR T cells and Mz CAR T cells, e.g., at 4 hours M28z-KITv CAR T cells killed more tumor cells than M28z CAR T cells, and Mz-KITv CAR T cells killed more tumor cells than Mz CAR T cells (*see* Figures 7A and 8A), while at 18 hours no significant difference was seen (*see* Figures 7B and 8B).

However, for low MSLN expressing tumor cells, i.e., A549G cells, M28z-KITv CAR T cells and Mz-KITv CAR T cells exhibited increased killing capability. At 18 hours, M28z-KITv CAR T cells and Mz-KITv CAR T cells killed more low MSLN A549G tumor cells than M28z CAR T cells.

Example 4 – PD1 Expression and T Cells Status of the Constructs after Antigen Stimulation

The PD1 expression after antigen stimulation was assessed. After stimulation with A549GM cells for every 4 days (E:T=3:1), FACS were measured. The results for PD1 expression are shown in Figures 10A and 10B. As shown in Figures 10A and 10B, M28z-KITv CAR T cells and Mz-KITv CAR T cells showed less PD1+ expression after antigen stimulation in both $CD4^+$ T cells (*see* Figure 10A) and $CD8^+$ T cells (*see* Figure 10B) as compared to M28z CAR T cells. The results for the T cells status are shown in Figure 11A. As shown in Figure 11A, M28z-KITv CAR T cells and Mz-KITv CAR T cells showed more stem cell–like memory T cells (T_{SCM} cells) after antigen stimulation. T_{SCM} cells have higher potential to kill tumor cells. M28z-KITv CAR T cells and Mz-

KITv CAR T cells secreted higher IFN-γ and TNF-α than M28z and Mz CAR T cells, albeit less IL-2 (Figure 11B). Thus, the cKIT costimulatory constructs demonstrated successful effector-cytokine secretion.

P-ERK signal of CAR T cells after antigen stimulation was also examined. After co-culture with MGM cell (E:T=1:2) for 5 minutes, p-ERK levels of CD4+ and CD8+ CAR T cell were measured by FACS. Both CD4 and CD8 CAR T cells of M28z-KITv and Mz-KITv had stronger p-ERK activity than M28z (Figure 20).

Example 5 – In Vivo Activities of the Presently Disclosed Constructs

Low mesothelin-expressing lung cancer cells (A549G) and high mesothelin-expressing lung cancer cells (A549GM) were used in establishing lung tumors in NSG mice (Figure 14C). Mice with established low MSLN A549G lung tumor were treated with a single dose of 1×10^5 M28z, M28z-KITv and Mz-KITv CAR T cells. *In vivo* BLI was used to monitor tumor burden in NSG mice. The results are shown in Figure 12A-12D. As shown in Figures 12A-12D, while treating mice with tumor burden resulting from cancer cells with low-antigen (mesothelin) expression, M28z-KITv CAR T-cell treated mice showed better and long-lasting tumor regression compared to mice treated with M28z CAR T cells or Mz-KITv CAR T cells or untransduced T cells (UT). The mice survival data are presented as Kaplan-meier survival curves in Figure 14A. As shown in Figure 14A, while treating mice with low-antigen (mesothelin) expressing tumors, best survival was achieved in mice treated with a single dose of M28z-KITv CAR T cells, e.g., mice treated with M28z-KITv CAR T cells showed prolonged survival as compared to mice treated with M28z CAR T cells.

Mice with established high MSLN A549GM lung tumor were treated with a single dose of 1×10^5 M28z, M28z-KITv and Mz-KITv CAR T cells. *In vivo* BLI was used to monitor tumor burden in NSG mice. The results are shown in Figure 13A-13D. As shown in Figures 13A-13D, while treating mice with tumor burden resulting from cancer cells with high-antigen (mesothelin) expression, M28z-KITv CAR T-cell treated mice showed better and long-lasting tumor eradication. In addition, Mz-KITv CAR T-cell treated mice showed long-lasting tumor regression equivalent to mice treated with M28z CAR T cells. Mice treated with any of the mesothelin CAR T cells showed impressive tumor regressions compared to mice treated with untransduced (UT) T cells. The mice survival data are presented as Kaplan-meier survival curves in Figure 14B. As shown in Figure 14B, while treating mice with high-antigen (mesothelin) expressing tumors, best survival was achieved in mice treated with a single dose of either M28z-

KITv or Mz-KITv CAR T cells when compared to mice treated with M28z CAR T cells. In all the 3 groups of mice, median survival was not reached compared to mice treated with untransduced (UT) T cells.

Example 6 – Sensitivity to Tyrosine Kinase Inhibitors of the Constructs

After 7 days stimulation with A549GM cells (E:T=3:1), CAR T were treated with 500nM or 5μM of Dasatinib (Dasa), 500nM or 5μM of Ponatinib (Pona), or 100nM or 1μM of PKC412 for 72 hours, then viable cells were measured by FACS. As shown in Figure 15, M28z-KITv CAR T were more sensitive to tyrosine kinase inhibitors than M28z CAR T cells.

Example 7 - In Vivo Activities of the Presently Disclosed Constructs

Lung tumor was established in NSG mice using A549GM tumor cells, which had high expression level of mesothelin (MSLN), or A549G tumor cells, which had low expression level of mesothelin (MSLN). The mice were then treated with a single dose of 1×10^5 M28z, M28z-KITv or Mz-KITv CAR T cells. Both Mz-KITv and M28z-KITv CAR T cells had higher anti-tumor efficacy against high mesothelin expressing lung cancer cells than M28z CAR T cells (Figures 16A-16C). In low MSLN A549G lung cancer, M28z-KITv CAR T cells had enhanced antitumor activity as compared to M28z CAR T cells, although Mz-KITv CAR T cells had less antitumor activity than M28z CAR T cells (Figures 17A-17C).

MSTO cell were overexpressed with low and high MSLN protein to produce MG-LM and MGM cell respectively (Figure 19A). In pleural mesothelioma tumor model, mesothelioma was established in NSG mice through pleural injection of high MSLN-expressing mesothelioma (MGM) tumor cells or low MSLN-expressing mesothelioma (MG-LM) tumor cells . These mice were then intrapleural administered with a single dose of 5×10^4 P28z, Mz, M28z, M28z-KITv or Mz-KITv CAR T cells. In high MSLN-expressing mesothelioma, Mz-KITv CAR T cells had increased antitumor activity than Mz CAR T cells, although no significant difference between M28z-KITv and M28z CAR T cells was detected (Figures 18A and 18B). CAR T-cell doses were purposefully reduced to mimic low E:T ratios seen in clinic. The reduced cKIT CAR T-cell dose resulted in equivalent anti-tumor efficacy as compared to CD28 CAR T cells. This may be due to PDL1/PD1 pathway related exhaustion at very low E:T ratio. M28z-KITv CAR T cells also had enhanced antitumor activity to low-MSLN expressing mesothelioma (Figure 19B).

CAR T cells obtained from mice were exposed to high mesothelin-expressing mesothelioma cells and were analyzed for PD1 expression. PD1 upregulation was low at high E:T ratios, but similar at low E:T ratios (Figures 21A and 21B).

Example 8: In Vitro Nano String Analysis

After co-cultured with $MSLN^+$ tumor cells for 24 hours, $CD8^+$ M28z and M28z-KITv CAR T cells were collected for nano string analysis of CAR T panel genes. 87 out of 780 detected genes had significant fold changes (Figure 22A). Heatmap of Pathway scores showed enriched gene sets relating to phenotypic and functional T cell features in M28z-KITv CAR T cells (Figure 22B). Upregulated gene pathways in M28z-KITv CAR T cells were listed in Figure 22C. The expression of Type I interferon signaling genes (Figure 23A) and Type II interferon signaling genes (Figure 23B) significantly increased in M28z-KITv CAR T cells.

Embodiments of the presently disclosed subject matter

From the foregoing description, it will be apparent that variations and modifications may be made to the presently disclosed subject matter to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or sub-combination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A cell comprising:
(a) an antigen-recognizing receptor that binds to an antigen, and
(b) a mutant of c-Kit comprising an activating mutation.

2. The cell of claim 1, wherein the c-Kit is human c-Kit.

3. The cell of claim 2, wherein the activating mutation is within the intracellular region of human c-Kit.

4. The cell of claim 3, wherein the intracellular region comprises amino acids 544 to 977 of human c-Kit.

5. The cell of any one of claims 2-4, wherein the activating mutation is within amino acids 816 to 826 of human c-Kit.

6. The cell of any one of claims 2-5, wherein the activating mutation is at amino acid position 816 or amino acid position 822.

7. The cell of any one of claims 2-4, wherein the activating mutation is within amino acids 550 to 570 of human c-Kit.

8. The cell of any one of claims 2-4 and 7, wherein the activating mutation is at amino acid position 560 of human c-Kit.

9. The cell of claim 2-4, wherein the activating mutation is selected from D816V, D816Y, D816H, D816F, N822K, V560G, or a combination thereof.

10. The cell any one of claims 2-4 and 9, wherein the activating mutation comprises or consists of D816V.

11. The cell of any one of claims 2-10, wherein the human c-Kit comprises or consists of the amino acid sequence set forth in SEQ ID NO:1.

12. The cell of any one of claims 1-11, wherein the mutant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2 or a portion thereof.

13. The cell of any one of claims 1-12, wherein the mutant consists of amino acids 543 to 976 of SEQ ID NO: 2.

14. The cell of any one of claims 1-13, wherein the c-Kit mutant is operably linked to an inducible promoter.

15. The cell of claim 14, wherein the inducible promoter is selected from nuclear factor of activated T cells (NFAT), transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter.

16. The cell of any one of claims 1-15, wherein the c-Kit mutant enhances cell persistence of the cell.

17. The cell of any one of claims 1-16, wherein the c-Kit mutant reduces apoptosis or anergy of the cell.

18. The cell of any one of claims 1-17, wherein said antigen-recognizing receptor is exogenous or endogenous.

19. The cell of any one of claims 1-18, wherein said antigen-recognizing receptor is recombinantly expressed.

20. The cell of any one of claim 1-19, wherein the antigen-recognizing receptor is expressed from a vector.

21. The cell of any one of claims 1-20, wherein the c-Kit mutant is expressed from a vector.

22. The cell of any one of claims 1-21, wherein the cell is an immunoresponsive cell.

23. The cell of any one of claims 1-22, wherein the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage.

24. The cell of claim 23, wherein the cell of the lymphoid lineage is selected from T cells, B cells, Natural Killer (NK) cells, dendritic cells.

25. The cell of any one of claims 1-24, wherein the cell is a T cell.

26. The cell of claim 24 or 25, wherein the T cell is a cytotoxic T lymphocyte (CTL), a γδ T cell, a tumor-infiltrating lymphocyte (TIL), a regulatory T cell, or a Natural Killer T (NKT) cell.

27. The cell of any one of claims 1-26, wherein the antigen is a tumor antigen or a pathogen antigen.

28. The cell of any one of claims 1-27, wherein said antigen is a tumor antigen.

29. The cell of claim 28, wherein the tumor antigen is selected from the group consisting of mesothelin, CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-B2, erb-B3, Erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, ERBB2, MAGEA3, CT83 (also known as KK-LC-1), p53, MART1,GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, HPV E6 oncoprotein, HPV E7 oncoprotein, and ERBB.

30. The cell of claim 29, wherein the antigen is mesothelin.

31. The cell of any one of claims 1-30, wherein said antigen-recognizing receptor is selected from a T cell receptor (TCR), a chimeric antigen receptor (CAR), and a TCR like fusion molecule.

32. The cell of any one of claim 1-31, wherein the antigen-recognizing receptor is a CAR.

33. The cell of claim 32, wherein the CAR comprises an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling region.

34. The cell of claim 33, wherein the intracellular signaling domain of the CAR further comprises at least one co-stimulatory signaling region.

35. The cell of claim 34, wherein the at least one co-stimulatory signaling region comprises a CD28 polypeptide.

36. The cell of claim 33, wherein the CAR does not comprise a co-stimulatory signaling region.

37. A method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into a cell (a) a first nucleic acid sequence encoding an antigen-recognizing receptor that binds to an antigen; and (b) a second nucleic sequence encoding a c-Kit mutant comprising an activating mutation.

38. The method of claim 37, wherein the first nucleic acid sequence is operably linked to a first promoter.

39. The method of claim 37 or 38, wherein the second nucleic acid sequence is operably linked to a second promoter.

40. The method of any one of claims 37-39, wherein one or both of the first and the second nucleic acid sequences are comprised in a vector.

41. The method of claim 341, wherein the vector is a retroviral vector.

42. A composition comprising: a) a mutant of human c-Kit comprising an activating mutation; and b) an antigen-recognizing receptor that binds to an antigen.

43. The composition of claim 42, wherein the -Kit mutant is operably linked to a first promoter.

44. The composition of claim 42 or 43, wherein the antigen-recognizing receptor is operably linked to a second promoter.

45. The composition of claim 43 or 44, wherein one or both of the first and second promoters are inducible promoters.

46. The composition of claim 45, wherein the inducible promoter is selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter.

47. A nucleic acid composition comprising (a) a first polynucleotide encoding an antigen-recognizing receptor and (b) a second polynucleotide encoding a mutant of human c-Kit comprising an activating mutation.

48. The nucleic acid composition of claim 47, further comprising a first promoter that is operably linked to the c-Kit mutant.

49. The nucleic acid composition of claim 47 or 48, further comprising a second promoter that is operably linked to the antigen-recognizing receptor.

50. The nucleic acid composition of claim 48 or 49, wherein one or both of the first and second promoters are inducible promoters.

51. The nucleic acid composition of claim 50, wherein the inducible promoter is selected from a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, and an IL-2 promoter.

52. The nucleic acid composition of any one of claims 47-51, wherein one or both of the first and second polynucleotides are comprised in a vector.

53. The nucleic acid composition of claim 52, wherein the vector is a retroviral vector.

54. A vector comprising the nucleic acid composition of any one of claims 47-53.

55. A cell comprising the composition of any one of claims 42-46, or a nucleic acid composition of any one of claims 47-53, or a vector of claim 54.

56. A pharmaceutical composition comprising a cell of any one of claims 1-36 and 55, and a pharmaceutically acceptable excipient.

57. The pharmaceutical composition of claim 56, further comprising an inhibitor of c-Kit.

58. The pharmaceutical composition of claim 57, wherein the inhibitor of c-Kit is selected from dasatinib (BMS-354825), midostaurin (PKC412), ponatinib, imatinib, and a combination thereof.

59. The pharmaceutical composition of any one of claims 56-58, which is for treating and/or preventing a neoplasia, a pathogen infection, or an infection disease.

60. A method of reducing tumor burden in a subject, the method comprising administering to the subject a cell of any one of claims 1-36 and 55 or a pharmaceutical composition of any one of claims 56-59.

61. The method of claim 60, wherein the method reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.

62. A method of treating and/or preventing a neoplasm, the method comprising administering to the subject a cell of any one of claims 1-36 and 55 or a pharmaceutical composition of any one of claims 56-59.

63. A method of lengthening survival of a subject having a neoplasm, the method comprising administering to the subject a cell of any one of claims 1-36 and 55 or a pharmaceutical composition of any one of claims 56-59.

64. The method of any one of claims 60-63, wherein the tumor or neoplasm is a solid tumor.

65. The method of claim 64, wherein the solid tumor is selected from the group consisting of mesothelioma, lung cancer, pancreatic cancer, ovarian cancer, breast cancer, colon cancer, pleural tumor, glioblastoma, esophageal cancer, gastric cancer, synovial sarcoma, thymic carcinoma, endometrial carcinoma, stomach cancer, cholangiocarcinoma, and a combination thereof.

66. The method of claim 64 or 65, wherein the solid tumor is mesothelioma.

67. The method of claim 64 or 65, wherein the solid tumor is lung cancer.

68. The method of any one of claims 50-67, further comprising administering an inhibitor of c-Kit.

69. The method of claim 68, wherein the inhibitor of c-Kit is selected from dasatinib (BMS-354825), midostaurin (PKC412), ponatinib, imatinib, and a combination thereof.

70. A kit comprising a cell of any one of claims 1-36 and 55, a composition of any one of claims 42-46, a nucleic acid composition of any one of claims 47-53, or a vector of claim 54.

71. The kit of claim 70, wherein the kit further comprises written instructions for treating and/or preventing a neoplasia, a pathogen infection, or an infectious disease.

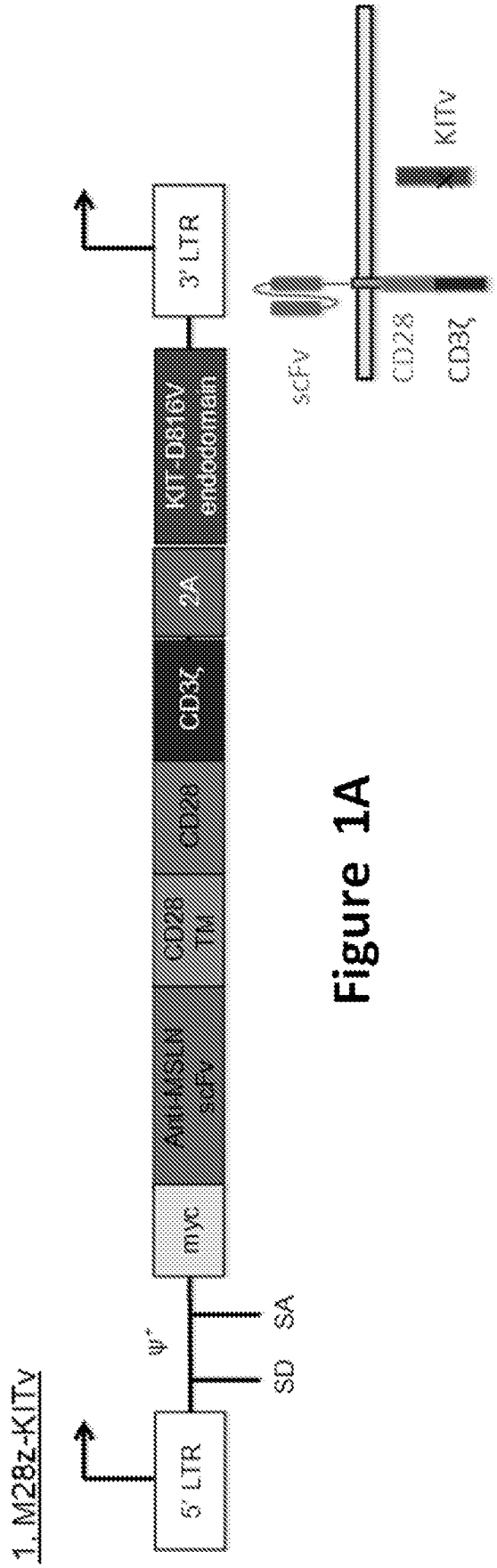


Figure 1A

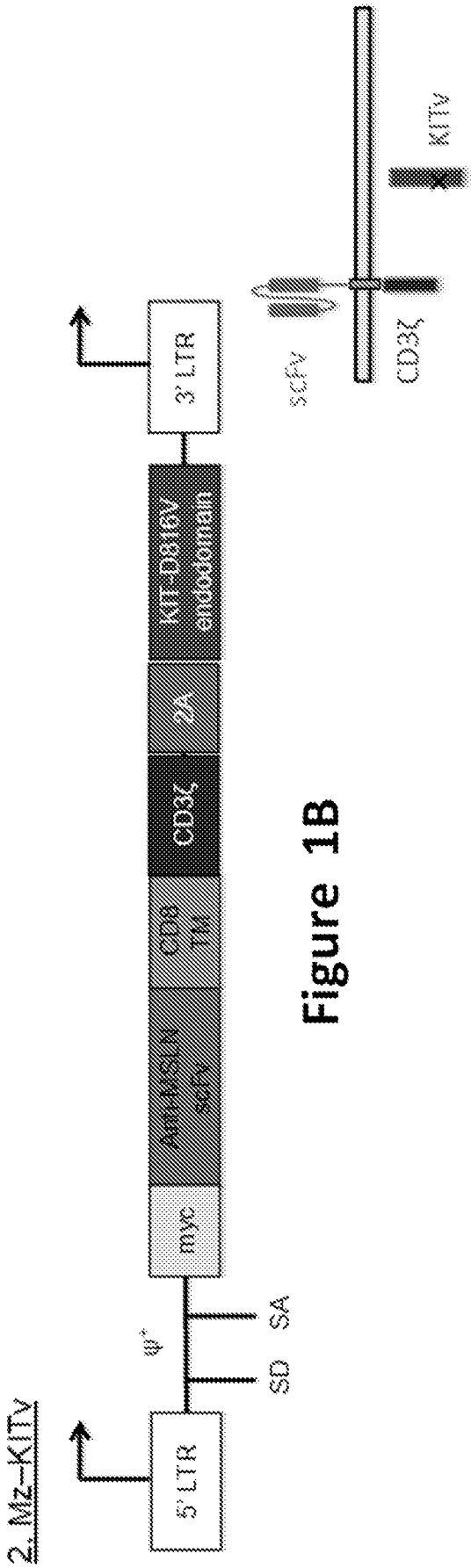


Figure 1B

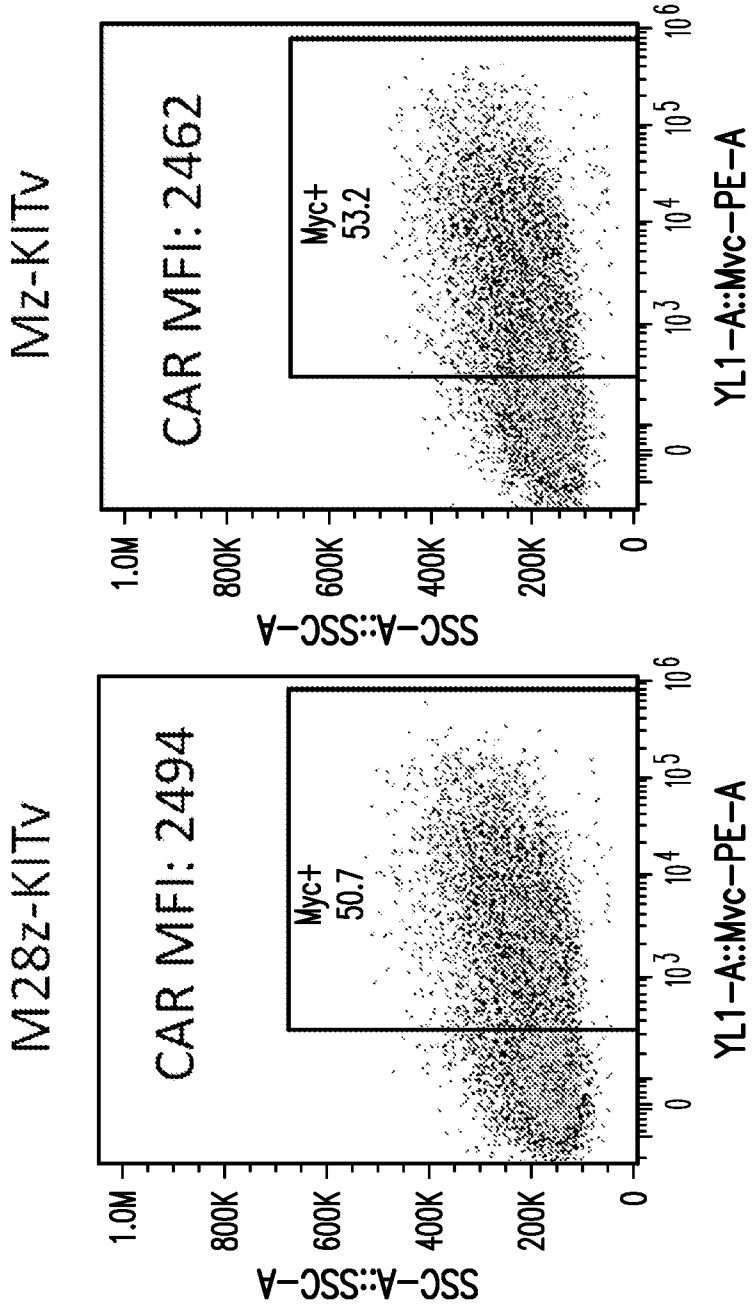

M28z
CAR MFI: 4200
Myc+
51.9
M28z-KITv
CAR MFI: 2494
Myc+
50.7
Mz-KITv
CAR MFI: 2462
Myc+
53.2
SSC-A::SSC-A
YL1-A::Myc-PE-A
1.0M
800K
600K
400K
200K
0
10^3
10^4
10^5
10^6

Figure 2

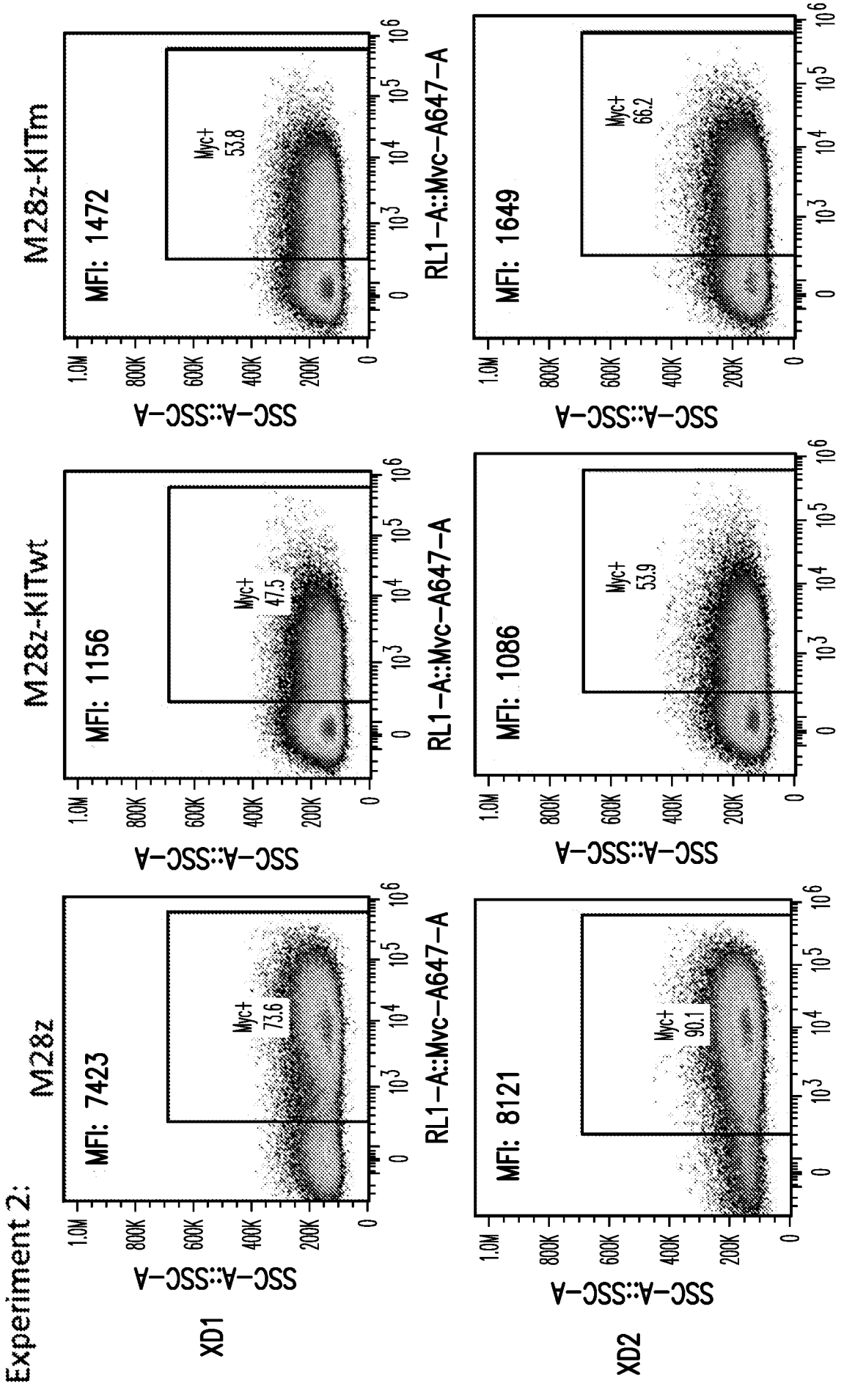


Figure 2 continued

MFI of M28z much higher than other CAR-T

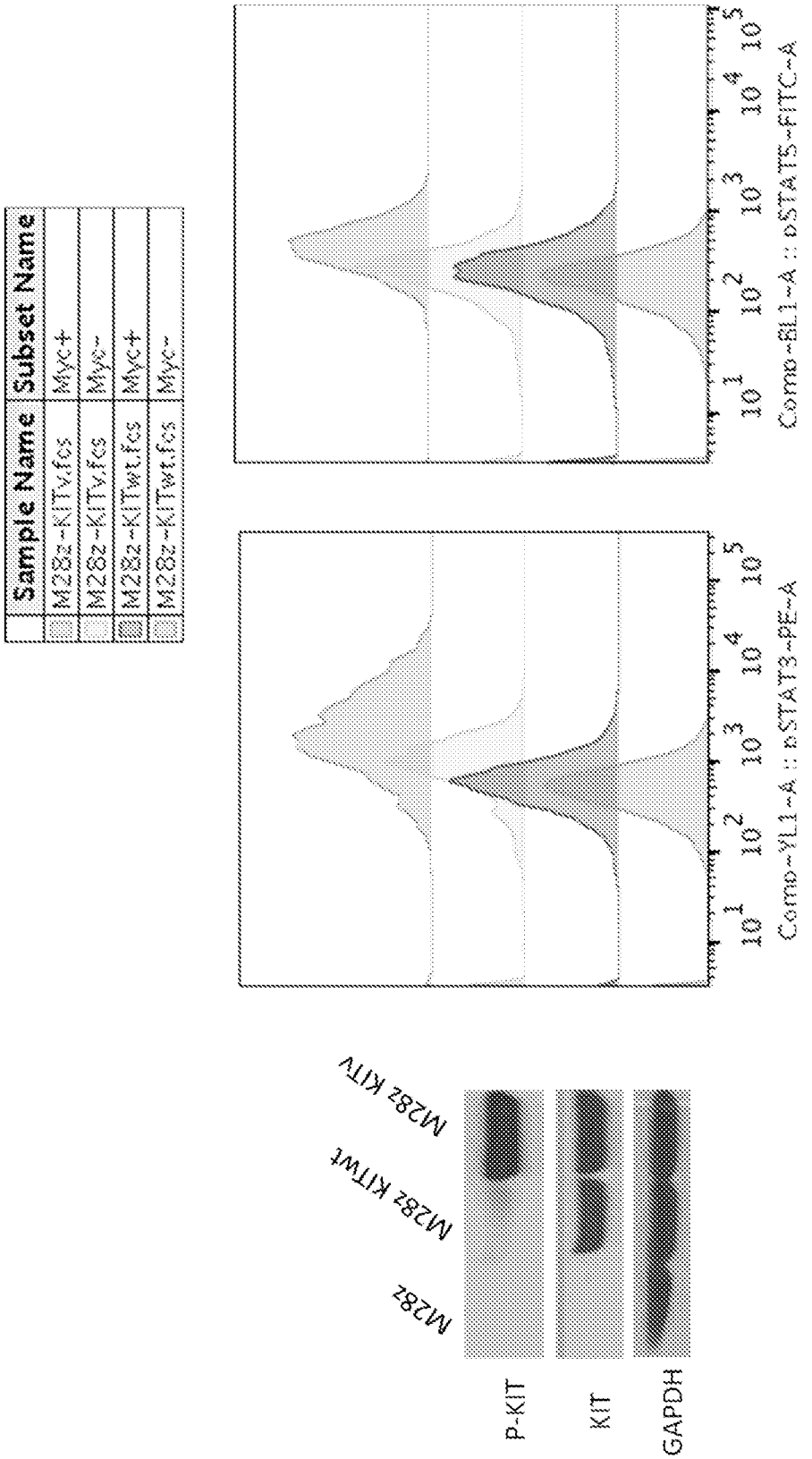


Figure 3B

Figure 3A

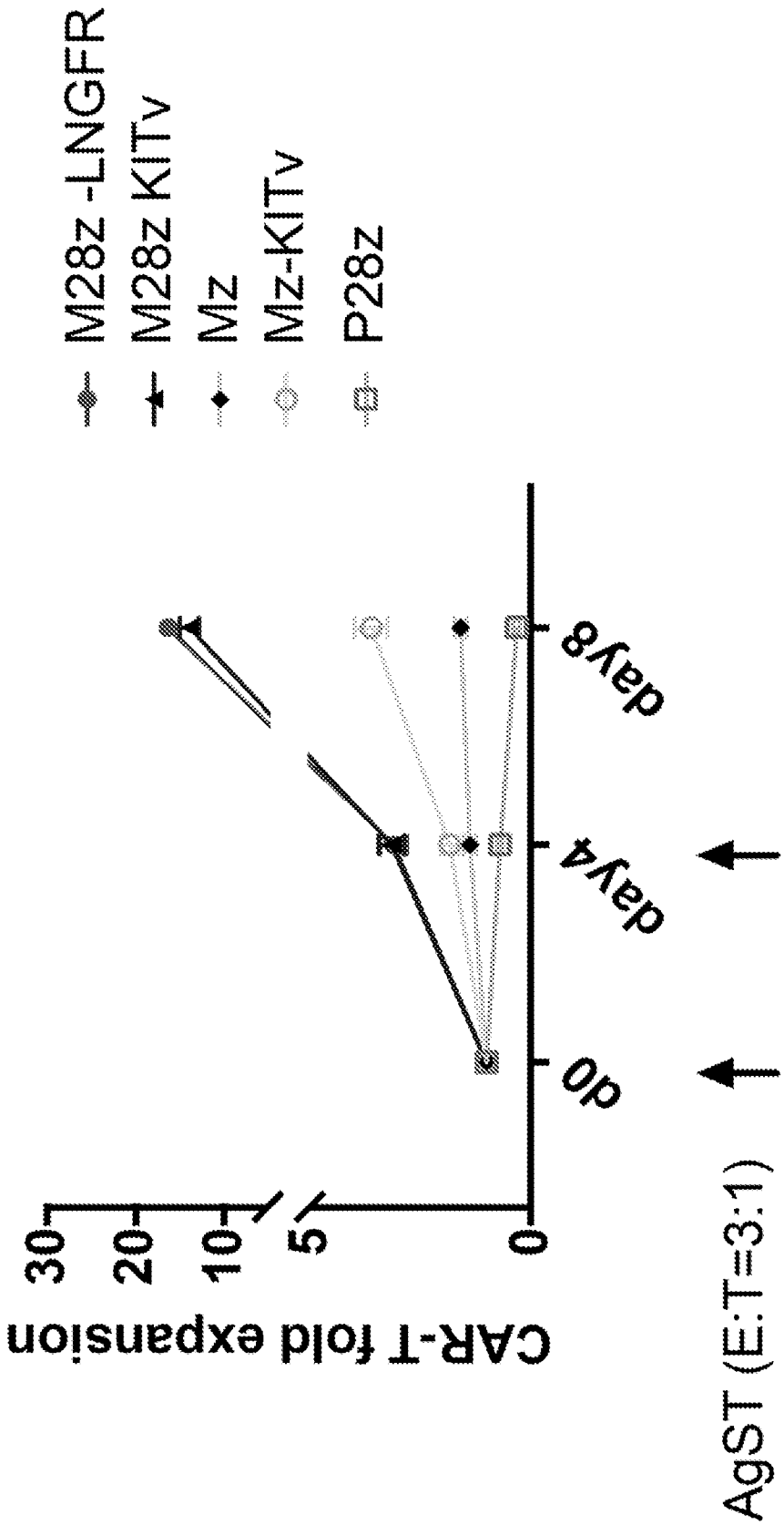

M28z -LNGFR
M28z KITv
Mz
Mz-KITv
P28z
CAR-T fold expansion
30
20
10
5
0
d0
day4
day8
AgST (E:T=3:1)

Figure 4

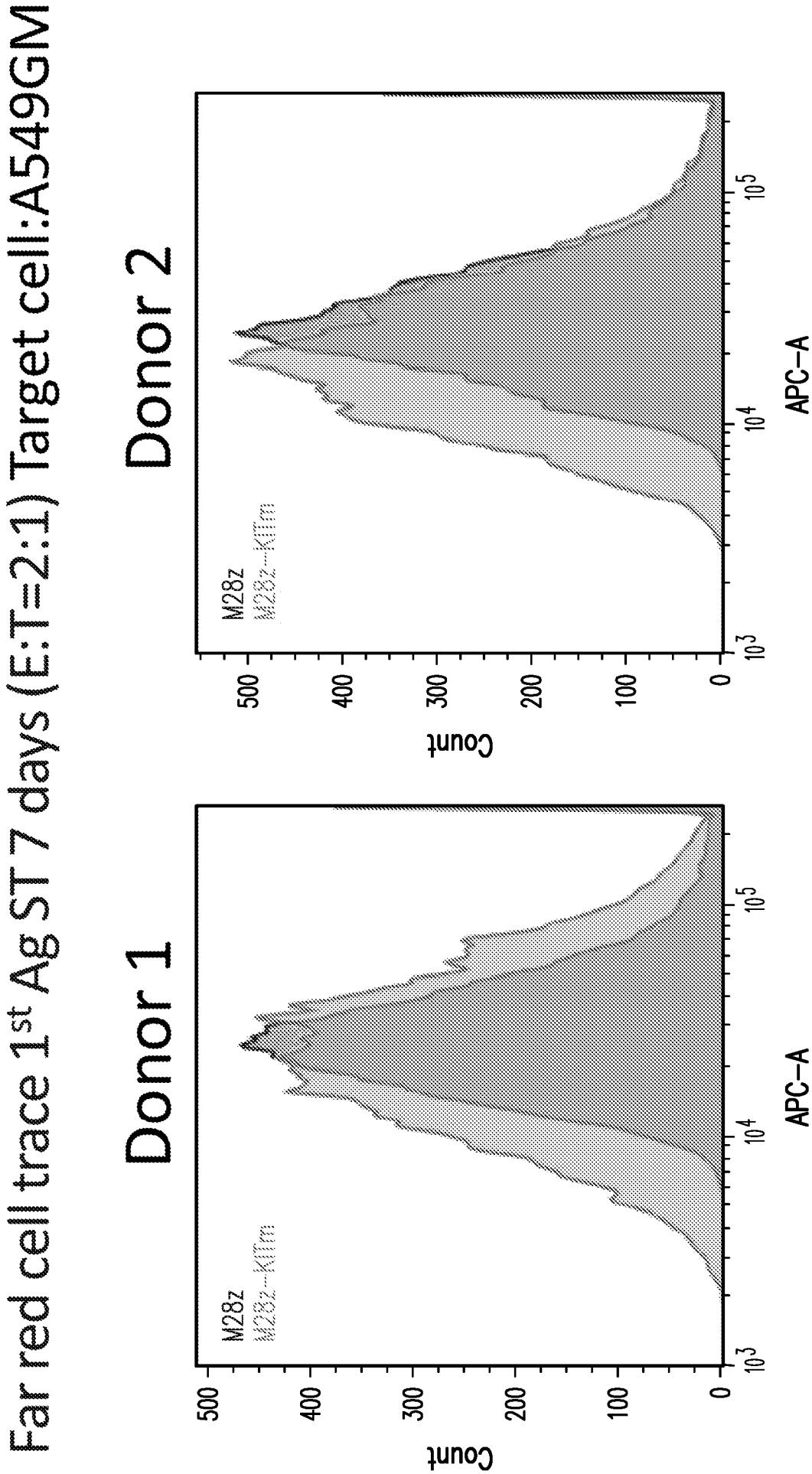

Far red cell trace 1st Ag ST 7 days (E:T=2:1) Target cell:A549GM
Donor 1
Donor 2
M28z
M28z--KITm
Count
APC-A
0
100
200
300
400
500
10^3
10^4
10^5

Figure 5

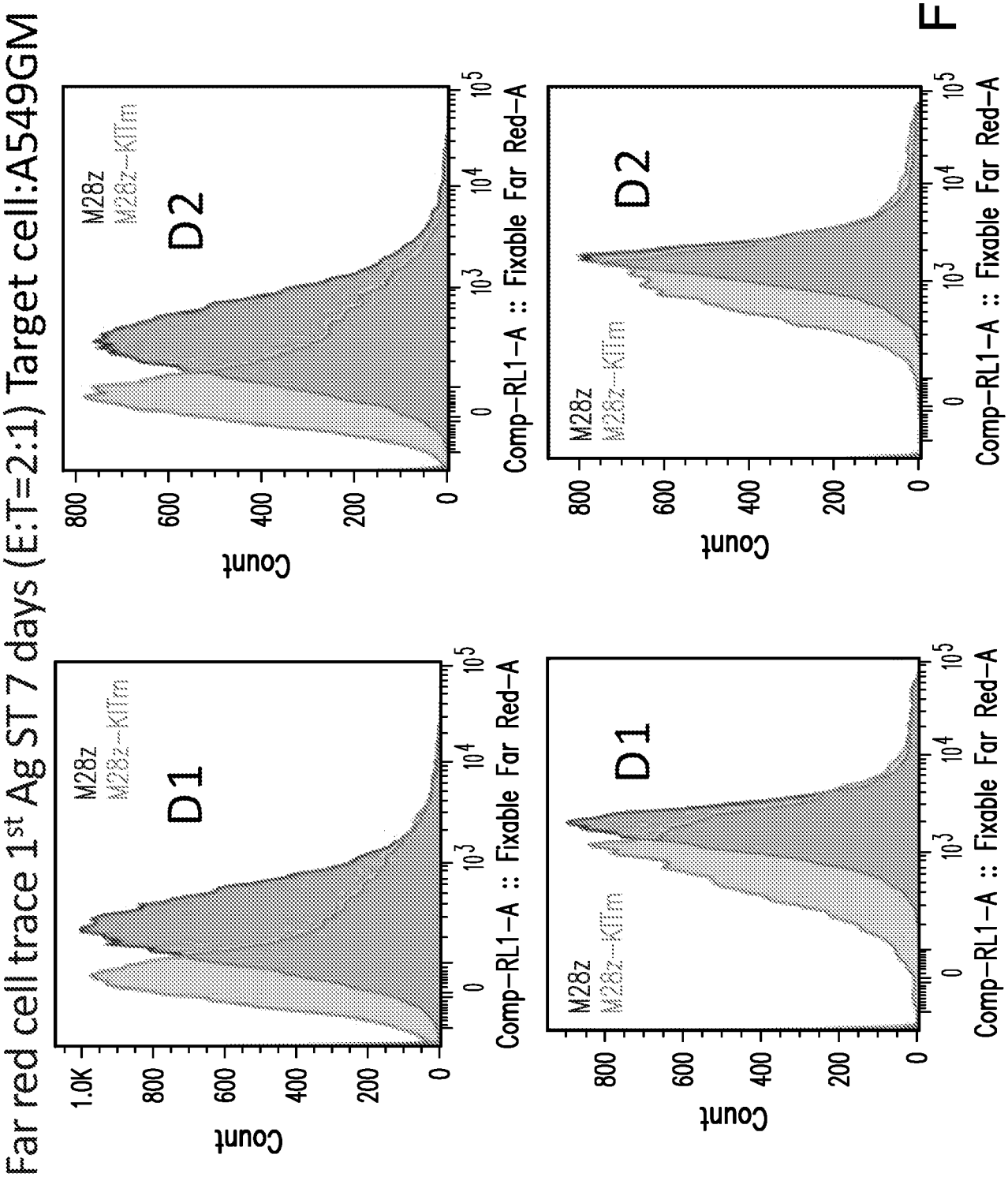

Far red cell trace 1st Ag ST 7 days (E:T=2:1) Target cell:A549GM
D1
D2
M28z
M28z-KITm
Count
Comp-RL1-A :: Fixable Far Red-A
1.0K
800
600
400
200
0
10^3
10^4
10^5

Figure 6

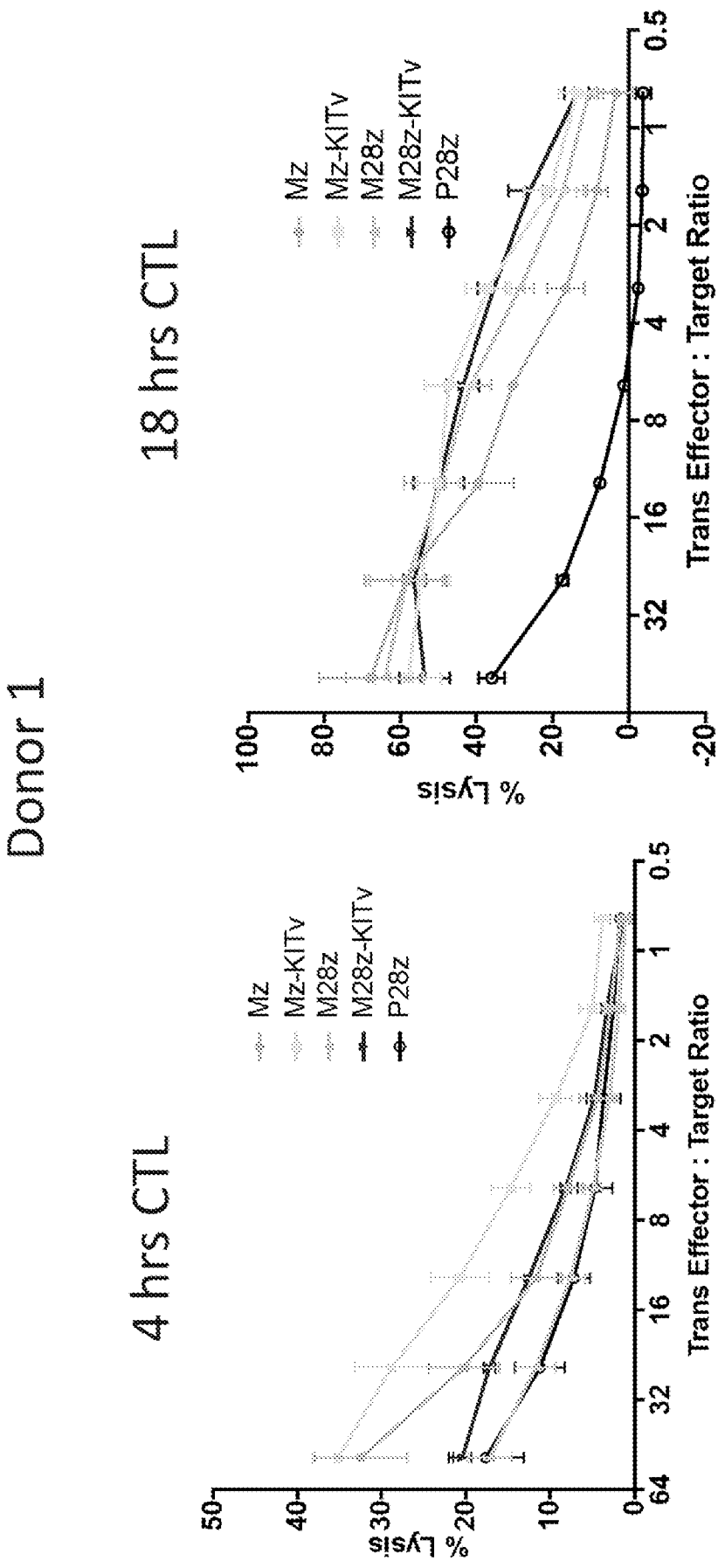


Figure 7A

Figure 7B

Donor 2

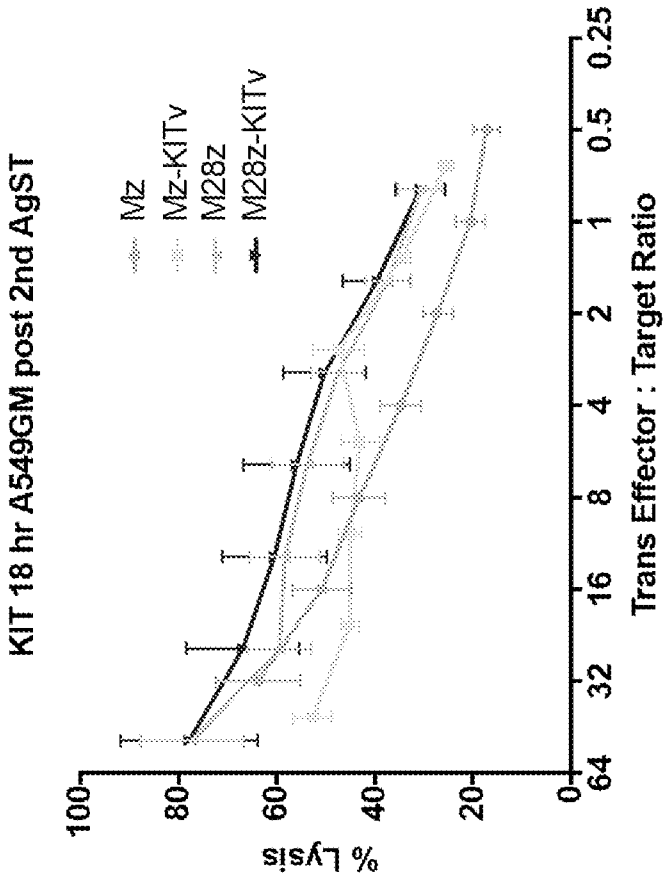


Figure 8B

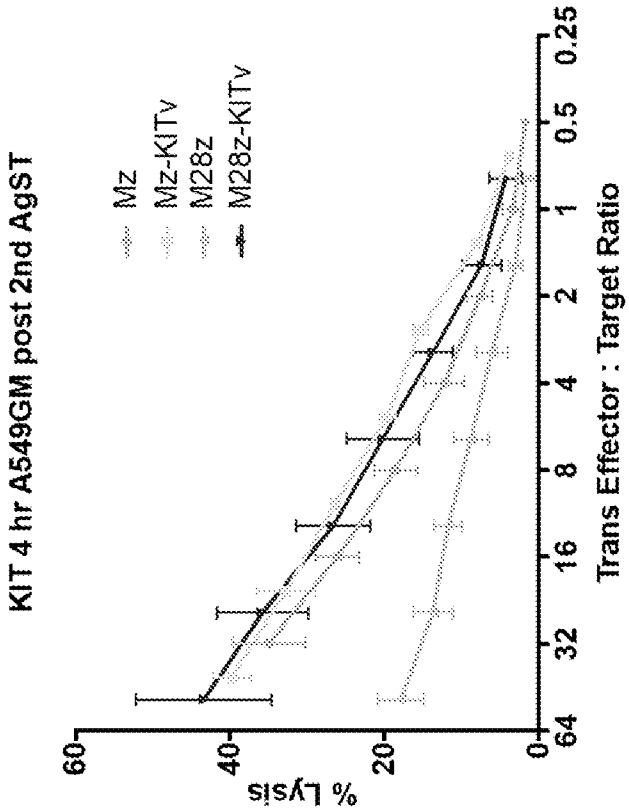
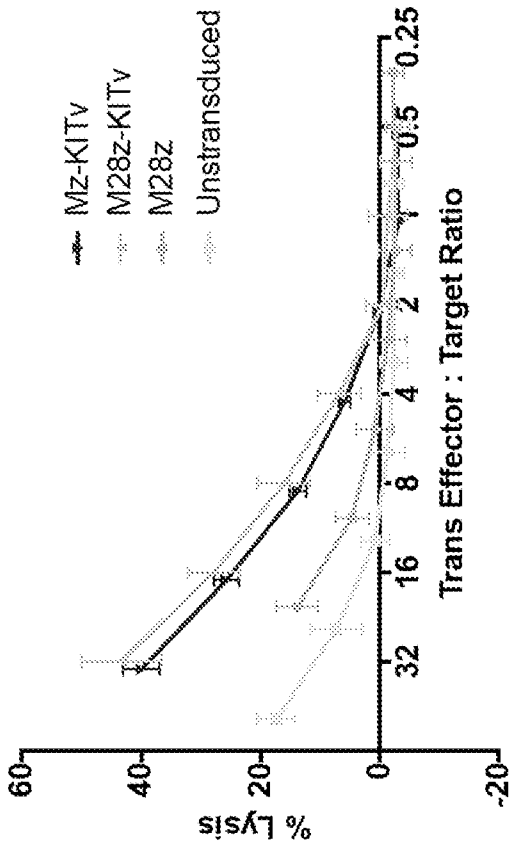


Figure 8A


Mz-KITv
M28z-KITv
M28z
Unstransduced
% Lysis
60
40
20
0
-20
32
16
8
4
2
1
0.5
0.25
Trans Effector : Target Ratio

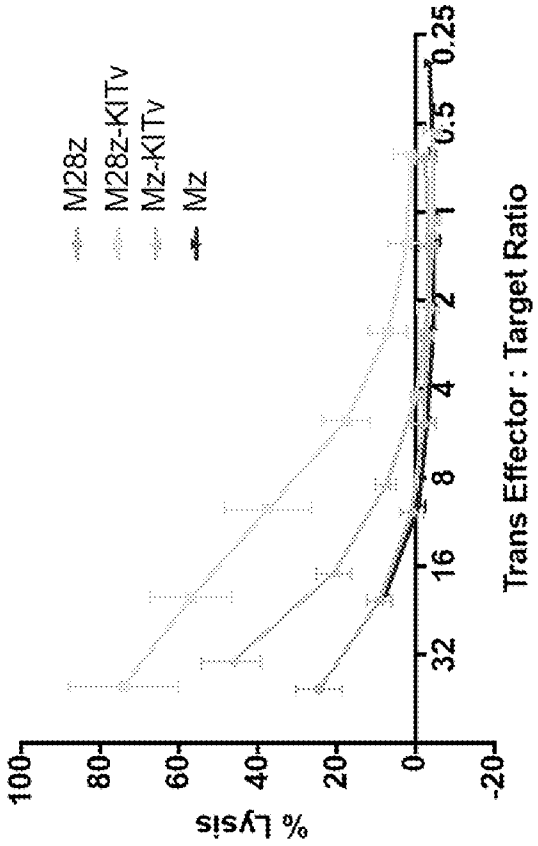

M28z
M28z-KITv
Mz-KITv
Mz
% Lysis
100
80
60
40
20
0
-20
32
16
8
4
2
1
0.5
0.25
Trans Effector : Target Ratio

Figure 9

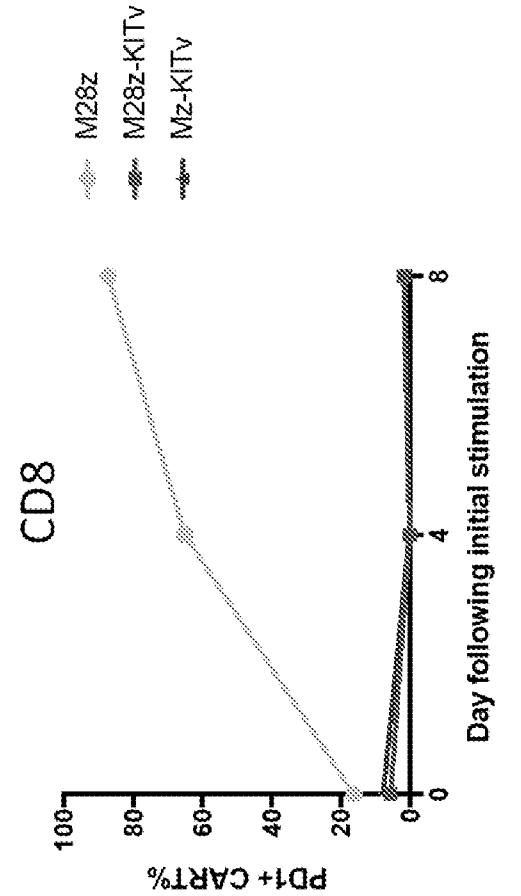


Figure 10B

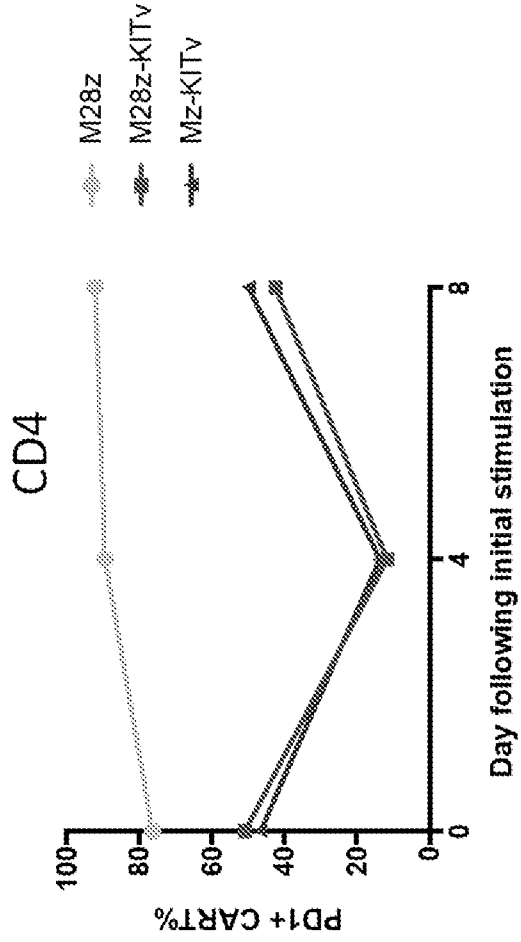


Figure 10A

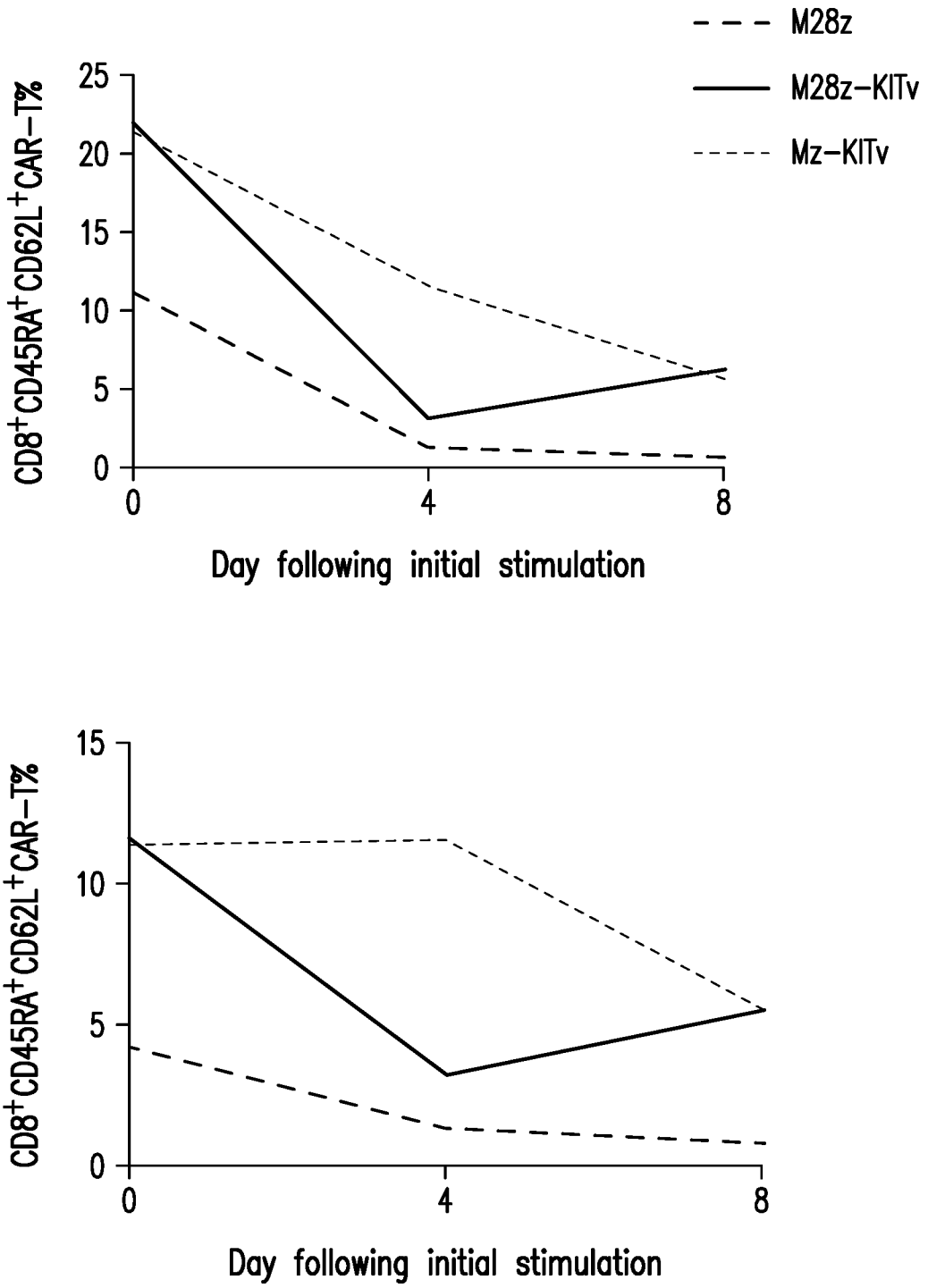

M28z
M28z-KITv
Mz-KITv
CD8+CD45RA+CD62L+CAR-T%
25
20
15
10
5
0
0
4
8
Day following initial stimulation
CD8+CD45RA+CD62L+CAR-T%
15
10
5
0
0
4
8
Day following initial stimulation

Figure 11A

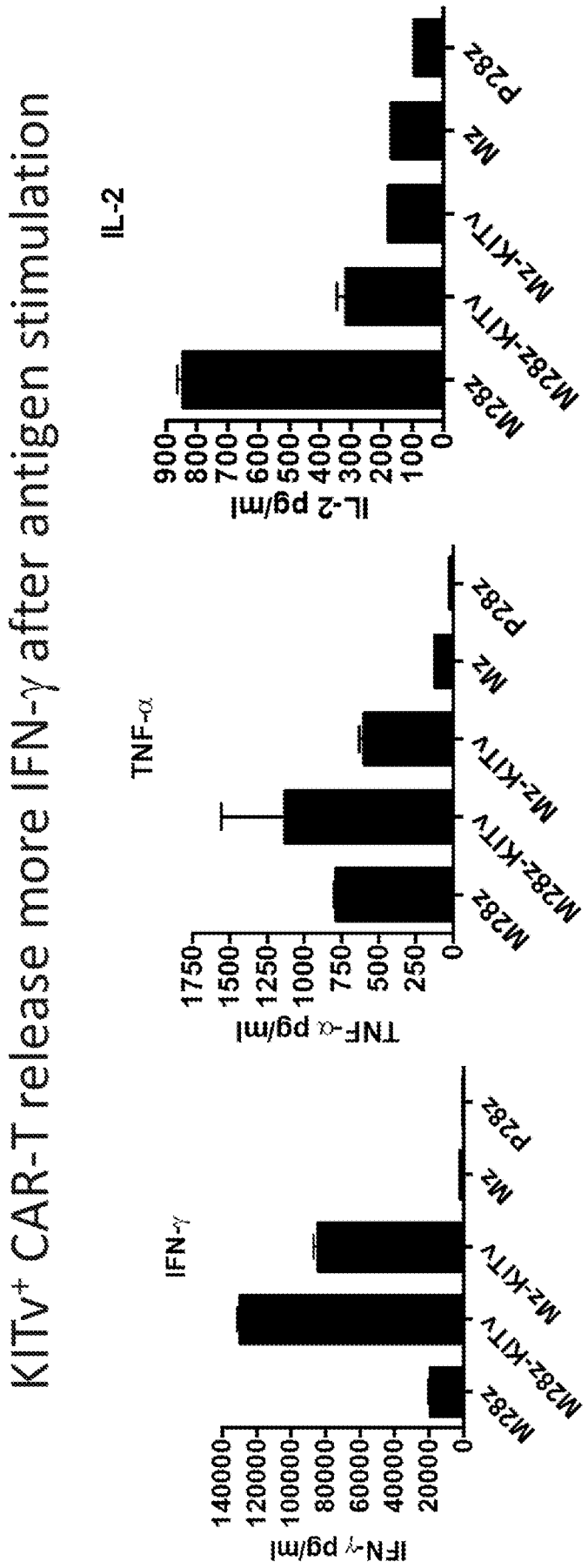

KITv+ CAR-T release more IFN-γ after antigen stimulation
IL-2
IL-2 pg/ml
900
800
700
600
500
400
300
200
100
0
M28z
M28z-KITv
Mz-KITv
Mz
P28z
TNF-α
TNF-α pg/ml
1750
1500
1250
1000
750
500
250
0
M28z
M28z-KITv
Mz-KITv
Mz
P28z
IFN-γ
IFN-γ pg/ml
140000
120000
100000
80000
60000
40000
20000
0
M28z
M28z-KITv
Mz-KITv
Mz
P28z

Figure 11B

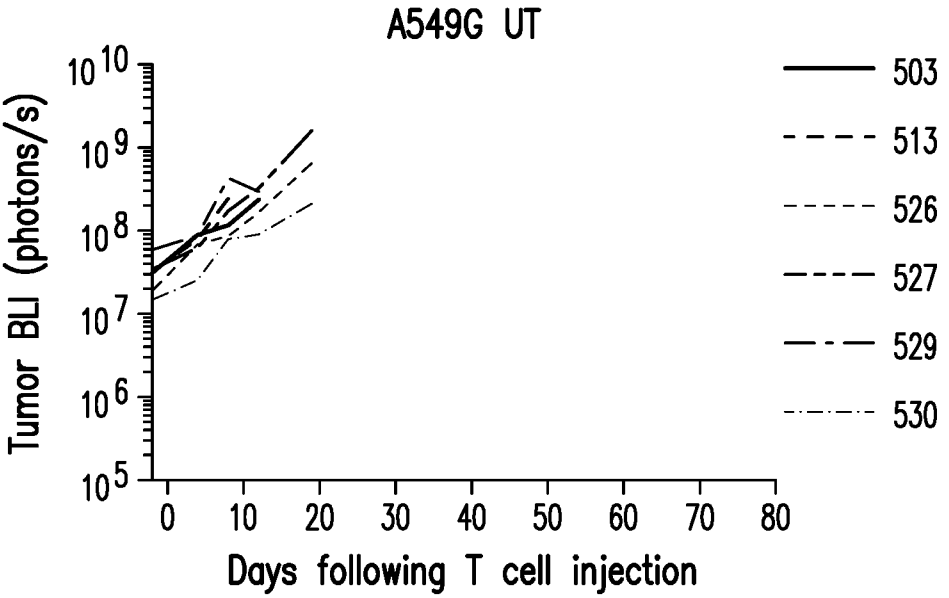


Figure 12A

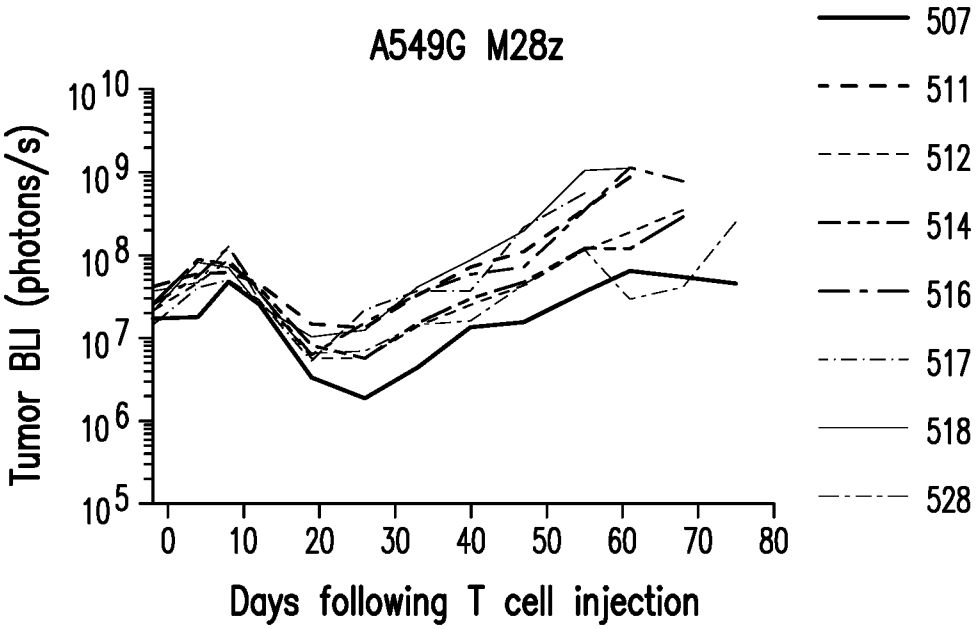


Figure 12B

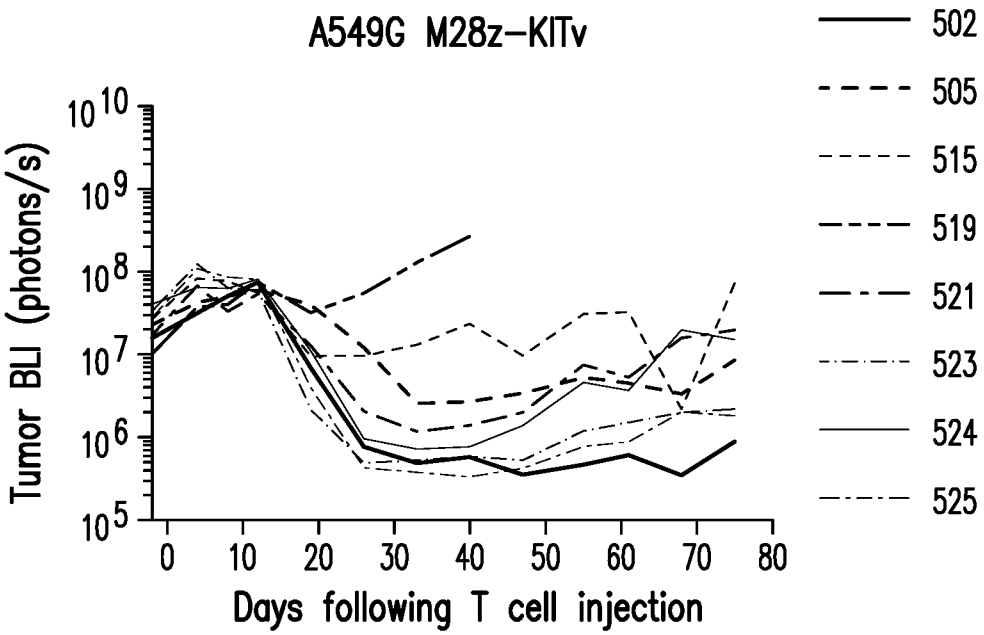


Figure 12C

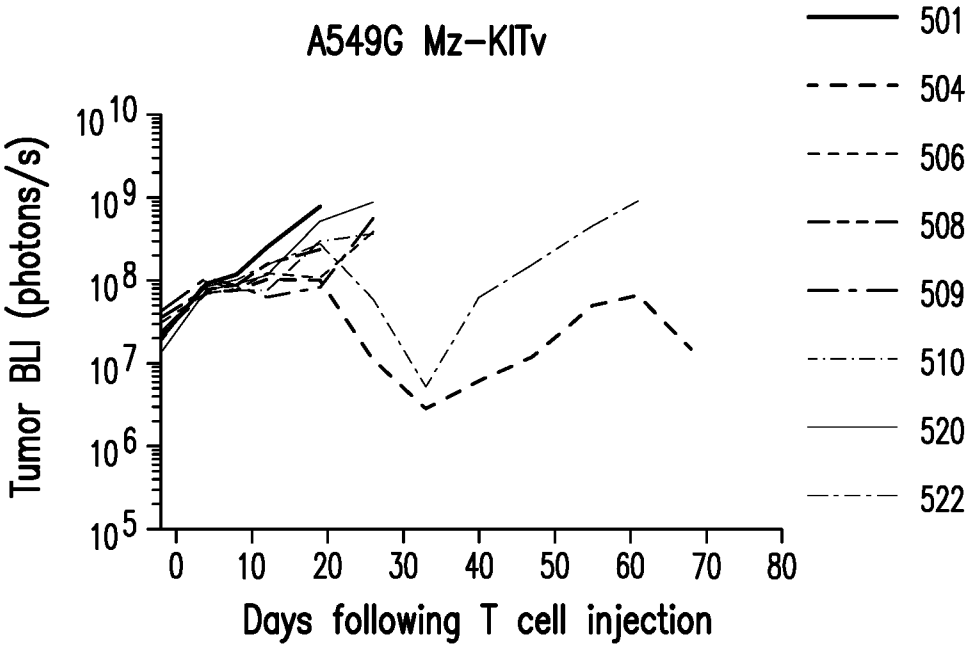


Figure 12D

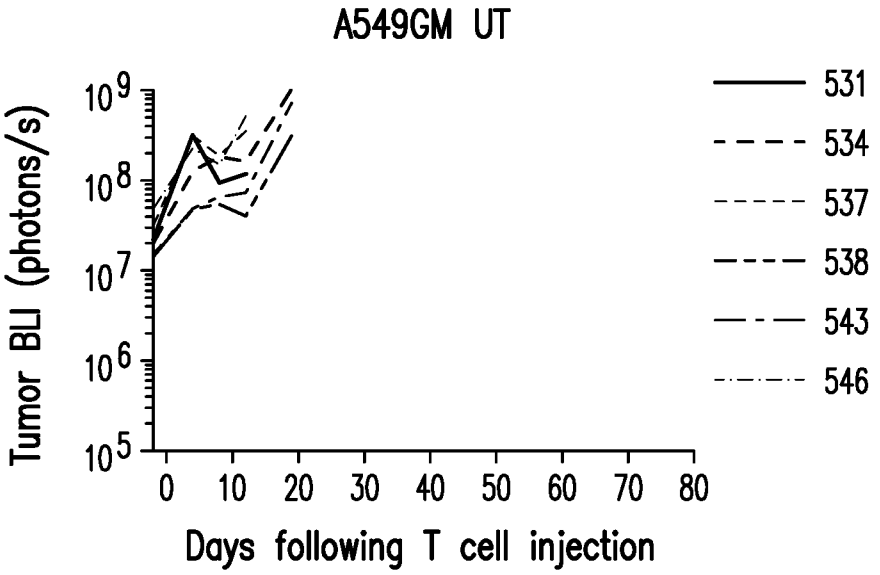


Figure 13A

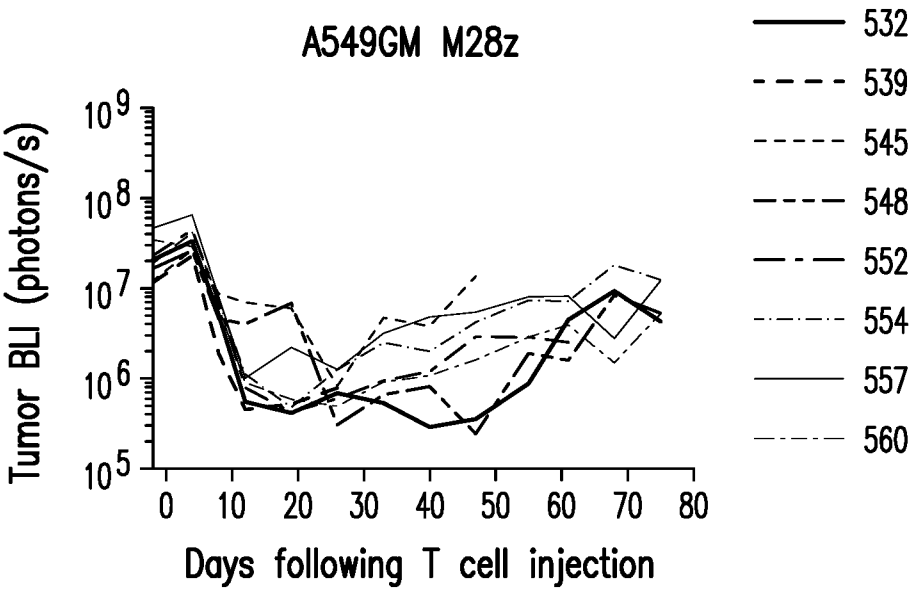


Figure 13B

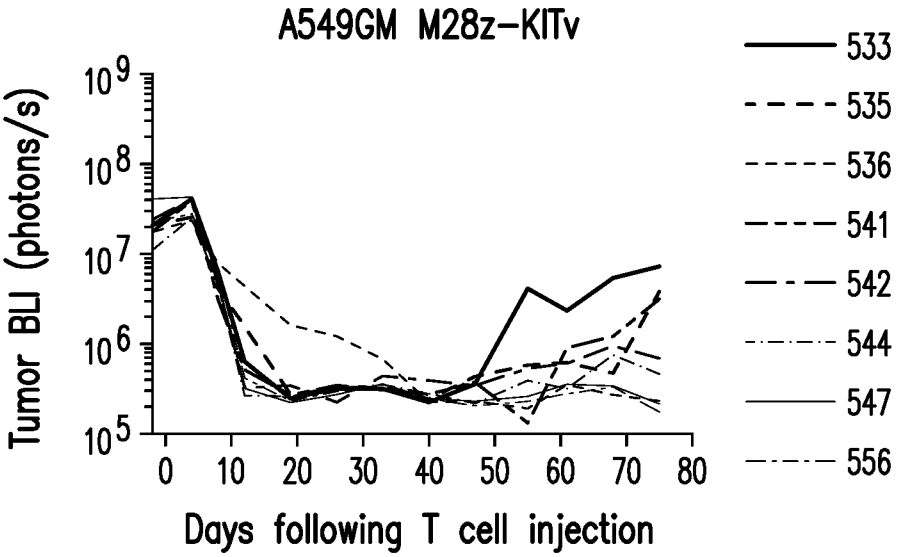


Figure 13C

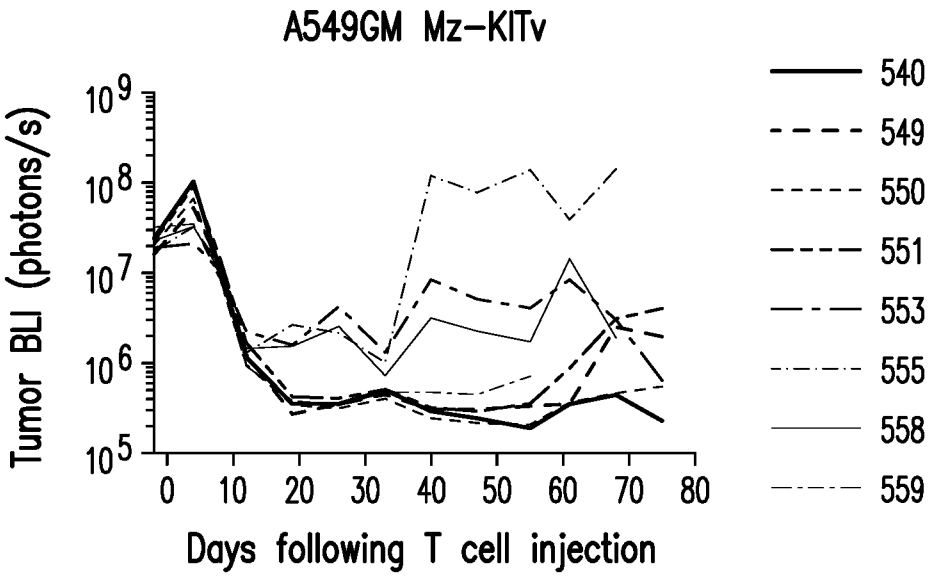


Figure 13D

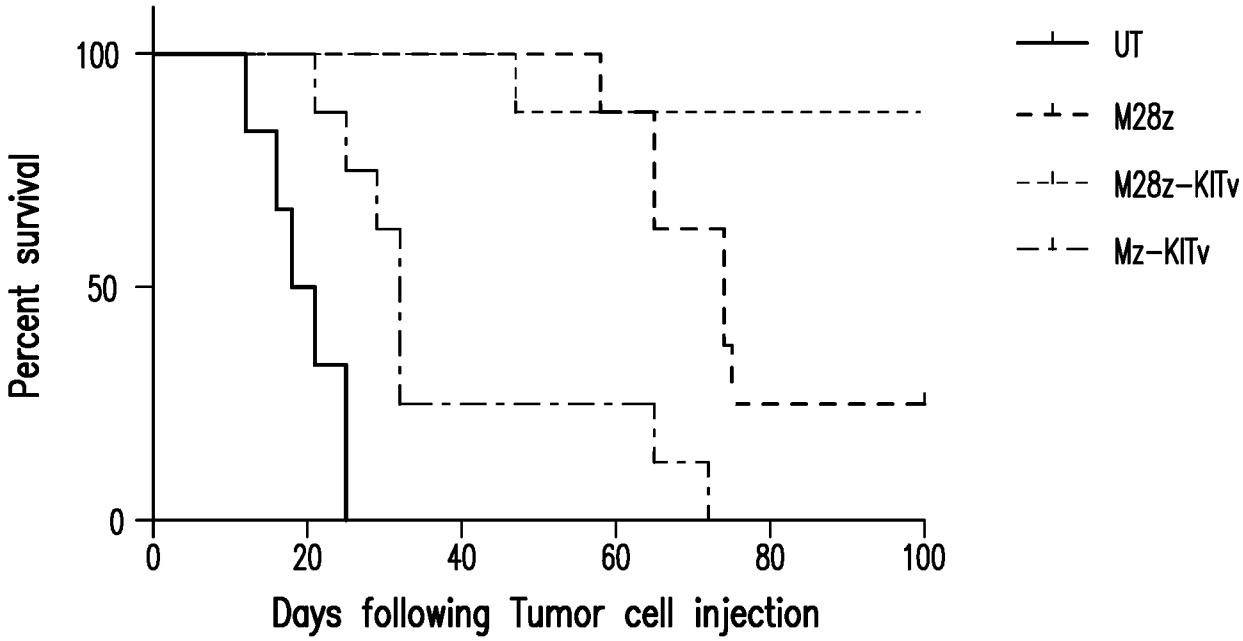


Figure 14A

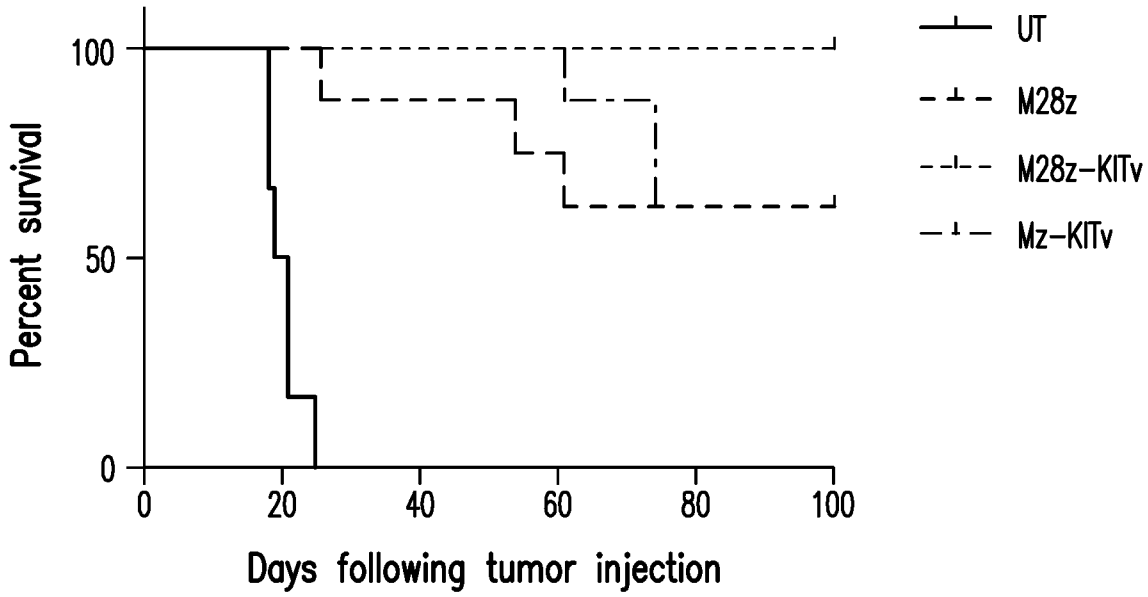
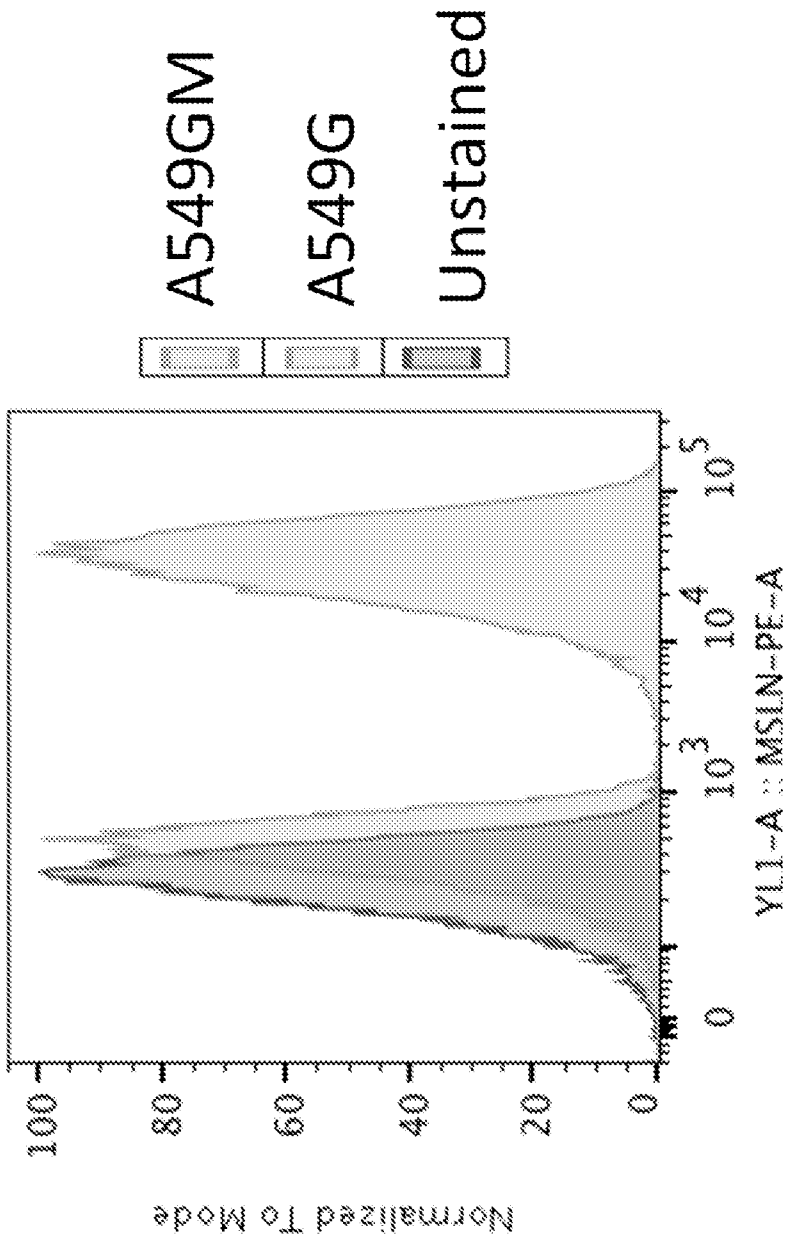


Figure 14B



	MFI
Unstained	280
A549G	439
A549GM	33708

Figure 14C

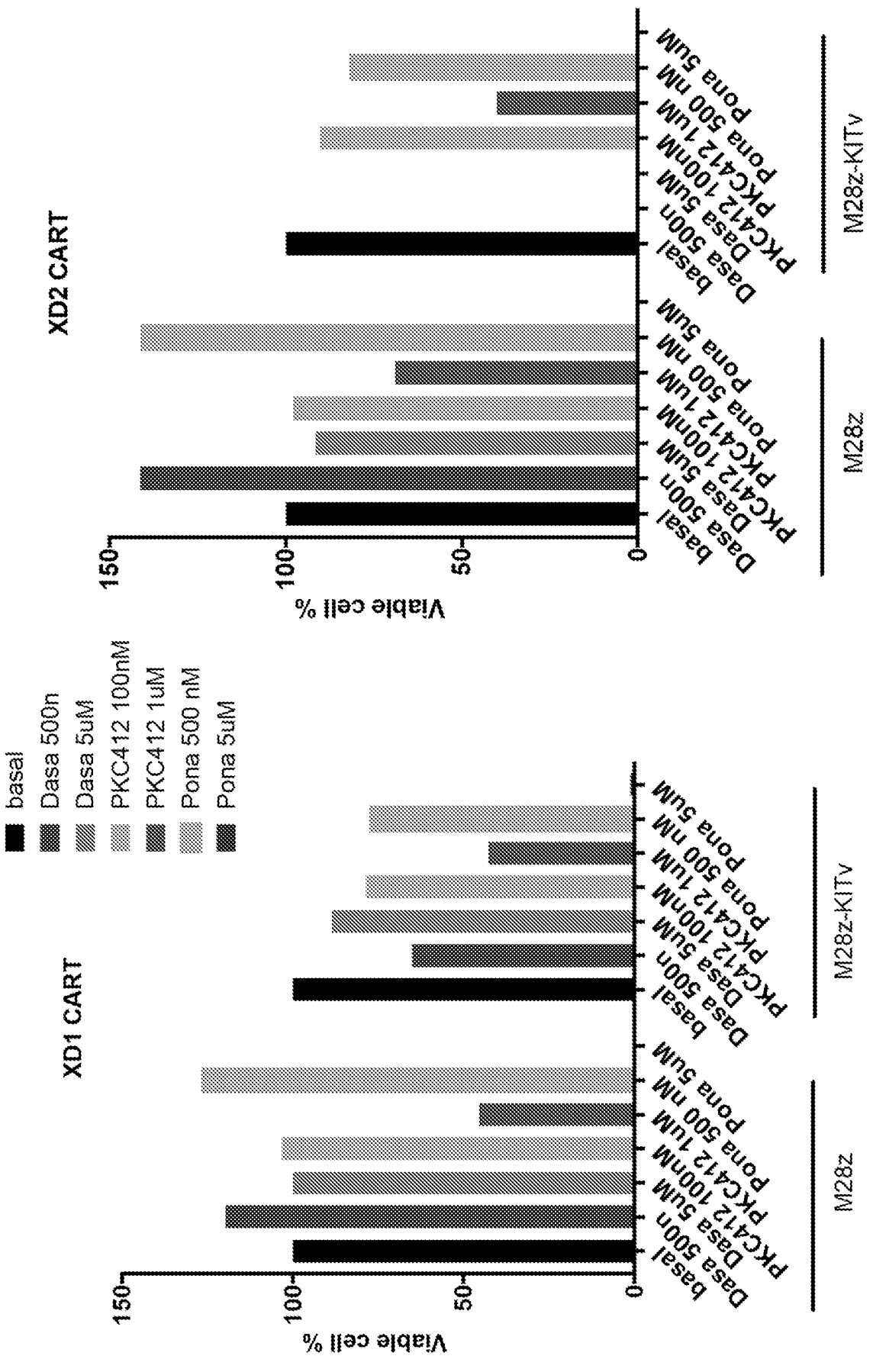

XD1 CART
XD2 CART
Viable cell %
150
100
50
0
basal
Dasa 500n
Dasa 5uM
PKC412 100nM
PKC412 1uM
Pona 500 nM
Pona 5uM
M28z
M28z-KITv

Figure 15

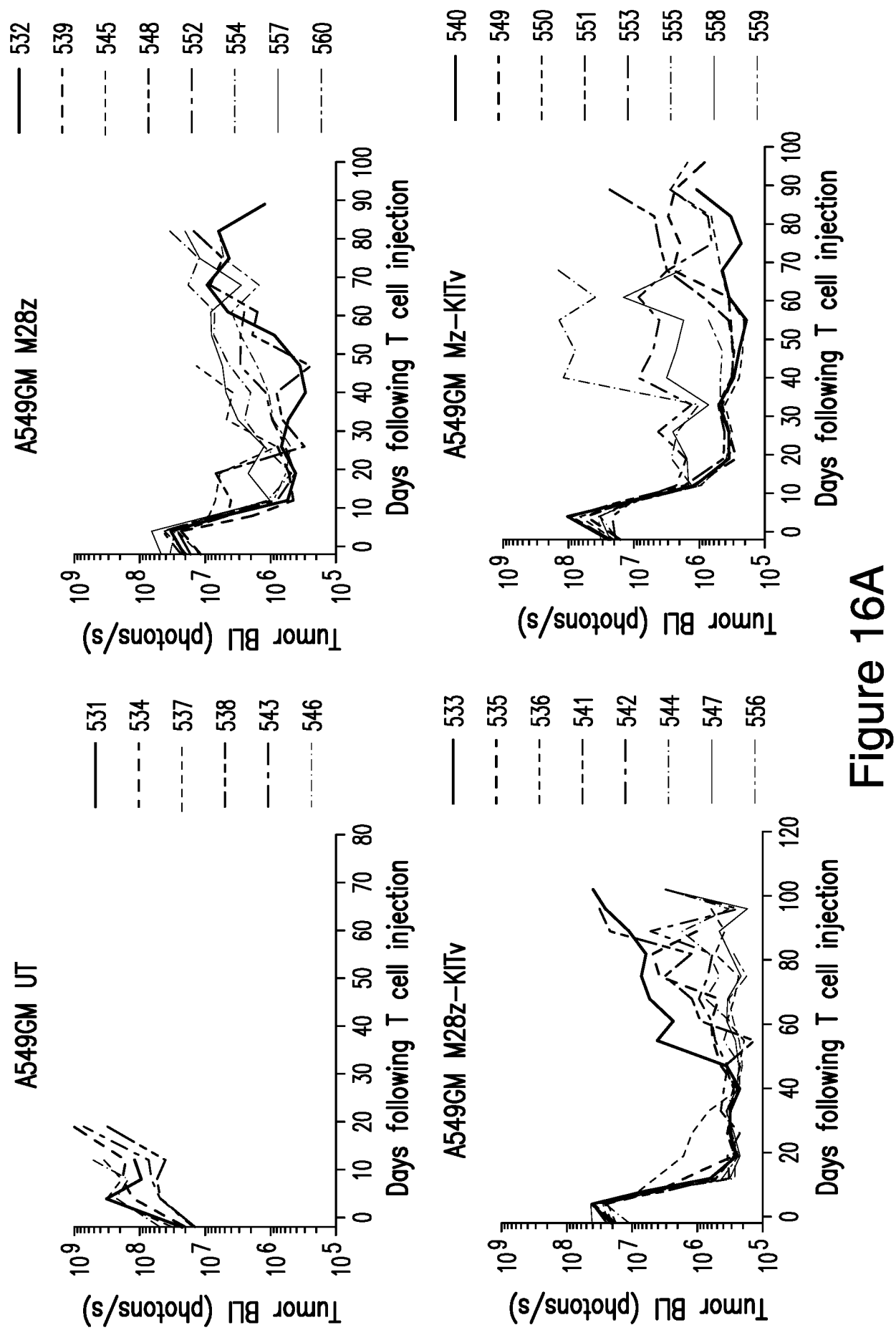
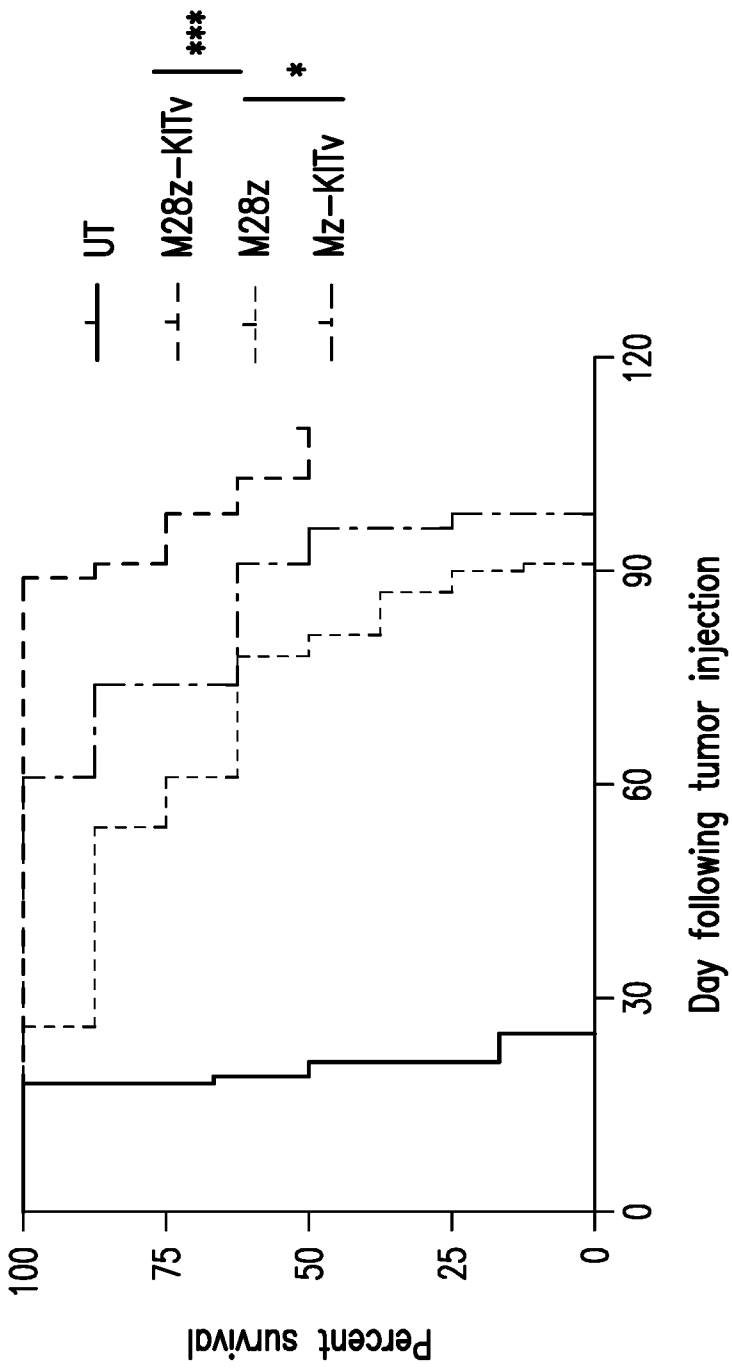

A549GM UT
A549GM M28z
A549GM Mz–KITv
A549GM M28z–KITv
Tumor BLI (photons/s)
Days following T cell injection
531
534
537
538
543
546
532
539
545
548
552
554
557
560
540
549
550
551
553
555
558
559
533
535
536
541
542
544
547
556

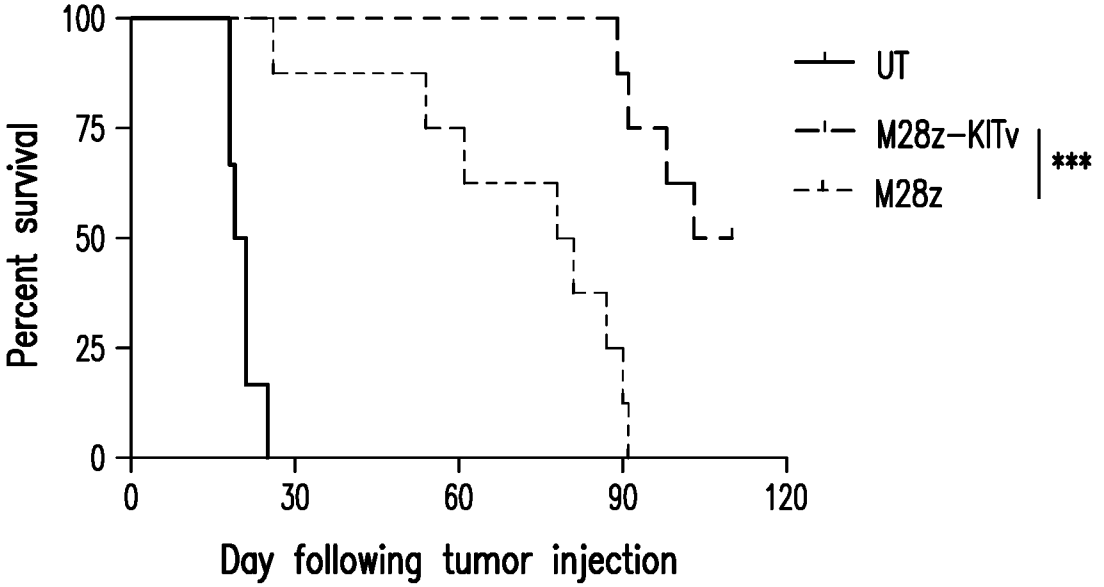
Figure 16A



Median survival (day)

UT	M28z	M28z-KITv	Mz-KITv
20	79.5	106.5	93.5

Figure 16B


100
75
50
25
0
Percent survival
0
30
60
90
120
Day following tumor injection
UT
M28z-KITv
M28z
***

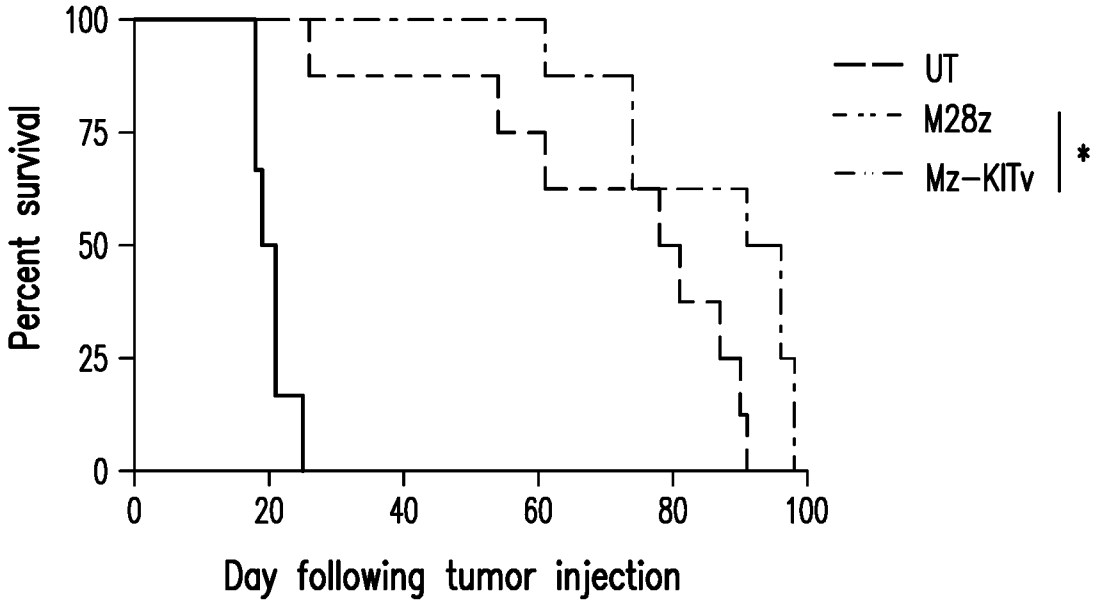

100
75
50
25
0
Percent survival
0
20
40
60
80
100
Day following tumor injection
UT
M28z
Mz-KITv
*

Figure 16C

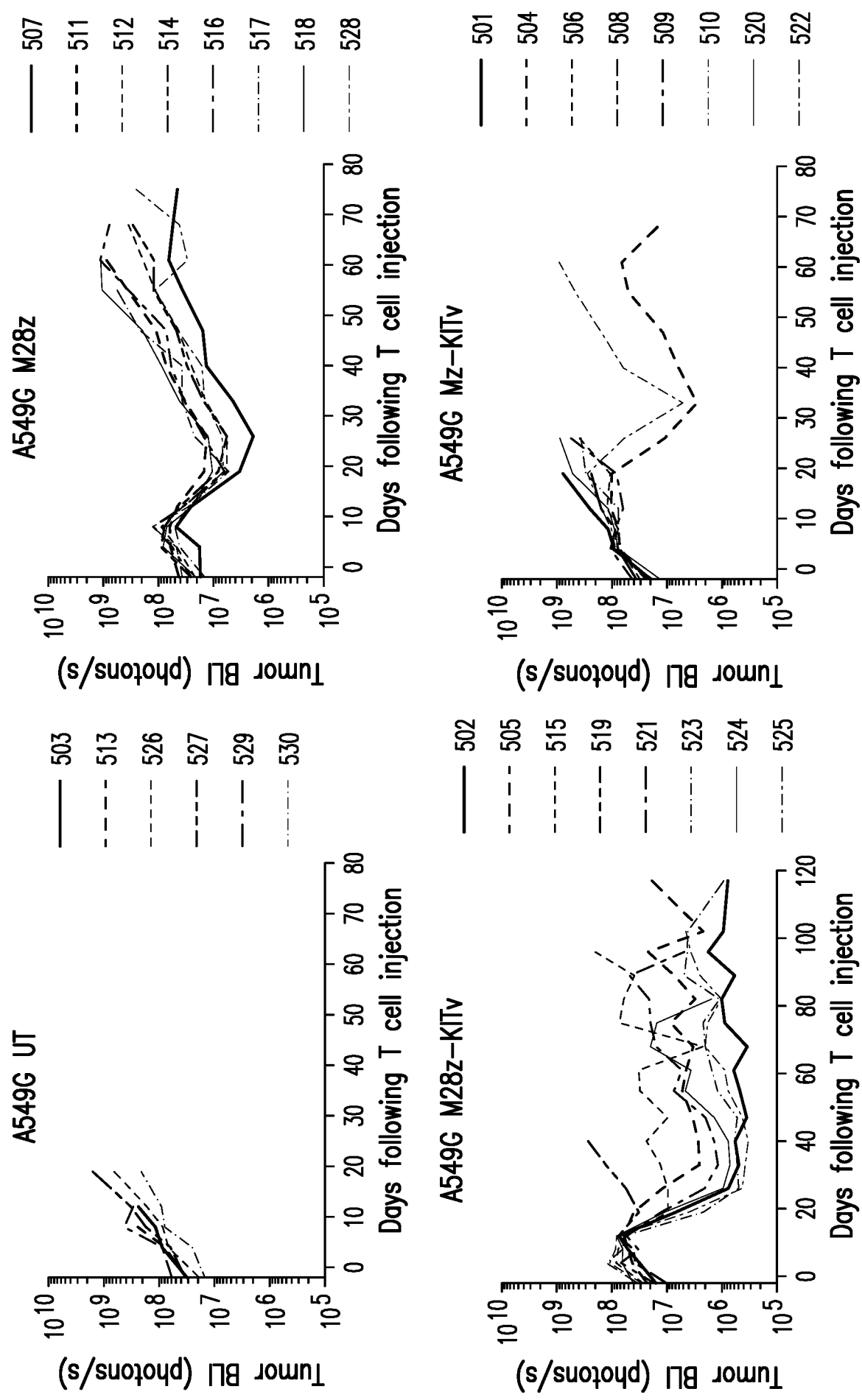
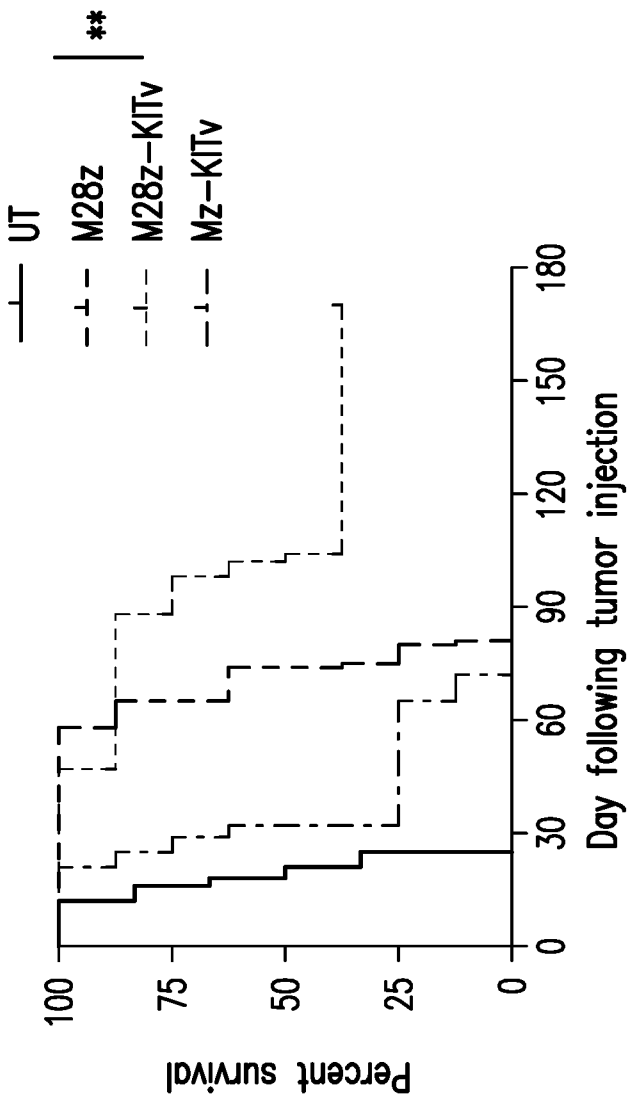

A549G UT
A549G M28z
A549G M28z–KITv
A549G Mz–KITv
Tumor BLI (photons/s)
Days following T cell injection
503
513
526
527
529
530
507
511
512
514
516
517
518
528
502
505
515
519
521
523
524
525
501
504
506
508
509
510
520
522

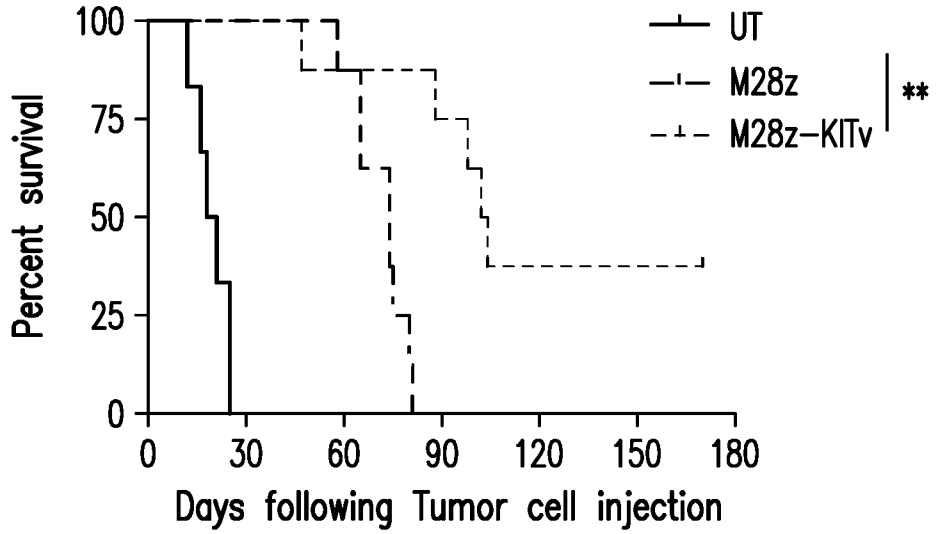
Figure 17A



Median survival (day)

UT	M28z	M28z-KITv	Mz-KITv
19.5	74	103	32

Figure 17B


100
75
50
25
0
Percent survival
0
30
60
90
120
150
180
Days following Tumor cell injection
UT
M28z
M28z-KITv
**

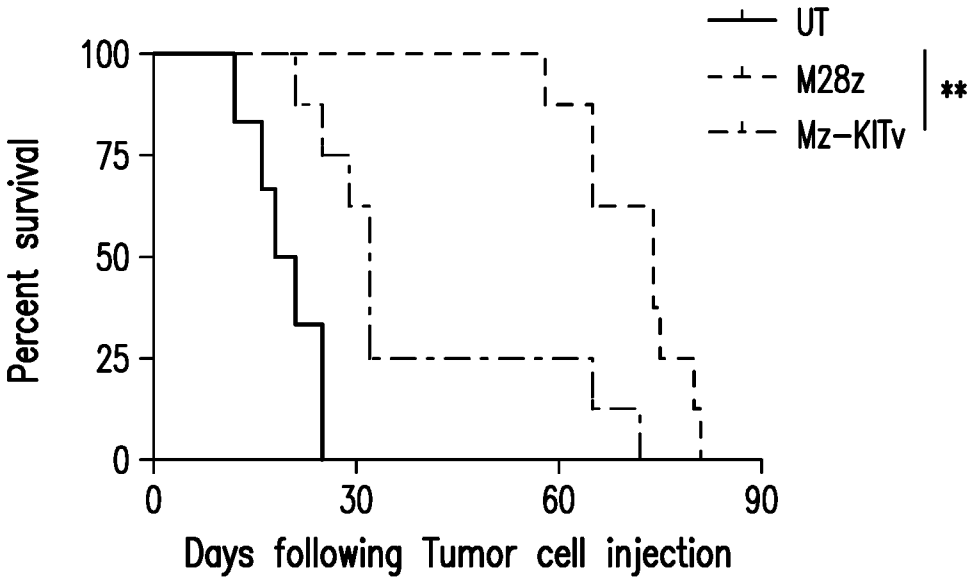
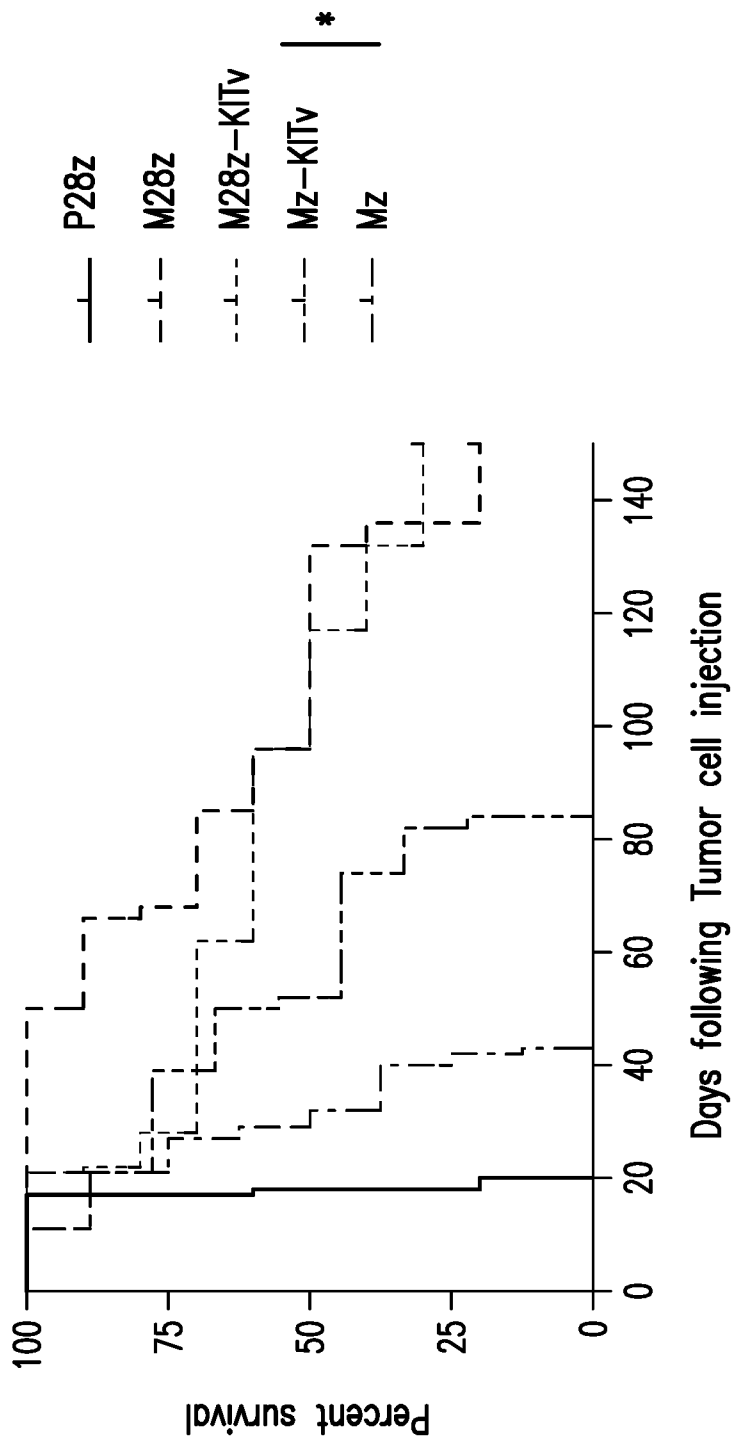

100
75
50
25
0
Percent survival
0
30
60
90
Days following Tumor cell injection
UT
M28z
Mz-KITv
**

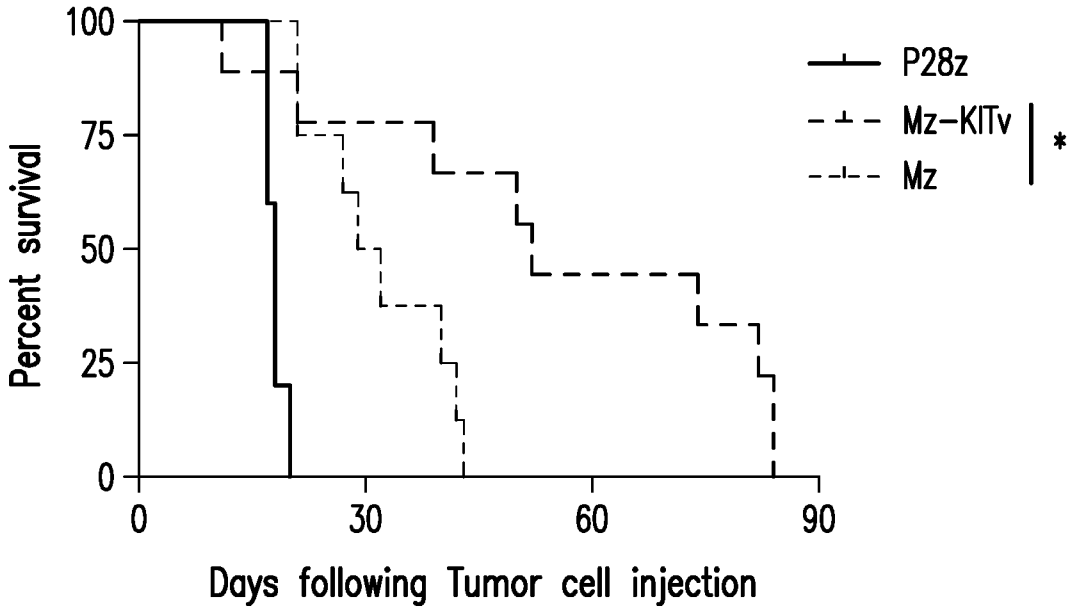
Figure 17C



Median survival (day)

UT	Mz	M28z	M28z-KITv	Mz-KITv
19.5	30.5	114	106.5	52

Figure 18A


100
75
50
25
0
Percent survival
0
30
60
90
Days following Tumor cell injection
P28z
Mz-KITv
Mz
*

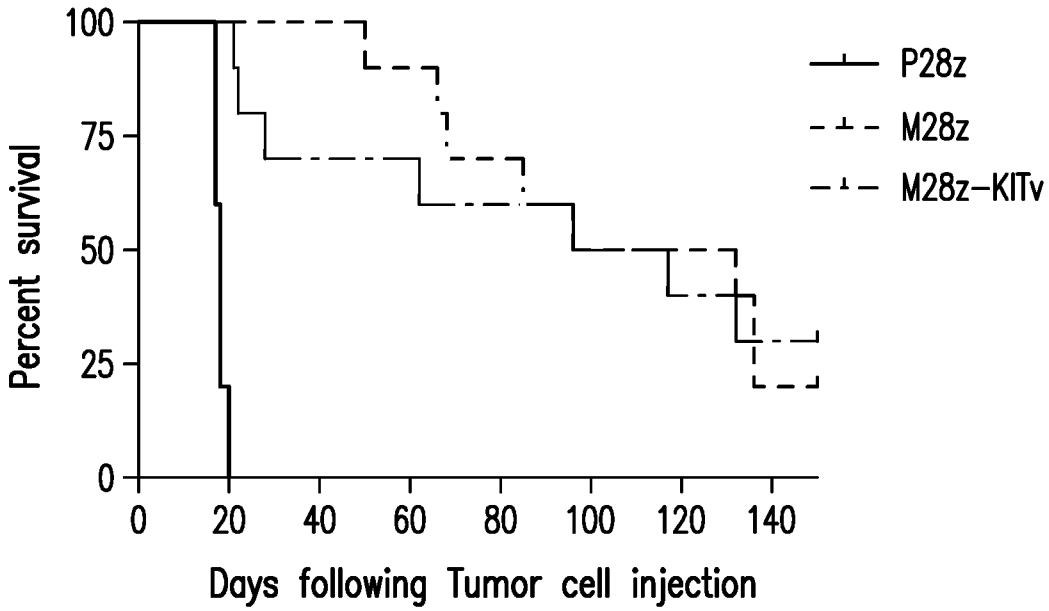

100
75
50
25
0
Percent survival
0
20
40
60
80
100
120
140
Days following Tumor cell injection
P28z
M28z
M28z-KITv

Figure 18B

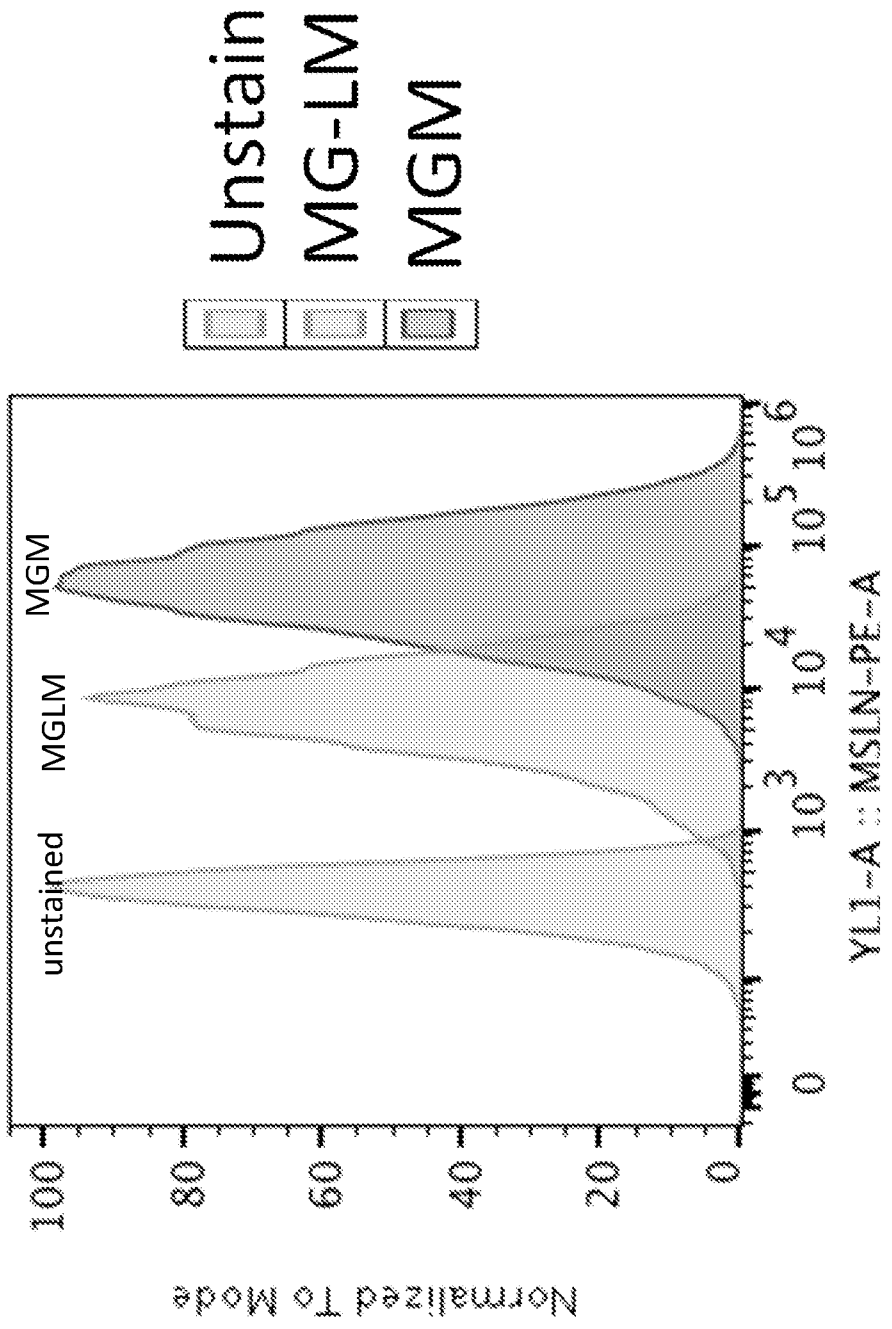
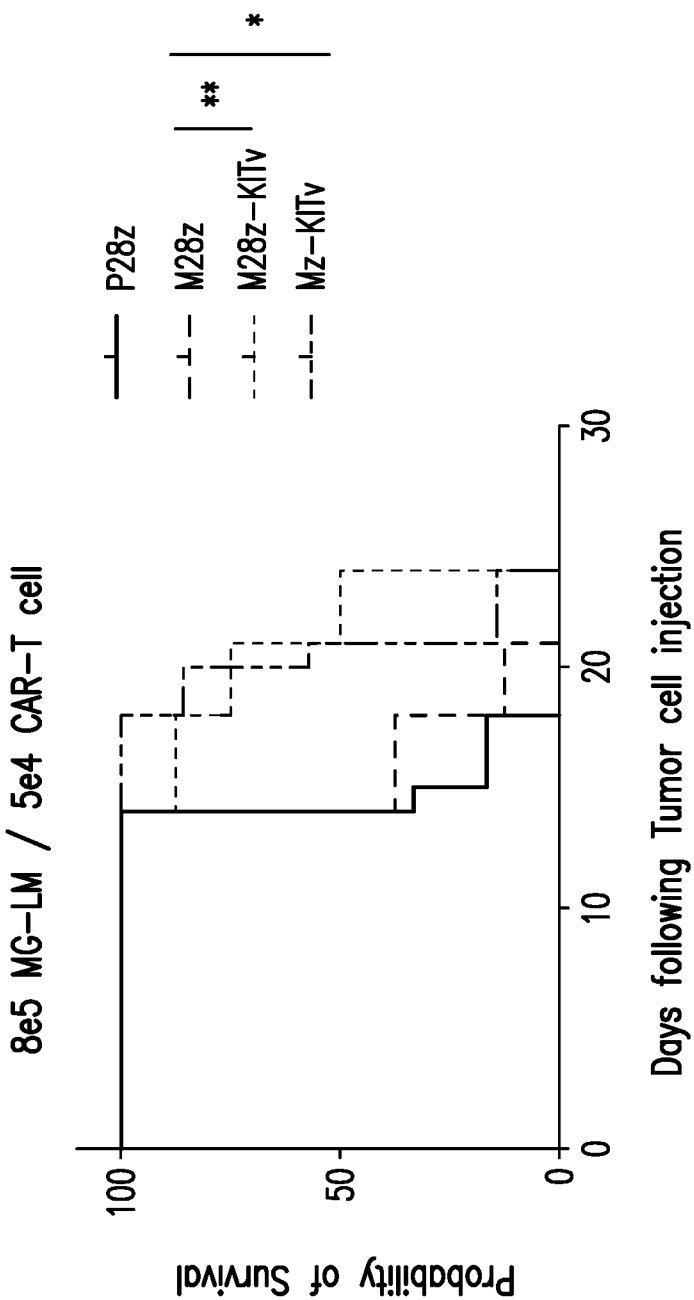

Unstain
MG-LM
MGM
unstained
MGLM
MGM
Normalized To Mode
100
80
60
40
20
0
0
10^3
10^4
10^5
10^6
YL1-A :: MSLN-PE-A

Figure 19A



Median survival (day)

P28z	M28z	M28z-KITv	Mz-KITv
14	14	22.5	21

Figure 19B

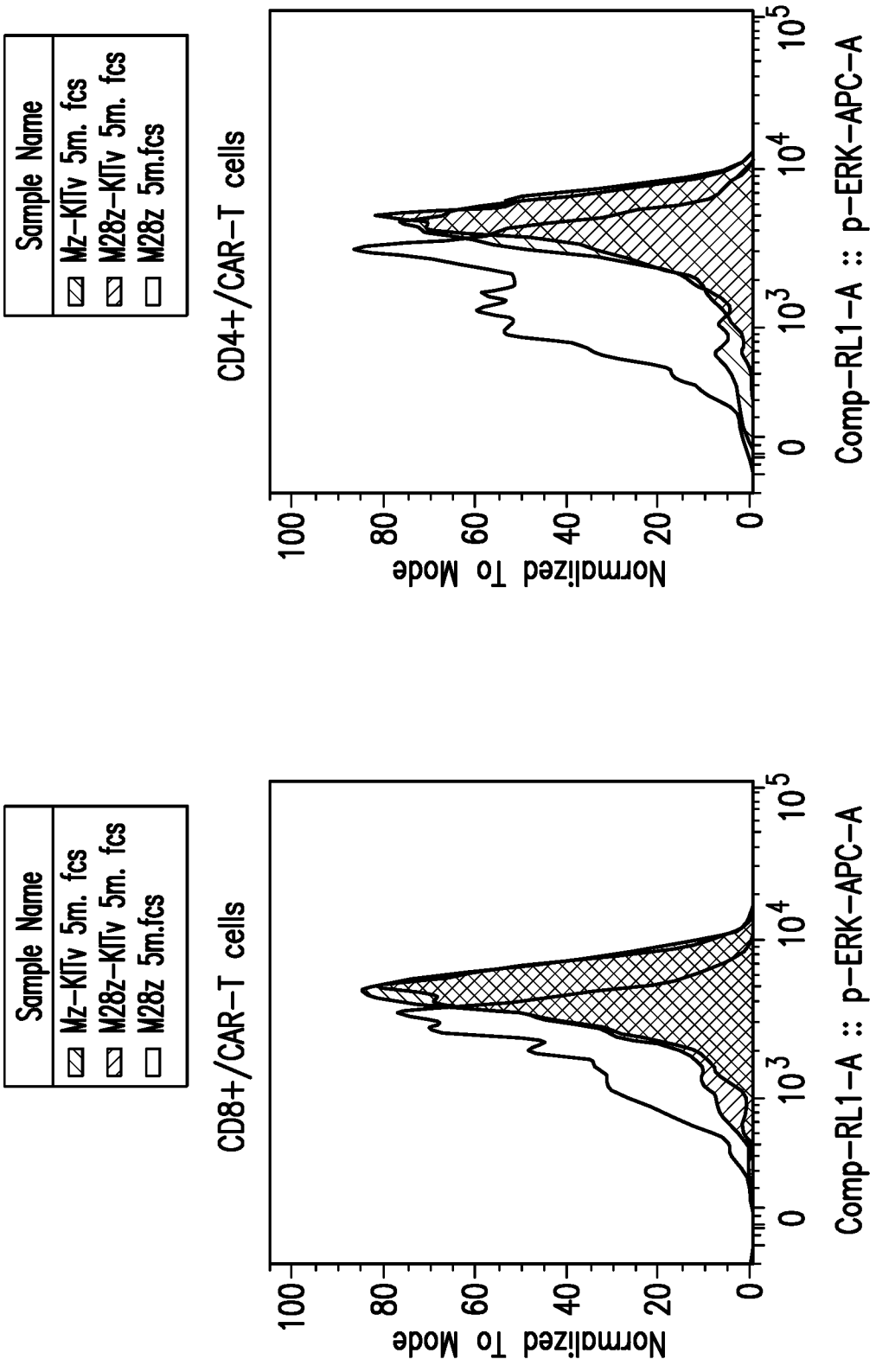

Sample Name
Mz-KITv 5m. fcs
M28z-KITv 5m. fcs
M28z 5m.fcs
CD4+/CAR-T cells
Normalized To Mode
Comp-RL1-A :: p-ERK-APC-A
Sample Name
Mz-KITv 5m. fcs
M28z-KITv 5m. fcs
M28z 5m.fcs
CD8+/CAR-T cells
Normalized To Mode
Comp-RL1-A :: p-ERK-APC-A

Figure 20

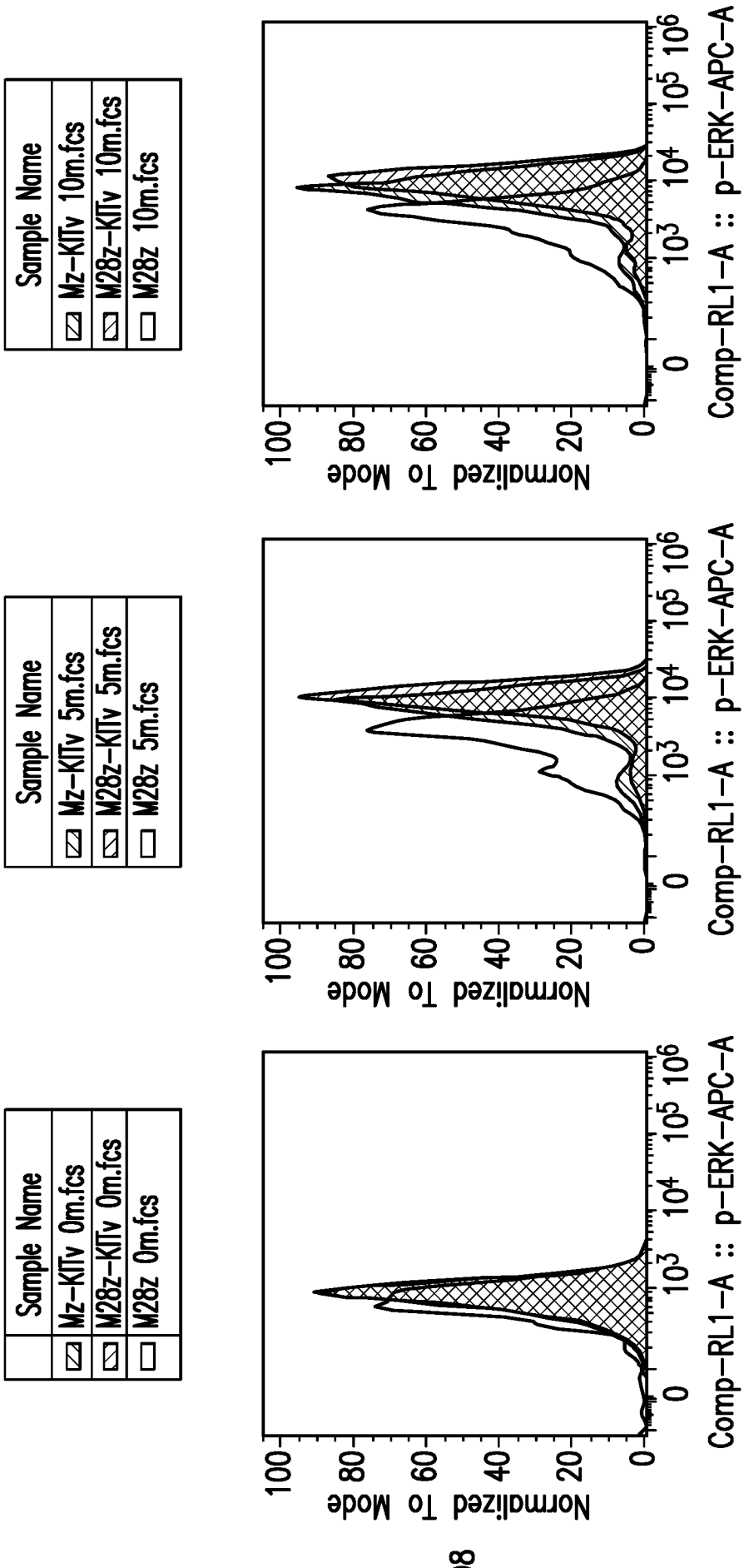


Figure 20 Continued

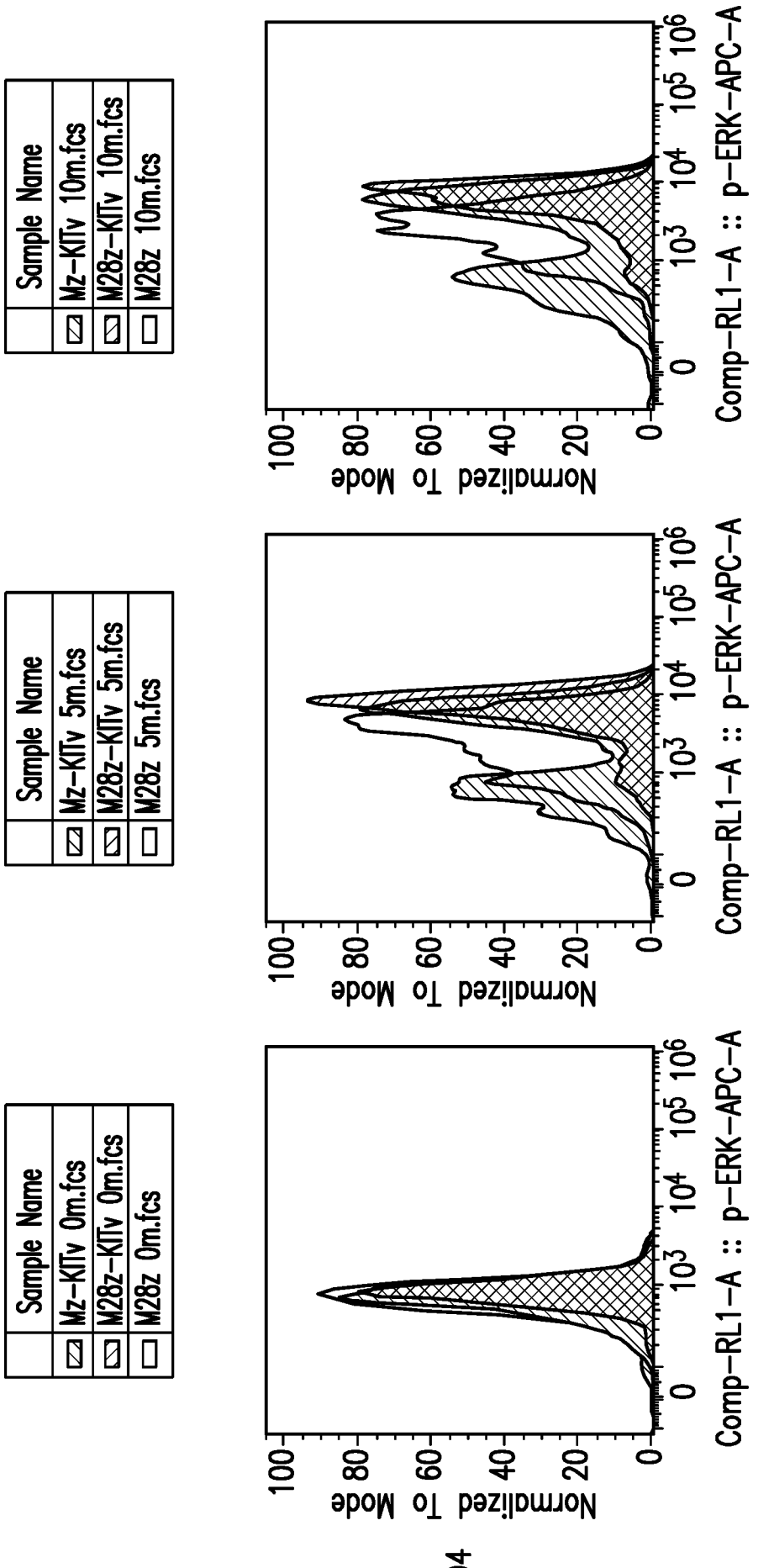


Figure 20 Continued

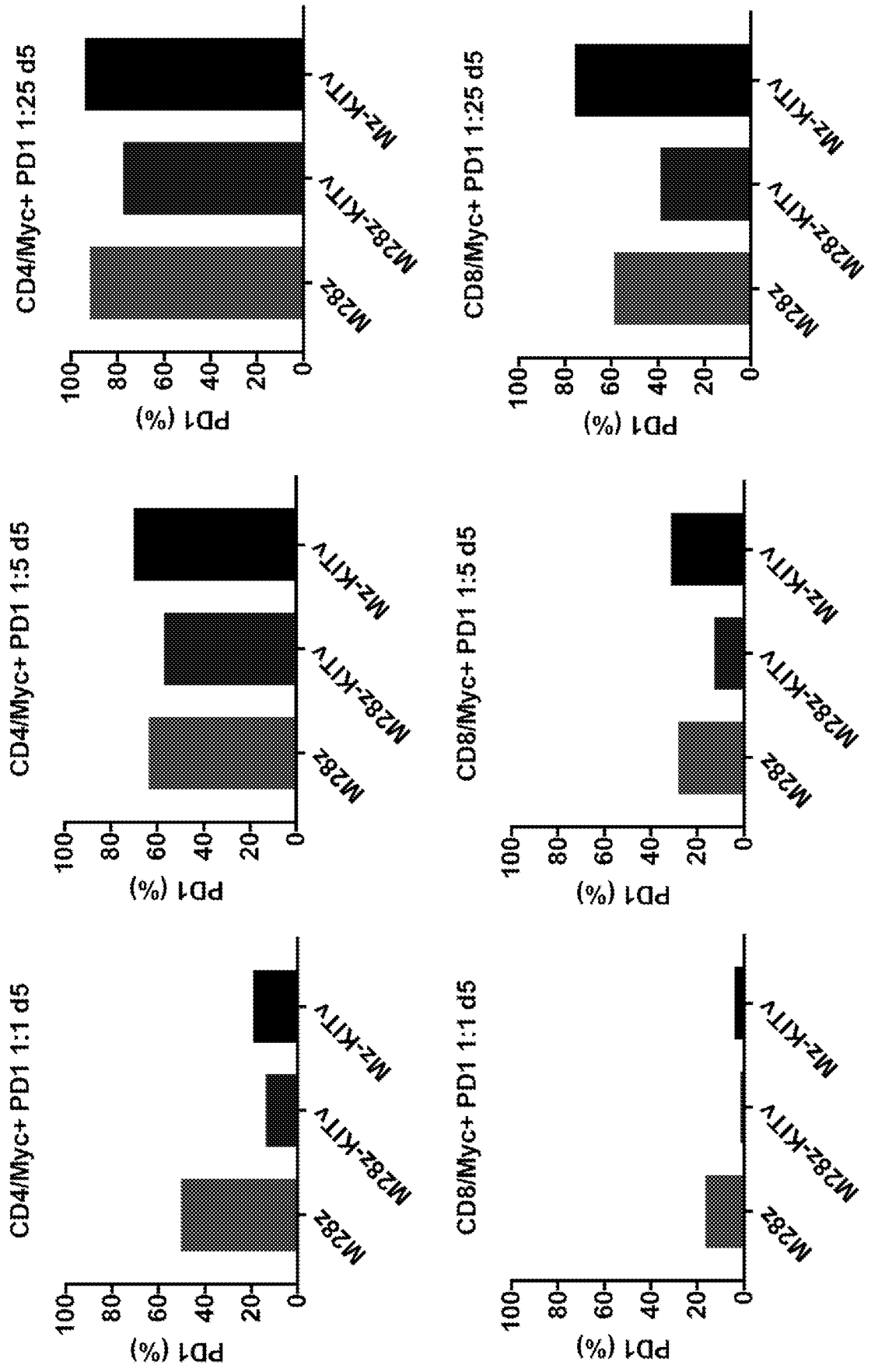

CD4/Myc+ PD1 1:25 d5
CD8/Myc+ PD1 1:25 d5
CD4/Myc+ PD1 1:5 d5
CD8/Myc+ PD1 1:5 d5
CD4/Myc+ PD1 1:1 d5
CD8/Myc+ PD1 1:1 d5
PD1 (%)
0
20
40
60
80
100
M28z
M28z-KITv
Mz-KITv

Figure 21A

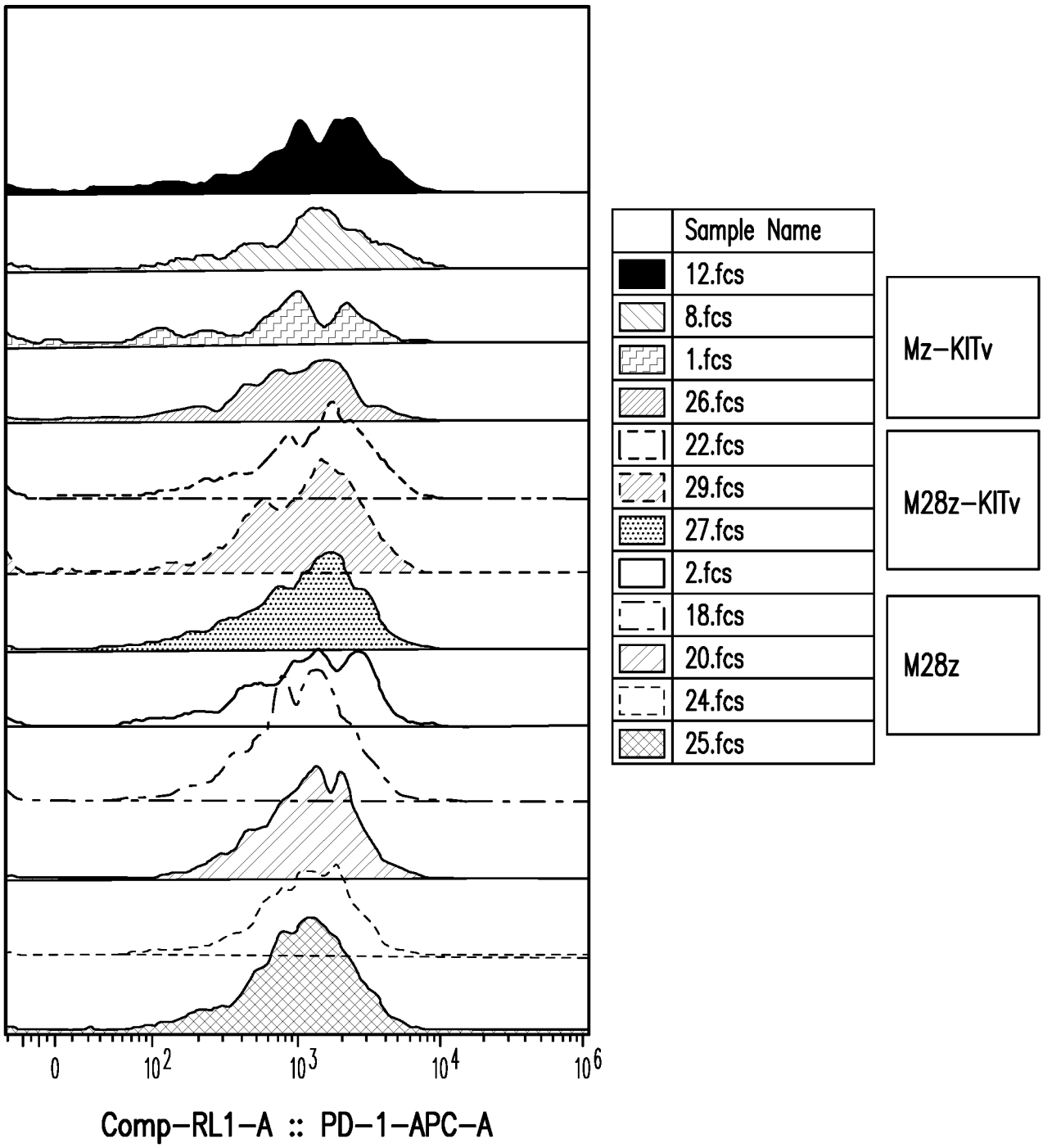

Sample Name
12.fcs
8.fcs
1.fcs
26.fcs
22.fcs
29.fcs
27.fcs
2.fcs
18.fcs
20.fcs
24.fcs
25.fcs
Mz-KITv
M28z-KITv
M28z
0
10^2
10^3
10^4
10^6
Comp-RL1-A :: PD-1-APC-A

Figure 21B

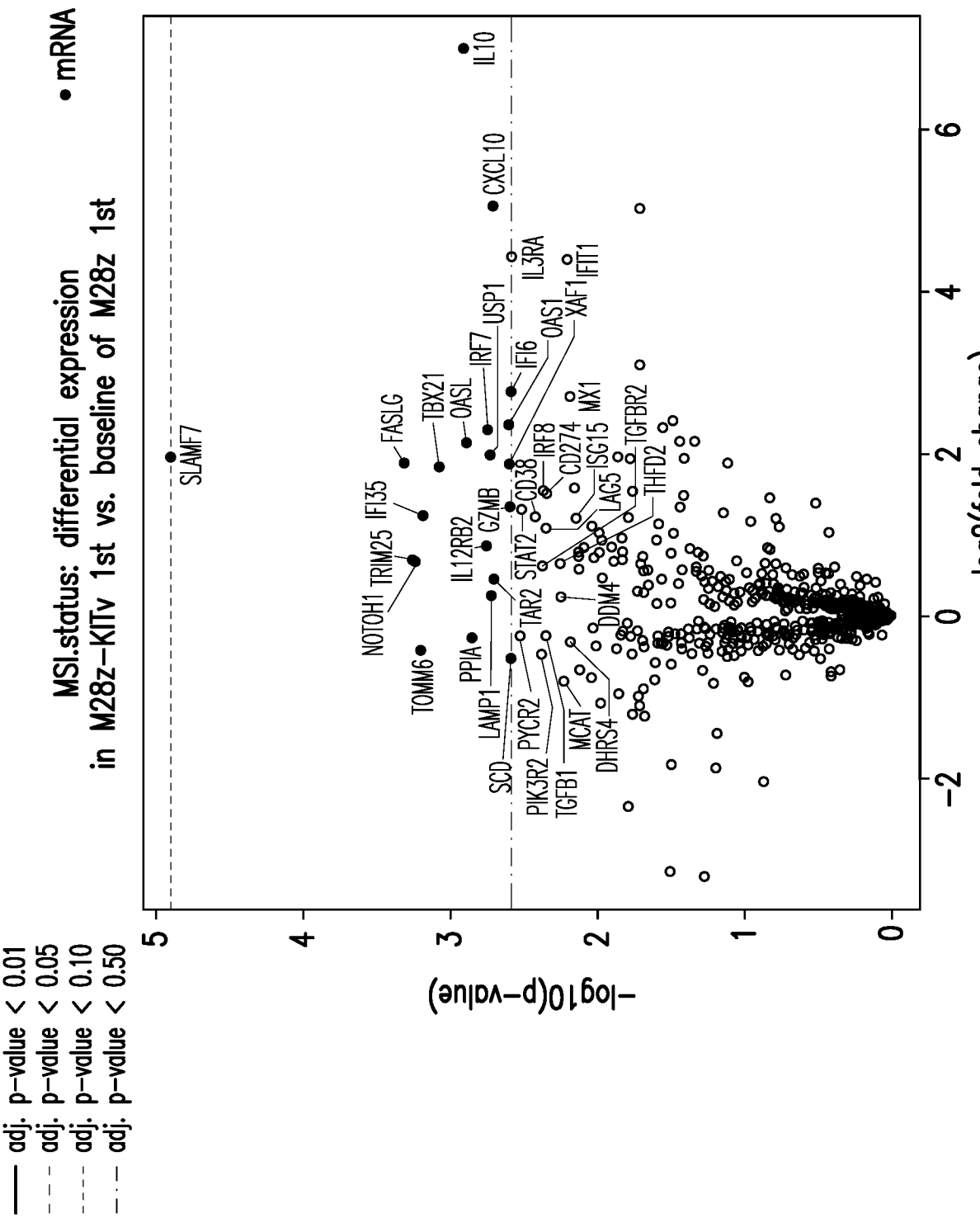

MSI.status: differential expression
in M28z−KITv 1st vs. baseline of M28z 1st
• mRNA
adj. p−value < 0.01
adj. p−value < 0.05
adj. p−value < 0.10
adj. p−value < 0.50
−log10(p−value)
log2(fold change)
SLAMF7
IL10
CXCL10
IL3RA
IFIT1
XAF1
OAS1
USP1
IRF7
IFI6
OASL
TBX21
FASLG
IFI35
TRIM25
NOTOH1
TOMM6
PPIA
IL12RB2
GZMB
CD38
IRF8
CD274
MX1
ISG15
LAG3
TGFBR2
THFD2
STAT2
TAR2
DDM4
LAMP1
SCD
PYCR2
PIK3R2
TGFB1
MCAT
DHRS4

Figure 22A

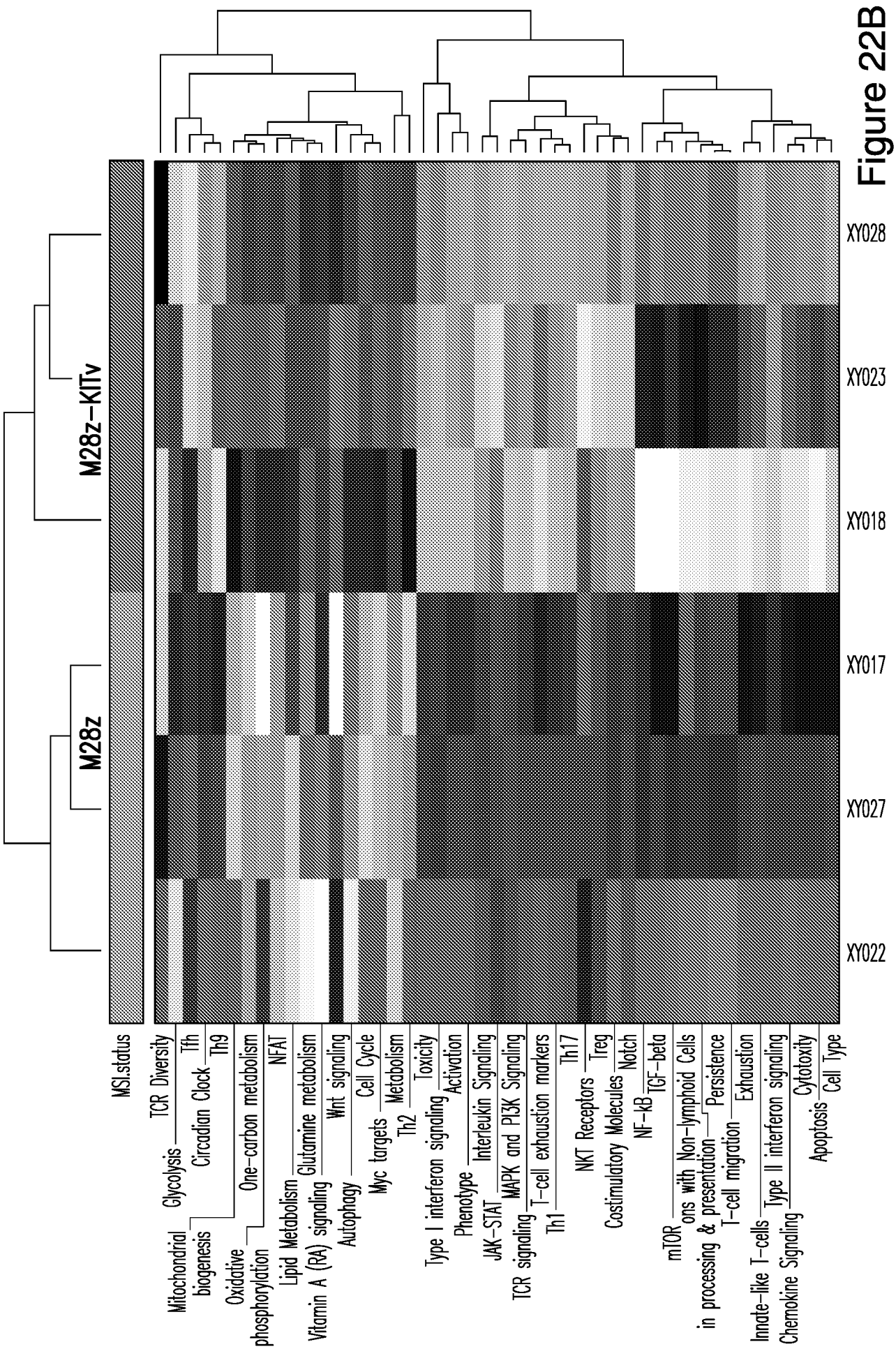

M28z
M28z-KITv
MSI.status
TCR Diversity
Glycolysis
Mitochondrial biogenesis
Tfh
Circadian Clock
Th9
Oxidative phosphorylation
One-carbon metabolism
NFAT
Lipid Metabolism
Glutamine metabolism
Vitamin A (RA) signaling
Wnt signaling
Autophagy
Cell Cycle
Myc targets
Metabolism
Th2
Toxicity
Type I interferon signaling
Activation
Phenotype
Interleukin Signaling
JAK-STAT
MAPK and PI3K Signaling
TCR signaling
T-cell exhaustion markers
Th1
Th17
NKT Receptors
Treg
Costimulatory Molecules
Notch
NF-kB
TGF-beta
mTOR
ons with Non-lymphoid Cells
in processing & presentation
Persistence
T-cell migration
Exhaustion
Innate-like T-cells
Type II interferon signaling
Chemokine Signaling
Cytotoxity
Apoptosis
Cell Type
XY028
XY023
XY018
XY017
XY027
XY022

Figure 22B

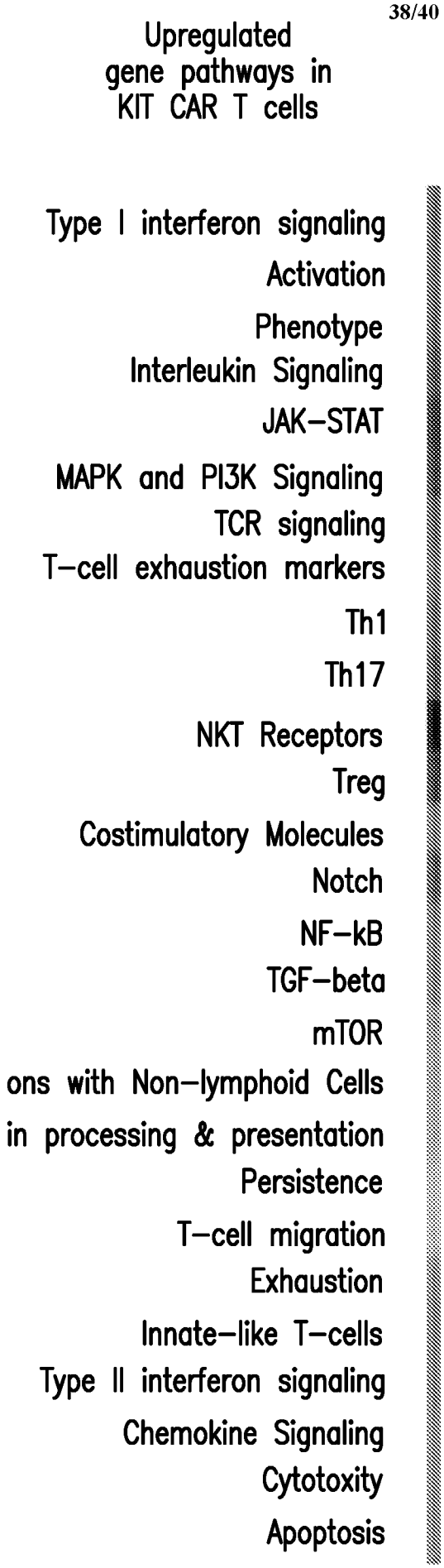

Upregulated
gene pathways in
KIT CAR T cells
Type I interferon signaling
Activation
Phenotype
Interleukin Signaling
JAK-STAT
MAPK and PI3K Signaling
TCR signaling
T-cell exhaustion markers
Th1
Th17
NKT Receptors
Treg
Costimulatory Molecules
Notch
NF-kB
TGF-beta
mTOR
ons with Non-lymphoid Cells
in processing & presentation
Persistence
T-cell migration
Exhaustion
Innate-like T-cells
Type II interferon signaling
Chemokine Signaling
Cytotoxity
Apoptosis

Figure 22C

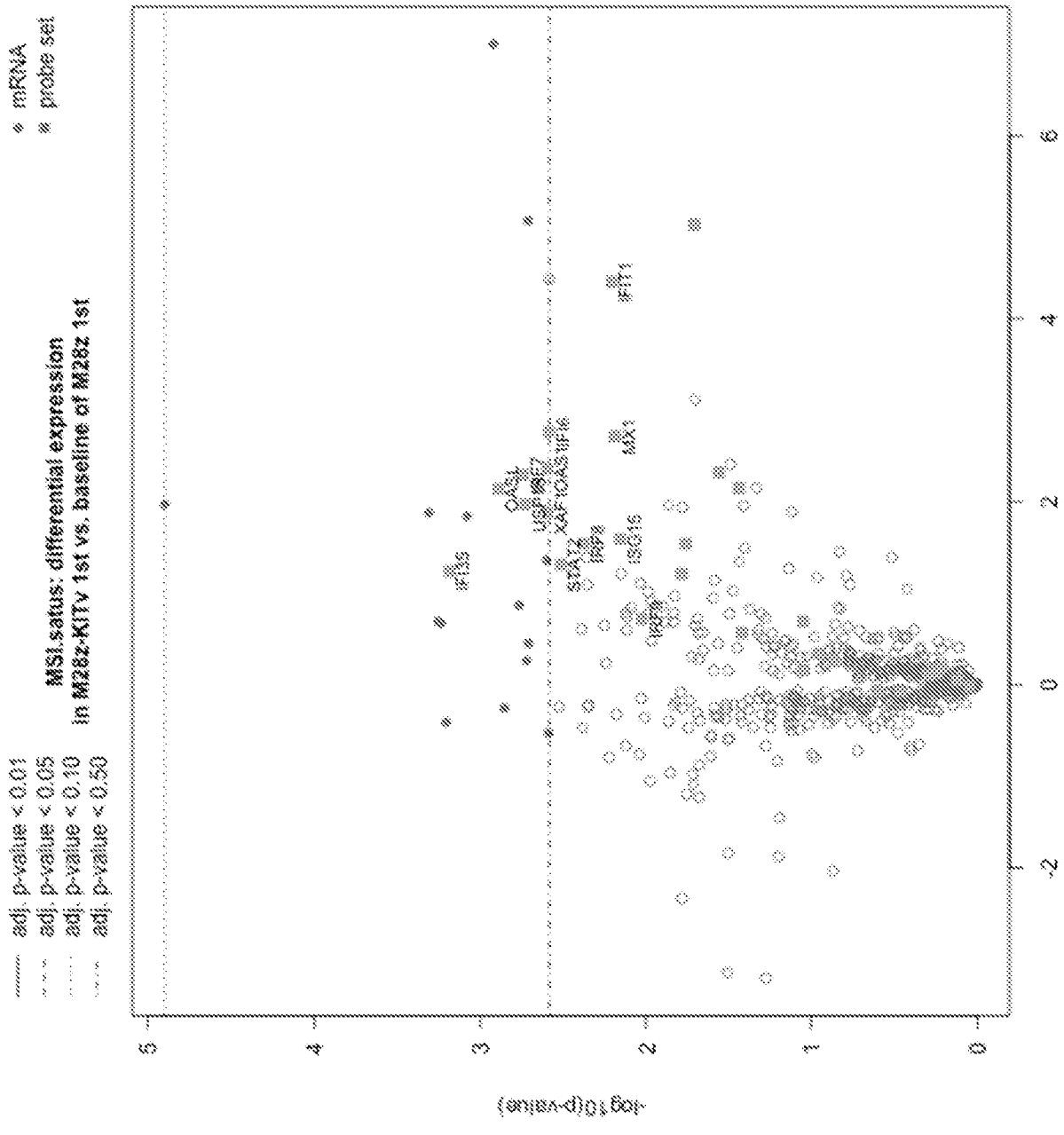


Figure 23A

Type I interferon signaling genes

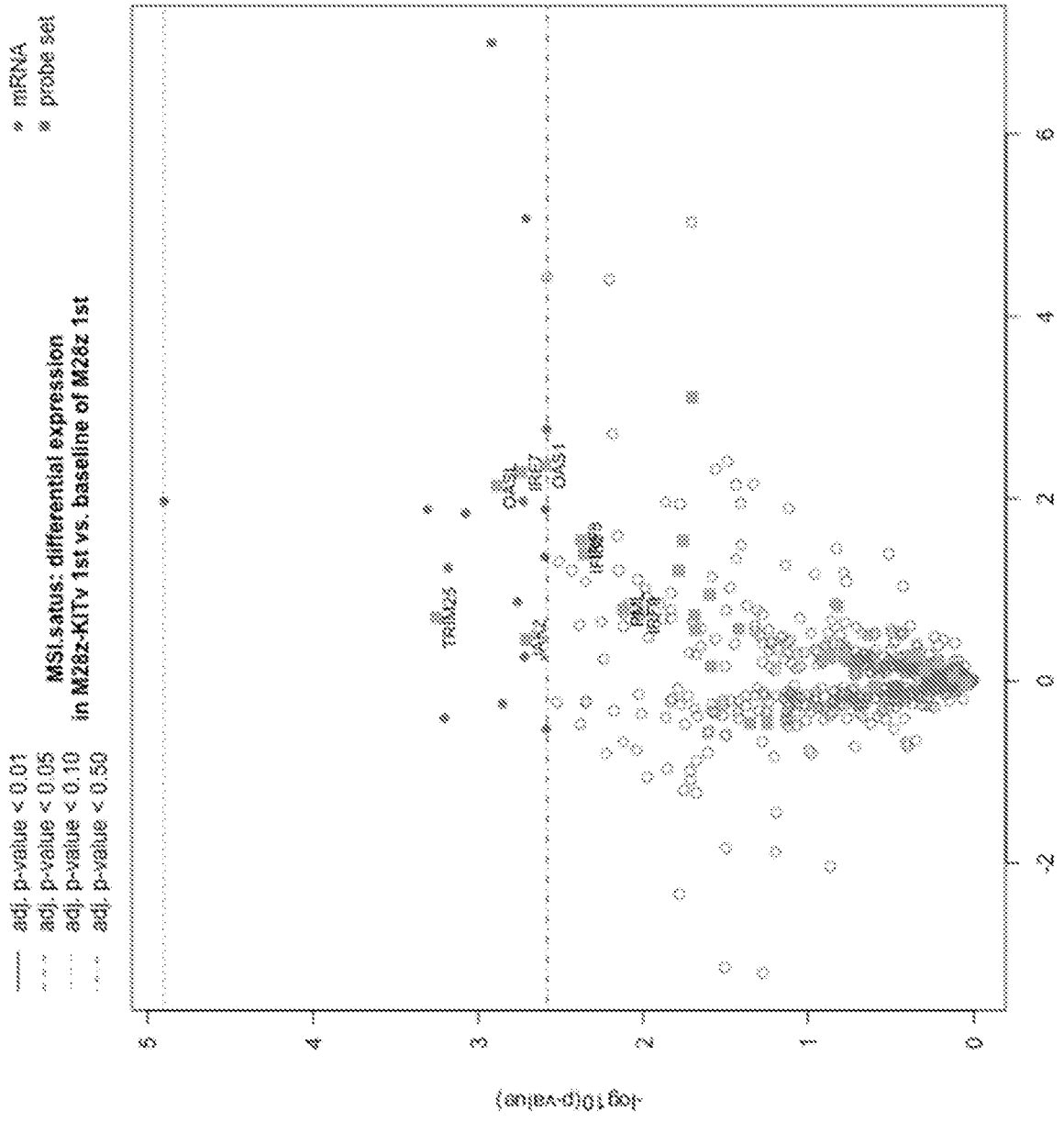


Figure 23B

Type II interferon signaling genes

SEQUENCE LISTING

<110> MEMORIAL SLOAN-KETTERING CANCER CENTER

<120> CELLS EXPRESSING C-KIT MUTATIONS AND USES THEREOF

<130> 072734.1174

<150> US 62/943,032
<151> 2019-12-03

<160> 52

<170> PatentIn version 3.5

<210> 1
<211> 976
<212> PRT
<213> Homo sapiens

<400> 1

Met Arg Gly Ala Arg Gly Ala Trp Asp Phe Leu Cys Val Leu Leu Leu
1 5 10 15

Leu Leu Arg Val Gln Thr Gly Ser Ser Gln Pro Ser Val Ser Pro Gly
20 25 30

Glu Pro Ser Pro Pro Ser Ile His Pro Gly Lys Ser Asp Leu Ile Val
35 40 45

Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val
50 55 60

Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn
65 70 75 80

Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr Asn Thr Gly Lys Tyr Thr
85 90 95

Cys Thr Asn Lys His Gly Leu Ser Asn Ser Ile Tyr Val Phe Val Arg
100 105 110

Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu
115 120 125

Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr
130 135 140

Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys Pro Leu Pro Lys Asp Leu
145 150 155 160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
165 170 175

Arg Ala Tyr His Arg Leu Cys Leu His Cys Ser Val Asp Gln Glu Gly
180 185 190

```
Lys Ser Val Leu Ser Glu Lys Phe Ile Leu Lys Val Arg Pro Ala Phe
        195                 200                 205

Lys Ala Val Pro Val Val Ser Val Ser Lys Ala Ser Tyr Leu Leu Arg
    210                 215                 220

Glu Gly Glu Glu Phe Thr Val Thr Cys Thr Ile Lys Asp Val Ser Ser
225                 230                 235                 240

Ser Val Tyr Ser Thr Trp Lys Arg Glu Asn Ser Gln Thr Lys Leu Gln
                245                 250                 255

Glu Lys Tyr Asn Ser Trp His His Gly Asp Phe Asn Tyr Glu Arg Gln
            260                 265                 270

Ala Thr Leu Thr Ile Ser Ser Ala Arg Val Asn Asp Ser Gly Val Phe
        275                 280                 285

Met Cys Tyr Ala Asn Asn Thr Phe Gly Ser Ala Asn Val Thr Thr Thr
    290                 295                 300

Leu Glu Val Val Asp Lys Gly Phe Ile Asn Ile Phe Pro Met Ile Asn
305                 310                 315                 320

Thr Thr Val Phe Val Asn Asp Gly Glu Asn Val Asp Leu Ile Val Glu
                325                 330                 335

Tyr Glu Ala Phe Pro Lys Pro Glu His Gln Gln Trp Ile Tyr Met Asn
            340                 345                 350

Arg Thr Phe Thr Asp Lys Trp Glu Asp Tyr Pro Lys Ser Glu Asn Glu
        355                 360                 365

Ser Asn Ile Arg Tyr Val Ser Glu Leu His Leu Thr Arg Leu Lys Gly
    370                 375                 380

Thr Glu Gly Gly Thr Tyr Thr Phe Leu Val Ser Asn Ser Asp Val Asn
385                 390                 395                 400

Ala Ala Ile Ala Phe Asn Val Tyr Val Asn Thr Lys Pro Glu Ile Leu
                405                 410                 415

Thr Tyr Asp Arg Leu Val Asn Gly Met Leu Gln Cys Val Ala Ala Gly
            420                 425                 430

Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe Cys Pro Gly Thr Glu Gln
        435                 440                 445

Arg Cys Ser Ala Ser Val Leu Pro Val Asp Val Gln Thr Leu Asn Ser
    450                 455                 460
```

```
Ser Gly Pro Pro Phe Gly Lys Leu Val Val Gln Ser Ser Ile Asp Ser
465                 470                 475                 480

Ser Ala Phe Lys His Asn Gly Thr Val Glu Cys Lys Ala Tyr Asn Asp
                485                 490                 495

Val Gly Lys Thr Ser Ala Tyr Phe Asn Phe Ala Phe Lys Gly Asn Asn
            500                 505                 510

Lys Glu Gln Ile His Pro His Thr Leu Phe Thr Pro Leu Leu Ile Gly
        515                 520                 525

Phe Val Ile Val Ala Gly Met Met Cys Ile Ile Val Met Ile Leu Thr
    530                 535                 540

Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln Trp Lys Val Val
545                 550                 555                 560

Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr Ile Asp Pro Thr Gln Leu
                565                 570                 575

Pro Tyr Asp His Lys Trp Glu Phe Pro Arg Asn Arg Leu Ser Phe Gly
            580                 585                 590

Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala
        595                 600                 605

Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met
    610                 615                 620

Leu Lys Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu
625                 630                 635                 640

Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val Asn Leu
                645                 650                 655

Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val Ile Thr Glu Tyr
            660                 665                 670

Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser
        675                 680                 685

Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys
    690                 695                 700

Asn Leu Leu His Ser Lys Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu
705                 710                 715                 720

Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala
                725                 730                 735
```

```
Asp Lys Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val
            740                 745                 750

Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp
        755                 760                 765

Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys Gly Met Ala Phe Leu Ala
    770                 775                 780

Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu
785                 790                 795                 800

Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp
                805                 810                 815

Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro
            820                 825                 830

Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe
        835                 840                 845

Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser
    850                 855                 860

Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr
865                 870                 875                 880

Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro
                885                 890                 895

Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu
            900                 905                 910

Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile
        915                 920                 925

Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro
    930                 935                 940

Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val
945                 950                 955                 960

Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
                965                 970                 975


<210>  2
<211>  976
<212>  PRT
<213>  Homo sapiens

<400>  2

Met Arg Gly Ala Arg Gly Ala Trp Asp Phe Leu Cys Val Leu Leu Leu
1               5                   10                  15
```

```
Leu Leu Arg Val Gln Thr Gly Ser Ser Gln Pro Ser Val Ser Pro Gly
            20                  25                  30

Glu Pro Ser Pro Pro Ser Ile His Pro Gly Lys Ser Asp Leu Ile Val
        35                  40                  45

Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val
    50                  55                  60

Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn
65                  70                  75                  80

Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr Asn Thr Gly Lys Tyr Thr
                85                  90                  95

Cys Thr Asn Lys His Gly Leu Ser Asn Ser Ile Tyr Val Phe Val Arg
            100                 105                 110

Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu
        115                 120                 125

Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr
    130                 135                 140

Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys Pro Leu Pro Lys Asp Leu
145                 150                 155                 160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
                165                 170                 175

Arg Ala Tyr His Arg Leu Cys Leu His Cys Ser Val Asp Gln Glu Gly
            180                 185                 190

Lys Ser Val Leu Ser Glu Lys Phe Ile Leu Lys Val Arg Pro Ala Phe
        195                 200                 205

Lys Ala Val Pro Val Val Ser Val Ser Lys Ala Ser Tyr Leu Leu Arg
    210                 215                 220

Glu Gly Glu Glu Phe Thr Val Thr Cys Thr Ile Lys Asp Val Ser Ser
225                 230                 235                 240

Ser Val Tyr Ser Thr Trp Lys Arg Glu Asn Ser Gln Thr Lys Leu Gln
                245                 250                 255

Glu Lys Tyr Asn Ser Trp His His Gly Asp Phe Asn Tyr Glu Arg Gln
            260                 265                 270

Ala Thr Leu Thr Ile Ser Ser Ala Arg Val Asn Asp Ser Gly Val Phe
        275                 280                 285
```

```
Met Cys Tyr Ala Asn Asn Thr Phe Gly Ser Ala Asn Val Thr Thr Thr
    290                 295                 300

Leu Glu Val Val Asp Lys Gly Phe Ile Asn Ile Phe Pro Met Ile Asn
305                 310                 315                 320

Thr Thr Val Phe Val Asn Asp Gly Glu Asn Val Asp Leu Ile Val Glu
                325                 330                 335

Tyr Glu Ala Phe Pro Lys Pro Glu His Gln Gln Trp Ile Tyr Met Asn
            340                 345                 350

Arg Thr Phe Thr Asp Lys Trp Glu Asp Tyr Pro Lys Ser Glu Asn Glu
        355                 360                 365

Ser Asn Ile Arg Tyr Val Ser Glu Leu His Leu Thr Arg Leu Lys Gly
    370                 375                 380

Thr Glu Gly Gly Thr Tyr Thr Phe Leu Val Ser Asn Ser Asp Val Asn
385                 390                 395                 400

Ala Ala Ile Ala Phe Asn Val Tyr Val Asn Thr Lys Pro Glu Ile Leu
                405                 410                 415

Thr Tyr Asp Arg Leu Val Asn Gly Met Leu Gln Cys Val Ala Ala Gly
            420                 425                 430

Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe Cys Pro Gly Thr Glu Gln
        435                 440                 445

Arg Cys Ser Ala Ser Val Leu Pro Val Asp Val Gln Thr Leu Asn Ser
    450                 455                 460

Ser Gly Pro Pro Phe Gly Lys Leu Val Val Gln Ser Ser Ile Asp Ser
465                 470                 475                 480

Ser Ala Phe Lys His Asn Gly Thr Val Glu Cys Lys Ala Tyr Asn Asp
                485                 490                 495

Val Gly Lys Thr Ser Ala Tyr Phe Asn Phe Ala Phe Lys Gly Asn Asn
            500                 505                 510

Lys Glu Gln Ile His Pro His Thr Leu Phe Thr Pro Leu Leu Ile Gly
        515                 520                 525

Phe Val Ile Val Ala Gly Met Met Cys Ile Ile Val Met Ile Leu Thr
    530                 535                 540

Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln Trp Lys Val Val
545                 550                 555                 560
```

```
Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr Ile Asp Pro Thr Gln Leu
                565                 570                 575

Pro Tyr Asp His Lys Trp Glu Phe Pro Arg Asn Arg Leu Ser Phe Gly
            580                 585                 590

Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala
        595                 600                 605

Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met
    610                 615                 620

Leu Lys Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu
625                 630                 635                 640

Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val Asn Leu
                645                 650                 655

Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val Ile Thr Glu Tyr
            660                 665                 670

Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser
        675                 680                 685

Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys
    690                 695                 700

Asn Leu Leu His Ser Lys Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu
705                 710                 715                 720

Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala
                725                 730                 735

Asp Lys Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val
            740                 745                 750

Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp
        755                 760                 765

Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys Gly Met Ala Phe Leu Ala
    770                 775                 780

Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu
785                 790                 795                 800

Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Val
                805                 810                 815

Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro
            820                 825                 830

Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe
```

```
        835                 840                 845

Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser
    850                 855                 860

Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr
865                 870                 875                 880

Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro
                885                 890                 895

Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu
            900                 905                 910

Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile
        915                 920                 925

Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro
    930                 935                 940

Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val
945                 950                 955                 960

Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
                965                 970                 975


<210>  3
<211>  622
<212>  PRT
<213>  Homo sapiens

<400>  3

Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
1               5                   10                  15

Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
            20                  25                  30

Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
        35                  40                  45

Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
    50                  55                  60

Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65                  70                  75                  80

Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
                85                  90                  95

Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
            100                 105                 110
```

```
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
        115                 120                 125

Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr His Phe Phe Ser Arg Ile
    130                 135                 140

Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145                 150                 155                 160

Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
                165                 170                 175

Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
            180                 185                 190

Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
        195                 200                 205

Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
    210                 215                 220

Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225                 230                 235                 240

Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
                245                 250                 255

Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
            260                 265                 270

Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
        275                 280                 285

Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
    290                 295                 300

Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305                 310                 315                 320

Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
                325                 330                 335

Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
            340                 345                 350

Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
        355                 360                 365

Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
    370                 375                 380
```

```
Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385                 390                 395                 400


Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
                405                 410                 415


Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
            420                 425                 430


Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
        435                 440                 445


Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
    450                 455                 460


Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465                 470                 475                 480


Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
                485                 490                 495


Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
            500                 505                 510


Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
        515                 520                 525


Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
    530                 535                 540


Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545                 550                 555                 560


Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
                565                 570                 575


Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
            580                 585                 590


Val Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
        595                 600                 605


Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
    610                 615                 620


<210>  4
<211>  10
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  4
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Gly Gly Ser Val Ser Ser Gly Ser Tyr Tyr
1 5 10

<210> 5
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 5

Ile Tyr Tyr Ser Gly Ser Thr
1 5

<210> 6
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 6

Ala Arg Glu Gly Lys Asn Gly Ala Phe Asp Ile Trp
1 5 10

<210> 7
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 7

Gln Ser Ile Ser Ser Tyr
1 5

<210> 8
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 8

Ala Ala Ser Ser
1

<210> 9
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 9

Gln Gln Ser Tyr Ser Thr Pro Leu Thr Phe
1 5 10

<210> 10
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 10

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30

Ser Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Glu Gly Lys Asn Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110

Thr Met Val Thr Val Ser Ser
115

<210> 11
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 11

Arg His Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

```
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Leu
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
            100                 105
```

<210> 12
<211> 357
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 12
caggttcagc ttcaggagag tggcccaggc ctggtgaagc caagtgagac tctcagcttg 60

acttgcacag tttctggagg cagtgtctcc tcaggcagct attattggtc ctggattcgg 120

cagccccctg ggaaaggcct ggagtggatt gggtacatat attacagtgg cagcacaaat 180

tacaatccat ccctgaagtc tcgagtaact atcagtgtgg acacaagcaa gaatcagttt 240

tcactcaaac tgtcttctgt gactgctgct gacactgctg tttattattg tgccagggag 300

gggaaaaatg gggcatttga tatttggggt cagggcacaa tggtgacagt cagctct 357

<210> 13
<211> 327
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 13
cgccatcaga tgactcagtc cccctccagt ctttctgcct cagttgggga tagagtgacc 60

atcacatgca gagcaagtca gagcatatca tcctatctga actggtacca gcagaagcca 120

gggaaagccc ccaaattgct gatttatgca gcctcaagtc tccagagtgg ggtgccaagc 180

aggttctcag gcagtggcag tgggacagat ttcacattga caatcagctc cctccaacct 240

gaagattttg ccacctacta ttgccagcaa tcctacagca cgcccctgac ttttggaggt 300

ggcacaaagg tagagatcaa gaggact 327

<210> 14
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 14

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30

Ser Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Glu Gly Lys Asn Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110

Thr Met Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Arg His Gln Met Thr Gln Ser Pro Ser Ser
130 135 140

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
145 150 155 160

Gln Ser Ile Ser Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
165 170 175

Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val
180 185 190

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
195 200 205

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
210 215 220

Ser Tyr Ser Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
225 230 235 240

Lys Arg Thr

<210> 15
<211> 729
<212> DNA

<213> Artificial Sequence

<220>
<223> Synthetic

<400> 15
caggttcagc ttcaggagag tggcccaggc ctggtgaagc caagtgagac tctcagcttg 60

acttgcacag tttctggagg cagtgtctcc tcaggcagct attattggtc ctggattcgg 120

cagccccctg ggaaaggcct ggagtggatt gggtacatat attacagtgg cagcacaaat 180

tacaatccat ccctgaagtc tcgagtaact atcagtgtgg acacaagcaa gaatcagttt 240

tcactcaaac tgtcttctgt gactgctgct gacactgctg tttattattg tgccagggag 300

gggaaaaatg gggcatttga tatttggggt cagggcacaa tggtgacagt cagctctgga 360

ggtggaggct caggaggagg aggcagtgga ggtggtgggt cacgccatca gatgactcag 420

tccccctcca gtctttctgc ctcagttggg gatagagtga ccatcacatg cagagcaagt 480

cagagcatat catcctatct gaactggtac cagcagaagc cagggaaagc ccccaaattg 540

ctgatttatg cagcctcaag tctccagagt ggggtgccaa gcaggttctc aggcagtggc 600

agtgggacag atttcacatt gacaatcagc tccctccaac ctgaagattt tgccacctac 660

tattgccagc aatcctacag cacgcccctg acttttggag gtggcacaaa ggtagagatc 720

aagaggact 729

<210> 16
<211> 20
<212> PRT
<213> Homo sapiens

<400> 16

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1   5   10   15

Asp Thr Thr Gly
            20

<210> 17
<211> 20
<212> PRT
<213> Mus musculus

<400> 17

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1   5   10   15

Gly Ser Thr Gly
            20

<210> 18
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic

<400> 18

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 19
ggaggtggag gctcaggagg aggaggcagt ggaggtggtg ggtca 45

<210> 20
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 20
ggtggaggcg gttcaggcgg aggtggctct ggcggtggcg gatca 45

<210> 21
<211> 235
<212> PRT
<213> Homo sapiens

<400> 21

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Ser Gln Phe Arg Val Ser Pro Leu Asp Arg Thr
20 25 30

Trp Asn Leu Gly Glu Thr Val Glu Leu Lys Cys Gln Val Leu Leu Ser
35 40 45

Asn Pro Thr Ser Gly Cys Ser Trp Leu Phe Gln Pro Arg Gly Ala Ala
50 55 60

Ala Ser Pro Thr Phe Leu Leu Tyr Leu Ser Gln Asn Lys Pro Lys Ala
65 70 75 80

Ala Glu Gly Leu Asp Thr Gln Arg Phe Ser Gly Lys Arg Leu Gly Asp
85 90 95

Thr Phe Val Leu Thr Leu Ser Asp Phe Arg Arg Glu Asn Glu Gly Tyr
100 105 110

Tyr Phe Cys Ser Ala Leu Ser Asn Ser Ile Met Tyr Phe Ser His Phe
115 120 125

```
Val Pro Val Phe Leu Pro Ala Lys Pro Thr Thr Thr Pro Ala Pro Arg
    130                 135                 140

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg
145                 150                 155                 160

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
                165                 170                 175

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr
            180                 185                 190

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His
        195                 200                 205

Arg Asn Arg Arg Arg Val Cys Lys Cys Pro Arg Pro Val Val Lys Ser
    210                 215                 220

Gly Asp Lys Pro Ser Leu Ser Ala Arg Tyr Val
225                 230                 235


<210>  22
<211>  247
<212>  PRT
<213>  Homo sapiens

<400>  22

Met Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Met
1               5                   10                  15

Gly Glu Ser Ile Ile Leu Gly Ser Gly Glu Ala Lys Pro Gln Ala Pro
            20                  25                  30

Glu Leu Arg Ile Phe Pro Lys Lys Met Asp Ala Glu Leu Gly Gln Lys
        35                  40                  45

Val Asp Leu Val Cys Glu Val Leu Gly Ser Val Ser Gln Gly Cys Ser
    50                  55                  60

Trp Leu Phe Gln Asn Ser Ser Ser Lys Leu Pro Gln Pro Thr Phe Val
65                  70                  75                  80

Val Tyr Met Ala Ser Ser His Asn Lys Ile Thr Trp Asp Glu Lys Leu
                85                  90                  95

Asn Ser Ser Lys Leu Phe Ser Ala Val Arg Asp Thr Asn Asn Lys Tyr
            100                 105                 110

Val Leu Thr Leu Asn Lys Phe Ser Lys Glu Asn Glu Gly Tyr Tyr Phe
        115                 120                 125
```

Cys Ser Val Ile Ser Asn Ser Val Met Tyr Phe Ser Ser Val Val Pro
    130   135   140

Val Leu Gln Lys Val Asn Ser Thr Thr Thr Lys Pro Val Leu Arg Thr
145   150   155   160

Pro Ser Pro Val His Pro Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu
                165   170   175

Asp Cys Arg Pro Arg Gly Ser Val Lys Gly Thr Gly Leu Asp Phe Ala
            180   185   190

Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Ile Cys Val Ala Pro
        195   200   205

Leu Leu Ser Leu Ile Ile Thr Leu Ile Cys Tyr His Arg Ser Arg Lys
    210   215   220

Arg Val Cys Lys Cys Pro Arg Pro Leu Val Arg Gln Glu Gly Lys Pro
225   230   235   240

Arg Pro Ser Glu Lys Ile Val
                245

<210> 23
<211> 69
<212> PRT
<213> Homo sapiens

<400> 23

Ser Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
1   5   10   15

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
            20   25   30

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp
        35   40   45

Ala Pro Leu Ala Gly Ile Cys Val Ala Leu Leu Leu Ser Leu Ile Ile
    50   55   60

Thr Leu Ile Cys Tyr
65

<210> 24
<211> 207
<212> DNA
<213> Homo sapiens

<400> 24
tctactacta ccaagccagt gctgcgaact ccctcacctg tgcaccctac cgggacatct   60

cagccccaga gaccagaaga ttgtcggccc cgtggctcag tgaaggggac cggattggac   120

ttcgcctgtg atatttacat ctgggcaccc ttggccggaa tctgcgtggc ccttctgctg 180

tccttgatca tcactctcat ctgctac 207

<210> 25
<211> 220
<212> PRT
<213> Homo sapiens

<400> 25

Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro Ser Ile Gln Val
1 5 10 15

Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
20 25 30

Asp Asn Ala Val Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
35 40 45

Arg Glu Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu
50 55 60

Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
65 70 75 80

Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
85 90 95

Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys
100 105 110

Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
115 120 125

Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
130 135 140

Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
145 150 155 160

Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
165 170 175

Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
180 185 190

Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
195 200 205

Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
210 215 220

<210> 26

<211> 81
<212> DNA
<213> Homo sapiens

<400> 26
ttttgggtgc tggtggtggt tggtggagtc ctggcttgct atagcttgct agtaacagtg 60

gcctttatta ttttctgggt g 81

<210> 27
<211> 27
<212> PRT
<213> Homo sapiens

<400> 27

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1   5   10   15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
            20   25

<210> 28
<211> 81
<212> DNA
<213> Homo sapiens

<400> 28
ttttgggtgc tggtggtggt tggtggagtc ctggcttgct atagcttgct agtaacagtg 60

gcctttatta ttttctgggt g 81

<210> 29
<211> 164
<212> PRT
<213> Homo sapiens

<400> 29

Met Lys Trp Lys Ala Leu Phe Thr Ala Ala Ile Leu Gln Ala Gln Leu
1   5   10   15

Pro Ile Thr Glu Ala Gln Ser Phe Gly Leu Leu Asp Pro Lys Leu Cys
            20   25   30

Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala
        35   40   45

Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
    50   55   60

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
65   70   75   80

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
                85   90   95

Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
            100   105   110

```
Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
        115                 120                 125

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
    130                 135                 140

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
145                 150                 155                 160

Leu Pro Pro Arg
```

<210> 30
<211> 112
<212> PRT
<213> Homo sapiens

<400> 30

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Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
1               5                   10                  15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            20                  25                  30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        35                  40                  45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Phe Asn Glu Leu Gln Lys
    50                  55                  60

Asp Lys Met Ala Glu Ala Phe Ser Glu Ile Gly Met Lys Gly Glu Arg
65                  70                  75                  80

Arg Arg Gly Lys Gly His Asp Gly Leu Phe Gln Gly Leu Ser Thr Ala
                85                  90                  95

Thr Lys Asp Thr Phe Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            100                 105                 110
```

<210> 31
<211> 336
<212> DNA
<213> Homo sapiens

<400> 31

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agagtgaagt tcagcaggag cgcagacgcc cccgcgtacc agcagggcca gaaccagctc       60

tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc      120

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgttcaat      180

gaactgcaga aagataagat ggcggaggcc ttcagtgaga ttgggatgaa aggcgagcgc      240

cggaggggca aggggcacga tggccttttc caggggctca gtacagccac caaggacacc      300
```

ttcgacgccc ttcacatgca ggccctgccc cctcgc 336

<210> 32
<211> 123
<212> DNA
<213> Homo sapiens

<400> 32
aggagtaaga ggagcaggct cctgcacagt gactacatga acatgactcc ccgccgcccc 60

gggcccaccc gcaagcatta ccagccctat gccccaccac gcgacttcgc agcctatcgc 120

tcc 123

<210> 33
<211> 218
<212> PRT
<213> Mus musculus

<400> 33

Met Thr Leu Arg Leu Leu Phe Leu Ala Leu Asn Phe Phe Ser Val Gln
1 5 10 15

Val Thr Glu Asn Lys Ile Leu Val Lys Gln Ser Pro Leu Leu Val Val
20 25 30

Asp Ser Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
35 40 45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
50 55 60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
65 70 75 80

Ser Asn Ala Glu Phe Asn Cys Asp Gly Asp Phe Asp Asn Glu Thr Val
85 90 95

Thr Phe Arg Leu Trp Asn Leu His Val Asn His Thr Asp Ile Tyr Phe
100 105 110

Cys Lys Ile Glu Phe Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Arg
115 120 125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Thr
130 135 140

Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Val Val Val Ala Gly Val
145 150 155 160

Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala Leu Cys Val Ile Trp
165 170 175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser Asp Tyr Met Asn Met
180 185 190

```
Thr Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala
        195                 200                 205


Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
    210                 215


<210>  34
<211>  63
<212>  DNA
<213>  Mus musculus

<400>  34
aatagtagaa ggaacagact ccttcaaagt gactacatga acatgactcc ccggaggcct       60

ggg                                                                     63


<210>  35
<211>  288
<212>  PRT
<213>  Homo sapiens

<400>  35

Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln
1               5                   10                  15


Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp
            20                  25                  30


Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp
        35                  40                  45


Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val
    50                  55                  60


Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala
65                  70                  75                  80


Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
                85                  90                  95


Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg
            100                 105                 110


Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
        115                 120                 125


Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
    130                 135                 140


Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro
145                 150                 155                 160


Arg Pro Ala Gly Gln Phe Gln Thr Leu Val Val Gly Val Val Gly Gly
```

```
                165                 170                 175

Leu Leu Gly Ser Leu Val Leu Leu Val Trp Val Leu Ala Val Ile Cys
            180                 185                 190

Ser Arg Ala Ala Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln Pro
        195                 200                 205

Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr Gly
    210                 215                 220

Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val Pro
225                 230                 235                 240

Cys Val Pro Glu Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser Gly
                245                 250                 255

Met Gly Thr Ser Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro Arg
            260                 265                 270

Ser Ala Gln Pro Leu Arg Pro Glu Asp Gly His Cys Ser Trp Pro Leu
        275                 280                 285
```

<210> 36
<211> 435
<212> DNA
<213> Homo sapiens

<400> 36
ccaggatggt tcttagactc cccagacagg ccctggaacc cccccacctt ctccccagcc 60

ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg 120

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc 180

gctttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg 240

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc 300

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca 360

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc 420

aggccagccg gccag 435

<210> 37
<211> 60
<212> DNA
<213> Homo sapiens

<400> 37
atgcagatcc cacaggcgcc ctggccagtc gtctgggcgg tgctacaact gggctggcgg 60

<210> 38
<211> 495
<212> DNA
<213> Homo sapiens

<400> 38

atgcagatcc cacaggcgcc ctggccagtc gtctgggcgg tgctacaact gggctggcgg 60

ccaggatggt tcttagactc cccagacagg ccctggaacc cccccacctt ctccccagcc 120

ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg 180

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc 240

gctttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg 300

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc 360

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca 420

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc 480

aggccagccg gccag 495

<210> 39
<211> 213
<212> DNA
<213> Homo sapiens

<400> 39
cccaccacga cgccagcgcc gcgaccacca accccggcgc ccacgatcgc gtcgcagccc 60

ctgtccctgc gcccagaggc gtgccggcca gcggcggggg gcgcagtgca cacgaggggg 120

ctggacttcg cctgtgatat ctacatctgg gcgcccctgg ccgggacttg tggggtcctt 180

ctcctgtcac tggttatcac cctttactgc aac 213

<210> 40
<211> 219
<212> DNA
<213> Homo sapiens

<400> 40
cccaccacga cgccagcgcc gcgaccacca accccggcgc ccacgatcgc gtcgcagccc 60

ctgtccctgc gcccagaggc gtgccggcca gcggcggggg gcgcagtgca cacgaggggg 120

ctggacttcg cctgtgatat ctacatctgg gcgcccctgg ccgggacttg tggggtcctt 180

ctcctgtcac tggttatcac cctttactgc aaccacagg 219

<210> 41
<211> 239
<212> PRT
<213> Homo sapiens

<400> 41

Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln
1 5 10 15

Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp
20 25 30

Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp
35 40 45

Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val

```
    50                  55                  60

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala
65                  70                  75                  80

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
                85                  90                  95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg
            100                 105                 110

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
        115                 120                 125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
    130                 135                 140

Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro
145                 150                 155                 160

Arg Pro Ala Gly Gln Ala Ala Ala Pro Thr Thr Thr Pro Ala Pro Arg
                165                 170                 175

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg
            180                 185                 190

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
        195                 200                 205

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr
    210                 215                 220

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn
225                 230                 235


<210>  42
<211>  717
<212>  DNA
<213>  Homo sapiens

<400>  42
atgcagatcc cacaggcgcc ctggccagtc gtctgggcgg tgctacaact gggctggcgg       60

ccaggatggt tcttagactc cccagacagg ccctggaacc cccccacctt ctccccagcc      120

ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg      180

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc      240

gctttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg      300

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc      360

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca      420

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc      480
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aggccagccg gccaggcggc cgcacccacc acgacgccag cgccgcgacc accaaccccg 540

gcgcccacga tcgcgtcgca gcccctgtcc ctgcgcccag aggcgtgccg gccagcggcg 600

gggggcgcag tgcacacgag ggggctggac ttcgcctgtg atatctacat ctgggcgccc 660

ctggccggga cttgtggggt ccttctcctg tcactggtta tcacccttta ctgcaac 717

<210> 43
<211> 241
<212> PRT
<213> Homo sapiens

<400> 43

Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln
1 5 10 15

Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp
20 25 30

Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp
35 40 45

Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val
50 55 60

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala
65 70 75 80

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
85 90 95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg
100 105 110

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
115 120 125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
130 135 140

Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro
145 150 155 160

Arg Pro Ala Gly Gln Ala Ala Ala Pro Thr Thr Thr Pro Ala Pro Arg
165 170 175

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg
180 185 190

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
195 200 205

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr

```
    210                 215                 220

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His
225                 230                 235                 240

Arg
```

<210> 44
<211> 723
<212> DNA
<213> Homo sapiens

<400> 44

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atgcagatcc cacaggcgcc ctggccagtc gtctgggcgg tgctacaact gggctggcgg       60

ccaggatggt tcttagactc cccagacagg ccctggaacc cccccacctt ctccccagcc      120

ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg      180

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc      240

gctttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg      300

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc      360

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca      420

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc      480

aggccagccg gccaggcggc cgcacccacc acgacgccag cgccgcgacc accaaccccg      540

gcgcccacga tcgcgtcgca gcccctgtcc ctgcgcccag aggcgtgccg gccagcggcg      600

gggggcgcag tgcacacgag ggggctggac ttcgcctgtg atatctacat ctgggcgccc      660

ctggccggga cttgtggggt ccttctcctg tcactggtta tcacccttta ctgcaaccac      720

agg                                                                    723
```

<210> 45
<211> 20
<212> PRT
<213> Homo sapiens

<400> 45

```
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                   10                  15

Val Thr Asn Ser
            20
```

<210> 46
<211> 20
<212> PRT
<213> Mus musculus

<400> 46

```
Met Tyr Ser Met Gln Leu Ala Ser Cys Val Thr Leu Thr Leu Val Leu
1               5                   10                  15
```

Leu Val Asn Ser
            20

<210> 47
<211> 20
<212> PRT
<213> Homo sapiens

<400> 47

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1   5   10   15

Asp Thr Thr Gly
            20

<210> 48
<211> 20
<212> PRT
<213> Mus musculus

<400> 48

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1   5   10   15

Gly Ser Thr Gly
            20

<210> 49
<211> 21
<212> PRT
<213> Homo sapiens

<400> 49

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1   5   10   15

His Ala Ala Arg Pro
            20

<210> 50
<211> 18
<212> PRT
<213> Homo sapiens

<400> 50

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1   5   10   15

His Ala

<210> 51
<211> 16
<212> PRT
<213> Homo sapiens

<400> 51

Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Ser Ser Ala Tyr Ser
1   5   10   15

<210> 52
<211> 30
<212> PRT
<213> Homo sapiens

<400> 52

Met Asp Ser Lys Gly Ser Ser Gln Lys Gly Ser Arg Leu Leu Leu Leu
1   5   10   15

Leu Val Val Ser Asn Leu Leu Leu Cys Gln Gly Val Val Ser
            20   25   30