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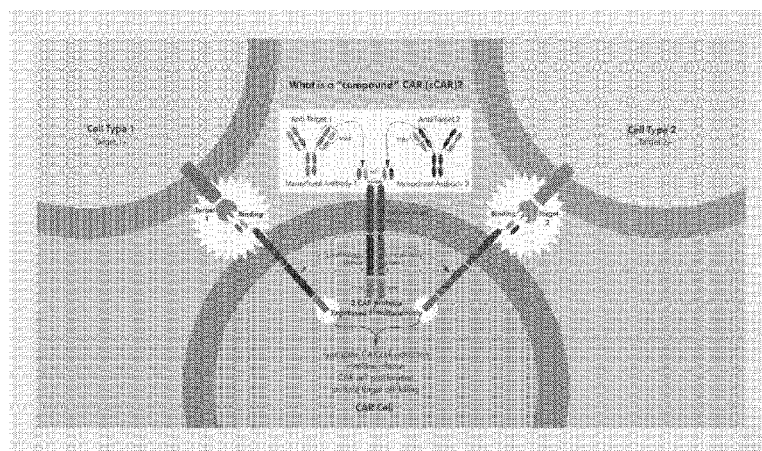
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(54) Title: CHIMERIC ANTIGEN RECEPTORS (CARs), COMPOSITIONS AND METHODS OF USE THEREOF

FIG. 1



(57) Abstract: The present invention relates to compositions and methods relating to chimeric antigen receptor (CAR) polypeptides and methods relating thereto. In one embodiment, the present invention relates to engineered cells having chimeric antigen receptor polypeptides directed to at least two targets. In another embodiment, the present invention relates to engineered cells having chimeric antigen receptor polypeptides and an enhancer moiety.



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## **CHIMERIC ANTIGEN RECEPTORS (CARs), COMPOSITIONS AND METHODS THEREOF**

### **CROSS-REFERENCE TO RELATED APPLICATIONS**

5           This application is an International PCT Application claiming priority from US Provisional Application Nos. 62/184,321, filed June 25, 2015; 62/235,840, filed on October 1, 2015; and 62/244, 435, filed October 21, 2015 all of which are incorporated herein by reference in its entirety.

### **BACKGROUND**

10           T cells, a type of lymphocyte, play a central role in cell-mediated immunity. They are distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. T helper cells, also called CD4+ T or CD4 T cells, express CD4 glycoprotein on their surface. Helper T cells are activated when exposed to peptide antigens presented by MHC (major histocompatibility complex) class II  
15 molecules. Once activated, these cells proliferate rapidly and secrete cytokines that regulate immune response. Cytotoxic T cells, also known as CD8+ T cells or CD8 T cells, express CD8 glycoprotein on the cell surface. The CD8+ T cells are activated when exposed to peptide antigens presented by MHC class I molecules. Memory T cells, a subset of T cells, persist long term and respond to their cognate antigen, thus providing the immune system with “memory”  
20 against past infections and/or tumor cells.

T cells can be genetically engineered to produce special receptors on their surface called chimeric antigen receptors (CARs). CARs are proteins that allow the T cells to recognize a specific protein (antigen) on tumor cells. These engineered CAR T cells are then grown in the



laboratory until they number in the billions. The expanded population of CAR T cells is then infused into the patient.

Clinical trials to date have shown chimeric antigen receptor (CAR) T cells to have great promise in hematologic malignancies resistant to standard chemotherapies. Most notably, CD19-specific CAR (CD19CAR) T-cell therapies have had remarkable results including long-term remissions in B-cell malignancies (Kochenderfer, Wilson et al. 2010, Kalos, Levine et al. 2011, Porter, Levine et al. 2011, Davila, Riviere et al. 2013, Grupp, Frey et al. 2013, Grupp, Kalos et al. 2013, Kalos, Nazimuddin et al. 2013, Kochenderfer, Dudley et al. 2013, Kochenderfer, Dudley et al. 2013, Lee, Shah et al. 2013, Park, Riviere et al. 2013, Maude, Frey et al. 2014).

Despite the success of CAR therapy in B-cell leukemia and lymphoma, the application of CAR therapy to T-cell malignancies has not yet been well established. Given that T-cell malignancies are associated with dramatically poorer outcomes compared to those of B-cell malignancies (Abramson, Feldman et al. 2014), CAR therapy in this respect has the potential to further address a great clinical need.

To date, current efforts have focused on CAR T-cells demonstrating efficacy in various B-cell malignancies. While initial remission rates of approximately 90% are common in B-ALL using CD19CAR, most of these relapse within a year. The relapse is at least in part due to the antigen escape. Thus, more effective CAR T cell treatments in order to prevent the relapse is urgently needed. Target discovery and selection are the initial step as there are no general rules to ensure or guide CAR design that are efficacious.

There are some roadblocks that hinder the broader adoption of CAR therapeutic approach. Among the most general challenges are: (1) selection of antigen target and chimeric

antigen receptor(s); (2)CAR design; (3)tumor heterogeneity, particularly the variance in the surface expression of tumor antigens. Targeting single antigen carries the risk of immune escape and this could be overcome by targeting multiple desired antigens.

Most CAR chimeric antigen receptors are scFvs derived from monoclonal antibodies and  
5 some of these monoclonal antibodies have been used in the clinical trials or treatment for diseases. However, they have limited efficacy, which suggests that alternative and more potent targeting approaches, such as CARs are required. scFvs are the most commonly used chimeric antigen receptor for CARs. However, CAR affinity binding and locations of the recognized epitope on the antigen could affect the function. Additionally the level of the surface CAR  
10 expression on the T cells or NK cells is affected by an appropriate leader sequence and promoter. Furthermore, overexpressed CAR proteins can be toxic to cells.

Therefore, there remains a need for improved chimeric antigen receptor-based therapies that allow for more effective, safe, and efficient targeting of T-cell associated malignancies.

### SUMMARY OF THE INVENTION

15 In one embodiment, the present disclosure provides an engineered cell having a first chimeric antigen receptor polypeptide including a first antigen recognition domain, a first signal peptide, a first hinge region, a first transmembrane domain, a first co-stimulatory domain, and a first signaling domain; and a second chimeric antigen receptor polypeptide including a second antigen recognition domain, a second signal peptide, a second hinge region, a second  
20 transmembrane domain, a second co-stimulatory domain, and a second signaling domain; wherein the first antigen recognition domain is different than the second antigen recognition domain.

In another embodiment, the present disclosure provides an engineered polypeptide including a chimeric antigen receptor and an enhancer.

In another embodiment, the present disclosure provides an engineered polypeptide including a chimeric antigen receptor polypeptide and an enhancer.

5           In another embodiment, the present disclosure provides an engineered chimeric antigen receptor polypeptide, the polypeptide including: a signal peptide, a CD45 antigen recognition domain, a hinge region, a transmembrane domain, at least one co-stimulatory domain, and a signaling domain. In another embodiment, the present disclosure provides a polynucleotide encoding for the aforementioned polypeptide.

10           In another embodiment, the present disclosure provides an engineered cell having the engineered polypeptide or polynucleotide described above.

          In another embodiment, the present disclosure provides a method of reducing the number of target cells including the steps of (i.)           contacting said target cells with an effective amount of an engineered cell having at least one chimeric antigen receptor polypeptide, for engineered  
15   cells having multiple chimeric antigen receptor polypeptides, each chimeric antigen receptor polypeptides are independent; and (ii.) optionally, assaying for the reduction in the number of said cells. The target cells include at least one cell surface antigen selected from the group consisting of interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3),  
BAFF-R, BCMA, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41,  
20   CD45, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, and CS1.

In another embodiment, the present disclosure provides methods for treating B-cell lymphoma, T-cell lymphoma, multiple myeloma, chronic myeloid leukemia, B-cell acute lymphoblastic leukemia (B-ALL), and cell proliferative diseases by administering any of the engineered cells described above to a patient in need thereof.

## BRIEF DESCRIPTION OF DRAWINGS

Figure 1. A schematic representation of cCAR construct (hereinafter, "multiple CAR or compound CAR"). Multiple or compound CAR targets multiple antigens (e.g. cell type 1 or cell type 2 or the same cell type). Multiple or cCAR T cell immunotherapies comprises individual component CAR comprising a different or same antigen recognition domain, a hinge region, a transmembrane domain, various co-stimulatory domain(s) and an intracellular signaling domain.

Figure 2A. A schematic representation of cCAR-T construct. The construct comprises a SFFV promoter driving the expression of-multiple modular units of CARs linked by a P2A peptide. Upon cleavage of the linker, the cCARs split and engage upon targets expressing CD33 and/or CD123. As a novel cCAR construct, the activation domains of the construct may include, but is not limited to, 4-1BB on the CD33 CAR segment and a CD28 region on the CD123 CAR.

Figure 2B. A Western blot depicting the expression of transduced CD33CD123 cCAR-T cells. The figure depicts expression of two different CAR proteins, i.e., CD33 CAR and CD123 CARs. The cCAR-T cells expressing both CD33 and CD123 CARs upon cleavage of the linker generate two distinct and consistently intense protein bands. Green Fluroscent Protein (GFP) is included as negative control.

Figure 2C. Flow cytometry representing the efficiency of transduction. Upper panel shows the lentiviral titer for CD33CD123 cCARs (also referred to as CD33CD123-2G-CAR)

tested on 293FT HEK (human embryonic kidney) cells to gauge maximum transduction efficiency before usage on UCB (umbilical cord blood) and PB (peripheral blood) T-cells.

Lower panel shows CD33CD123 cCAR (also referred to as CD33CD123-2G-CAR) T-cells transduced with lentiviral vectors comprising CD33CD123 cCAR construct and GFP-

5 transduced cells as control Percentages indicated by yellow circles are proxies for transduction efficiency.

Figure 3. Schematic showing a method of generating a high-efficiency compound CAR (cCAR).

Figure 4. A co-culture assay representing the incubation of CD33CD123-2G CAR-T cells  
10 (cCAR) with the promyelocytic leukemia cell line HL60. cCAR-T cell (lower panel) is compared to control GFP transduced T-cell (upper panel). The efficacy of the killing is measured by the population of CD33+ cells that is left over after incubation for about 24 hours (enclosed in yellow circles).

Figure 5. A co-culture assay representing incubation of cCAR-T cells with the  
15 myelogenous leukemia cell line KG-1a, which expresses about 100% CD33 and about 50-80% CD123. cCAR-T cell (lower panel) is compared to control GFP transduced T-cell (upper panel). The efficacy of the killing is measured by the population of CD33+ cells that is left over after incubation for about 24 hours.

Figure 6. A co-culture assay representing incubation of cCAR-T cells with AML patient  
20 samples (here referred to as AML-9). The patient cells include mixed populations of cells, such as for example, leukemia cells, monocytes, and other types of blasts. CD33 acts as a marker for CAR-T action as well as CD34, a specific marker for leukemia cells. The CAR-T panel (right) is

compared to control GFP transduced T-cells (middle). The efficacy of the killing is measured by the population of CD33+/CD34+ cells that is left over after incubation for at least 24 hours.

Figure 7. A co-culture assay representing incubation of cCAR-T cells with B-ALL patient samples (here referred to as Sp-BM-B6). The patient cells include mixed populations of cells, such as, for example, leukemia cells, monocytes, and other types of blasts. CD34 acts as a specific marker for leukemia cells. The CAR-T panel (right) is compared to control GFP transduced T-cells (middle). The efficacy of the killing is measured by the population of CD34+ cells left over after incubation for at least 24 hours.

Figure 8. CD33CD123 cCAR expression in NK-92 cells. The CD33CD123 cCAR expression are detected using goat-anti-mouse antibody, F(ab)2.

Figure 9. A co-culture assay representing incubation of cCAR NK-92 cells with HL-60. The cCAR NK-92 cells are compared with GFP transduced NK-92 cells. The efficacy of the killing is measured by the population of CD33+ cells left over after incubation for about 24 hours.

Figure 10. A co-culture assay representing incubation of cCAR NK-92 cells with KG1a. The cCAR NK cell panel is compared with GFP transduced NK-92 cells. The efficacy of the killing is measured by the population of CD33+ cells left over after incubation for about 24 hours.

Figure 11. Dose response of CD33CD123 cCAR (CAR-CD33/123) NK-92 cells with HL-60 or KG1a.

The efficacy of the killing is measured by the population of CD33+ cells left over after incubation for about 24 hours.

Figure 12. A comparison of CD33CD123 cCAR NK-92 cell killing ability with control in two populations of KG11 cells. Assays were performed at different ratios of CAR-CD33/123 (CD33CD123 cCAR NK-92 cells) and target cells, kG1a. The efficacy of the killing is measured by the population of CD33+CD123+ or CD33+CD123- cells left over after incubation for about 24 hours.

Figure 13. A schematic representation of cCAR. The construct comprises a SFFV promoter driving the expression of multiple modular units of CARs linked by a linker. Upon cleavage of the linker, the cCARs split and engage upon targets expressing combinations of various target antigens: CD19 and/or CD20, and/or CD22 and/or 138. Multiple cCARs utilize the same or different co-stimulatory domains, such as, without limiting 4-1BB (also labeled as 4-BB) and/or CD28.

Figures 14 A-C. BCMA-CS1 cCAR construct scheme (BC1cCAR). (A) The construct consists a SFFV promoter driving the expression of two modular units of CAR linked by a P2A peptide. Upon cleavage of this P2A peptide, the cCARs split and engage upon targets expressing BCMA and /or CS1. Two unit CARs use same co-stimulatory domain, 4-1BB. (B) Flow cytometry analysis of BC1cCAR expression on T cell surface for vector (left) and BC1cCAR (right, highlighted by a square) showing 15.3% positive for F(Ab)2. Gating done against isotype controls. (C) Preliminary functional validation of BC1cCAR-T cells by co-culturing K562 cells transduced with BCMA cDNA (BCMA-K562) (obtained from Kochenderfer, NIH). Bar graph shows lysis of the BCMA-K562 cell line vs. control T-cells as well as lysis of wild-type K562 (wt-K562) vs. control.

Figure 14D. BCMA-CS1-2G construct using two different co-stimulatory domains either 4-1BB or CD28 for each unit. The construct includes a SFFV promoter driving the expression of

two modular units of CAR linked by a P2A peptide. Upon cleavage of this P2A peptide, the cCARs split and engage targets expressing BCMA and /or CS1. Two unit CARs use a different co-stimulatory domain, either 4-1BB or CD28. Flow cytometry analysis of BC1cCAR expression on T cell surface for vector (left) and BC1cCAR (right, highlighted by a square) showing rare positive cells for F(Ab)2. Gating done against isotype controls.

Figure 14E. Protein expression of BC1cCAR and BCMA-CS1-2G in HEK-293FT cells. HEK-293FT cells were transfected with lentiviral plasmids for GFP (lane 1), BC1cCAR (lane 2), CD269-CS1-2G (lane 3) 48 hours after transfection, supernatant was removed, and cells were also removed. Cells were lysed for Western blot and probe with mouse anti-human CD3z antibody.

Figures 15A-B. MM1S cell line co-culture. Co-cultures were carried out under 24 hours and collected and analyzed via flow cytometry. Target MM1S cells (myeloma cells) were labeled with Cytotracker (CMTMR) dye to distinguish it from effector T-cells. Populations were gated by anti-BCMA (CD269) and anti-CS1 (CD319) antibodies. Figure 15A: Flow cytometry depictions of co-cultures. Figure 15B: right: graphical summary of lysis vs. E:T ratio.

Figures 16A-B. RPMI-8226 cell line co-culture. Co-cultures were carried out under 24 hours and collected and analyzed via flow cytometry. Target RPMI-8226 cells were labeled with Cytotracker (CMTMR) dye to distinguish it from effector T-cells. Populations were gated by anti-BCMA (CD269) and anti-CS1 (CD319) antibodies. Figure 16A: flow cytometry depictions of co-cultures. Figure 16B: graphical summary of lysis vs. E:T ratio.

Figures 17 A-B. U266 cell line co-culture. Co-cultures were carried out under 24 hours and collected, and analyzed via flow cytometry. Target U266 cells were labeled with Cytotracker



(CMTMR) dye to distinguish it from effector T-cells. Populations were gated by anti-BCMA (CD269) and anti-CS1 (CD319) antibodies. (A) flow cytometry depictions of co-cultures. (B) graphical summary of lysis vs. E:T ratio.

Figures 18A-B. MM10-G primary patient sample co-culture and specific lysis. Co-cultures were carried out under 24 hours and collected and analyzed via flow cytometry. Target MM10-G cells were labeled with Cytotracker (CMTMR) dye to distinguish it from effector T-cells. Populations were gated by anti-BCMA (CD269) and anti-CS1 (CD319) antibodies. Notably, gating shows MM10-G presenting with distinct BCMA<sup>+</sup> and CS1<sup>+</sup> populations. Figure 18A: flow cytometry depictions of co-cultures. Figure 18B: graphical summary of lysis vs. E:T ratio.

Figures 19A-B. MM7-G primary patient sample co-culture and specific lysis. Co-cultures were carried out under 24 hours and collected and analyzed via flow cytometry. Target MM7-G cells were labeled with Cytotracker (CMTMR) dye to distinguish it from effector T-cells. Populations were gated by anti-BCMA (CD269) and anti-CS1 (CD319) antibodies. Figure 19A: flow cytometry depictions of co-cultures. Figure 19B: graphical summary of lysis vs. E:T ratio.

Figures 20A-B. MM11-G primary patient sample co-culture and specific lysis. Co-cultures were carried out under 24 hours and collected and analyzed via flow cytometry. Target MM11-G cells were labeled with Cytotracker (CMTMR) dye to distinguish it from effector T-cells. Populations were gated by anti-BCMA (CD269) and anti-CS1 (CD319) antibodies. Figure 20A: flow cytometry depictions of co-cultures. Figure 20B: graphical summary of lysis vs. E:T ratio.

Figure 21. CD269-CS1-BBCAR NK cells demonstrate anti-leukemic effects *in vivo*.

NSG mice were sublethally irradiated and intravenously injected the following day with luciferase-expressing MM.1S multiple myeloma cells to induce measurable tumor formation.

After 3 days, the mice were intravenously injected with  $8 \times 10^6$  CD269-CS1-BBCAR NK cells or vector control NK control cells. On days 3, 6, and 8, mice were injected subcutaneously with RediJect D-Luciferin and subjected to IVIS imaging. Average light intensity measured for the CD269-CS1-BBCAR NK injected mice was compared to that of vector control NK injected mice.

Figure 22. Percent survival of mice was measured and compared between the two groups

based on the studies from Figure 21.

Figure 23. CRISPR/Cas9 interference system. The expression of sgRNA and Cas9 puromycin is driven by the U6 and SFFV promoters, respectively. The Cas9 is linked with puromycin resistant gene by E2A self-cleaving sequences.

Figure 24. A schematic providing an example of the steps for generation of CAR T or NK cell targeting hematologic malignancies.

Figure 25. Generation and cell sorting of stable CD45 knockdown NK-92 cells using CRISPR/Cas9 lentivirus system. Flow cytometry analysis indicated the CD45 expression levels on NK-92 cell surface (left panels). After transduction of sgCD45B CRISPR into NK-92 cells, transduced cells were cultured in medium containing puromycin for a few weeks. CD45 negative NK-92 cells were determined using CD45 antibody and were sorted. The purity of stable NK<sup>45i</sup>-92 (CD45 knockdown) NK-92 cells were determined by Flow cytometry analysis (right panel). This data showed that NK<sup>45i</sup>-92 cells were successfully generated and obtained.

Figure 26. Cell growth curve of wild type, GFP transduced NK-92 or NK<sup>45i</sup>-92NK cells.

To evaluate the effect for cell proliferation caused by CD45-knockdown (KD) in NK-92 cells, the number of cells of NK-92(●), GFP-transduced NK-92(■) and NK<sup>45i</sup>-92(▲) were counted at 48 h and 96 h after seeding into 24 well plates. IL-2 was added at 48 h time point. (n=3 independent experiments performed in duplicate). Data are mean  $\pm$  S.D. These data indicated that knockdown of CD45 receptor on NK-92 show similar cell growth curve compared to non-transduced NK-92 or GFP-transduced NK-92 cells.

Figures 27 A-B. Co-culture assay with CCRF-CEM (target: T) and GFP NK-92 or GFP NK<sup>45i</sup>-92 cells (effector: E), 5:1 (E:T) ratio. 16 hours incubation. (A) Flow cytometry analysis of CCRF-CEM only (blue dot in left panel), in co-culture with CCRF-CEM and control GFP transduced NK-92 cells (middle panel) or GFP NK<sup>45i</sup>-92 cells (right panel). Blue dots in all of panels indicates the leftover target CCRF-CEM cells and red dots shows effector cells by co-culture assay. All of incubation time were 16 h and the ratio of effector T-cells: target cell was 5:1. All experiments were performed in duplicate. (B) Bar graph indicates the percent of cell lysis by the GFP transduced NK<sup>45i</sup>-92 cells compared to the control GFP transduced NK92 cells in co-culture assay with CCRF-CEM. These data suggest that knockdown of CD45 in NK-92 cells does not show a significant difference for killing activity against CCRF-CEM cells compared to GFP-control NK-92 cells in vitro co-culture assay. Blue dots are in the upper left quadrant.

Figures 28 A-B. Co-culture assay with CCRF-CEM (target: T) and GFP NK-92, CD5CAR NK-92 or CD5CAR NK<sup>45i</sup>-92 cells (effector: E). 5:1 (E:T) ratio. 16 hours incubation (A) Flow cytometry analysis of CCRF-CEM only (left panel), in co-culture with CCRF-CEM and control GFP NK-92 cells (middle left panel), CD5CAR NK-92 cells (middle right panel),

CD5CAR NK<sup>45i</sup>-92 cells (right panel) from right to left. Blue dots in all of panels indicates the leftover target CCRF-CEM cells and red dots shows effector cells by co-culture assay. All of incubation times were 16 h and the ratio of effector T-cells: target cell is 5:1. All experiments were performed in duplicate. (B) Bar graph indicates the percent of cell lysis by the CD5CAR NK-92 cells or CD5CAR NK<sup>45i</sup>-92 cells compared to the control GFP NK92 cells in co-culture assay with CCRF-CEM. Data are mean  $\pm$  S.D. Both of CD5CAR NK-cells and CD5CAR NK<sup>45i</sup>-92 cells shows near to 100 % cell killing activity against CD5-positive CCRF-CEM compared to control GFP NK-92 cells. These data suggest that CD5CAR NK-cells and CD5CAR NK<sup>45i</sup>-92 cells can effectively lyse CCRF-CEM cells that express CD5 compared to GFP-control NK-92 cells in vitro co-culture assay, and provide proof that knockdown of CD45 does not affect cell function for killing activity in NK-92 cells. Blue dots are in the upper left quadrant of the first two panels starting from the left.

Figures 29A-B. Organization of the CD45CAR construct and its expression. (A) Schematic representation of the CD45CAR lentiviral vector. The CD45CAR construct is a modularized signaling domain containing: a leader sequence, an anti-CD45scFv, a hinge domain (H), a transmembrane domain (TM), two co-stimulatory domains (CD28 and 4-1BB) that define the construct as a 3<sup>rd</sup> generation CAR, and the intracellular signaling domain CD3 zeta. (B), HEK-293FT cells were transfected with lentiviral plasmids for GFP (lane 1) and CD45CAR (lane 2). 48 hours after transfection, supernatant was removed, and cells were also removed. Cells were lysed for Western blot and probe with mouse anti-human CD3z antibody.

Figures 30 A-B. Transduction of CD45CAR into NK<sup>45i</sup>-92 cells and cell sorting of CD45CAR transduced cells. (A) The expression levels of CD45CAR on NK<sup>45i</sup>-92 were determined by flow cytometry analysis (circled in blue at middle panel) compared to NK<sup>45i</sup>-92

cells (left panel) after CD45CAR lentviruses were transduced into NK<sup>45i</sup>-92 cells. CD45CAR expressed NK<sup>45i</sup>-92 cells were sorted and CD45 expression levels on cell surface were determined by Flow cytometry analysis (right panel). (B) About 87% of CD45CAR expression on cell surface was detected by flow cytometry analysis.

5            Figures 31A-B. Co-culture assay with CCRF-CEM (target: T) and GFP NK-92 or CD45CAR NK<sup>45i</sup>-92 cells (effector: E). 5:1 (E:T) ratio. 16 hours incubation. (A) Flow cytometry analysis of in co-culture with CCRF-CEM and control GFP transduced NK-92 cells (left panel) or CD45CAR NK<sup>45i</sup>-92 cells (right panel). Blue dots in all of panels indicates the leftover target CCRF-CEM cells and red dots shows effector NK-92 cells by co-culture assay.

10    All of incubation times were 16 h and the ratio of effector T-cells: target cell is 5:1. All experiments were performed in duplicate. (B) Bar graph indicates the percent of cell lysis by CD45CAR NK<sup>45i</sup>-92 cells compared to the control GFP NK92 cells in co-culture assay with CCRF-CEM. Data are mean  $\pm$  S.D. CD45CAR NK<sup>45i</sup>-92 cells shows about 70% cell lysis against CCRF-CEM cells compared to control GFP NK-92 cells. These data suggest that CD45CAR

15    NK<sup>45i</sup>-92 cells effectively lyse CCRF-CEM cells that express CD45 compared to GFP-control NK-92 cells in vitro co-culture assay.

             Figures 32 A-C. Co-culture assay with Jurkat cells (target: T) and GFP-control or CD45CAR NK<sup>45i</sup>-92 cells (effector: E). 5:1 or 2:1 (E:T) ratio. 6 hours incubation. (A) Flow cytometry analysis was carried out after Jurkat cells were stained by CMTMR cell tracker dye.

20    These data shows that Jurkat cells are CD45 positive (left panels) and mostly CD56 negative cells (right panel). (B) Flow cytometry analysis of co-culture assay with Jurkat cells (target: T) and control or CD45CAR NK<sup>45i</sup>-92 cells (effector: E). The ratio of co-culture assay was performed in 5:1 or 2:1 (E: T). Left panels showed that in co-culture with control GFP or

CD45CAR/CD45KD NK-92 cells in 5:1 (E:T) ratio and right panels indicated that in co-culture with control GFP or CD45CAR NK<sup>45i</sup>-92 cells in 2:1 (E:T) ratio. Blue dots in panels indicate the leftover target Jurkat cells and red dots represent effector cells by co-culture assay. All of incubation time were 6 h. All experiments were performed in duplicate. (C) Bar graph shows percent cell lysis by CD45CAR NK<sup>45i</sup>-92 cells compared to control GFP NK92 cells at in 5:1 or 2:1 (E: T) ratio. Data are mean  $\pm$  S.D. CD45CAR NK<sup>45i</sup>-92 cells shows about 60% cell lysis against Jurkat cells compared to control GFP NK-92 cells in both conditions. This data suggests that CD45CAR NK<sup>45i</sup>-92 cells effectively lyse Jurkat cells that express CD45 on cell surface compared to GFP-control NK-92 cells in vitro co-culture assay.

Figures 33A-C. Co-culture assay with GFP-NK-92 cells (target: T) and non-transduced NK-92 cells or CD45CAR NK<sup>45i</sup>-92 cells (effector: E). 5:1 or 2:1 (E:T) ratio. 6 hours incubation (A) Flow cytometry analysis was carried out using GFP control NK-92 cells. These data proof that GFP control NK-92 cells are about 99% GFP positive cells (green dots). (B) Flow cytometry analysis of co-culture assay with GFP control NK-92 cells (target: T) and non-transduced or CD45CAR NK<sup>45i</sup>-92 cells (effector: E). The ratio of co-culture assay was performed in 5:1 or 2:1 (E: T). Left panels showed that in co-culture with non-transduced or CD45CAR NK<sup>45i</sup>-92 cells in 5:1 (E:T) ratio and right panels indicated that in co-culture with non-transduced or CD45CAR NK<sup>45i</sup>-92 cells in 2:1 (E:T) ratio. Green dots in panels indicate the leftover target GFP NK-92 cells and red dots represent effector cells by co-culture assay. The incubation time was 6 h. All experiments were performed in duplicate. (C) Bar graph shows percent cell lysis of GFP NK-92 cells by CD45CAR NK<sup>45i</sup>-92 cells compared to non-transduced NK-92 cells at in 5:1 or 2:1 (E: T) ratio. Data are mean  $\pm$  S.D. CD45CAR NK<sup>45i</sup>-92 cells shows about 20% cell lysis in 2:1 (E:T) ratio and about 55% cell lysis in 5:1 (E:T) ratio against GFP NK-92 cells compared to non-

transduced NK-92 cells. This data suggests that CD45CAR NK<sup>45i</sup>-92 cells effectively lyse GFP NK-92 cells that express CD45 on cell surface compared to non-transduced NK-92 cells in vitro co-culture assay. Green dots are in the upper right quadrant of each panel.

Figures 33D-E. Transduction of CD45b-BB or CD45b-28 into NK<sup>45i</sup>-92 cells and cell  
 5 sorting of CD45b-BB or CD45b-28 transduced NK<sup>45i</sup>-92 cells. (D) The surface expression levels of CD45b-BB CAR or CD45b-28 CAR on NK<sup>45i</sup>-92 were determined by flow cytometry analysis (circled in blue at middle panel) compared to NK<sup>45i</sup>-92 cells (left panel) after CD45b-BB or CD45b-28 lentviruses transduced into NK<sup>45i</sup>-92 cells. (E) NK<sup>45i</sup>-92 cells expressing the CD45b-BB or CD45b-28 CAR were sorted by Flow cytometry analysis. About 74% of CD45b-  
 10 BB CAR or 82% of CD45b-28 CAR expression on cell surface was detected by flow cytometry analysis.

Figures 33 F-G. Co-culture assay with REH cells (target: T) and GFP NK-92 cells or CD45CAR NK<sup>45i</sup>-92 cells or CD45b-BB NK<sup>45i</sup>-92 cells or CD45b-28 NK<sup>45i</sup>-92 cells (effector: E). 5:1 (E:T) ratio. 20 hours incubation. (F) Flow cytometry analysis of REH cells only (left  
 15 panel), in co-culture with REH cells and control GFP transduced NK-92 cells (2nd left panel), CD45CAR NK<sup>45i</sup>-92 cells (middle panel), CD45b-BB NK<sup>45i</sup>-92 cells (4th from left panel) or CD45b-28 NK<sup>45i</sup>-92 cells (right panel). Blue dots in all of panels indicate the leftover target REH cells and red dots shows effector GFP or CARs-NK-92 cells by co-culture assay. REH is a B acute lymphoblastic cell line. All of incubation times were 20h and the ratio of effector NK-  
 20 cells: target cell is 5:1. All experiments were performed in duplicate. (G) Bar graph indicates the percent of cell lysis by CD45CAR NK<sup>45i</sup>-92 cells, CD45b-BB NK<sup>45i</sup>-92 cells or CD45b-28 NK<sup>45i</sup>-92 cells compared to the control GFP NK92 cells in co-culture assay with REH cells. Data are mean + S.D. CD45CAR NK<sup>45i</sup>-92 cells shows about 76% cell lysis, CD45b-BB NK<sup>45i</sup>-

92 cells shows about 79% cell lysis and CD45b-28 NK<sup>45i</sup>-92 shows 100% cell lysis against REH cells compared to control GFP NK-92 cells. These data suggest that all three CD45CARs effectively lyse REH cells.

Figures 34 A-B. Schematic diagram to elucidate the construct and its expression in T or NK cells. (A) a combination of CAR, (third generation), sushi/IL-15 is assembled on an expression vector and their expression is driven by the SFFV promoter. CAR with sushi/IL-15 is linked with the P2A cleaving sequence. The sushi/IL-15 portion is composed of IL-2 signal peptide fused to sushi domain and linked to IL-5 via a 26-amino acid poly-proline linker. (B) CAR and sushi/IL15 are present on the T or NK cells.

Figures 35 A-B. CD4IL15RA-CAR expression. (A) HEK-293FT cells were transfected with lentiviral plasmids for GFP (lane 1) and CD4IL15RA CAR (lane 2), and positive control, CD4CAR (lane 3). 48 hours after transfection, supernatant was removed, and cells were also removed for a Western blot with mouse anti-human CD3z antibody. (B) HEK-293 cells were transduced with either GFP (left) or CD4IL15RA-CAR(right) viral supernatant from transfected HEK-293FT cells. After 3 days incubation, cells were harvested, stained with goat-anti-mouse F(Ab')<sub>2</sub> and analyzed by flow cytometry.

Figure 36. Transduction of NK cells with CD4IL15RACAR. NK-92 cells were transduced with either GFP (left) or CD4IL15RACAR (right) viral supernatant from transfected HEK-293FT cells. A second transduction was performed 24 hours after the first. 24 hours after the second transduction, cells were harvested, washed and moved to tissue culture plates with fresh media and IL-2. After 3 days incubation, cells were harvested and stained with goat-anti-mouse F(Ab')<sub>2</sub> antibody or goat IgG (control) at 1:250 for 30 minutes. Cells were washed and stained with streptavidin-PE conjugate at 1:500, washed, suspended in 2% formalin, and



analyzed by flow cytometry.

Figure 37. Transduction of T cells with CD4IL15RACAR. Left is the Western blot.

HEK-293FT cells were transfected with lentiviral plasmids for GFP (lane 1) and CD4IL15RACAR (lane 2). 48 hours after transfection, supernatant was removed, and cells were also

collected for a Western blot with mouse anti-human CD3zeta antibody. Right is

CD4IL15RACAR expression. Activated T cells from cord blood buffy coat were transduced with either GFP (left) or CD4IL15RACAR (right) viral supernatant from transfected HEK-

293FT cells. A second transduction was performed 24 hours after the first. 24 hours after the second transduction, cells were harvested, washed and moved to tissue culture plates with fresh

media and IL-2. After 3 days incubation, cells were harvested and stained with goat-anti-mouse

F(Ab')<sub>2</sub> or isotype control for 30 minutes. Transduced with either GFP (left) or CD4IL15RA

(right). Cells were washed and stained with streptavidin-PE conjugate at 1:250, washed,

suspended in 2% formalin, and analyzed by flow cytometry

Figures 38 A-B. CD4CAR NK-92 cells and CD4IL15RA CAR NK-92 cells eliminate

KARPAS 299 T leukemic cells in co-culture. (A) NK-92 cells transduced with either GFP control (upper right), CD4CAR (lower left), or CD4IL15RA (lower right) lentiviral supernatant

were incubated with KARPAS 299 cells at a ratio of 5:1. After 4 hours co-culture, cells were

stained with mouse-anti-human CD4 (APC) and CD3 (PerCp) antibodies and analyzed by flow

cytometry (N=2). The upper left panel shows labeled Karpas 299 cells alone. (B) The percentage

of target cells lysed is shown in the graph.

Figure 39. CD4CAR NK-92 cells and CD4IL15RA CAR NK-92 cells eliminate MOLT4

T leukemic cells expressing CD4 in co-culture. NK-92 cells transduced with either GFP control

(left), CD4CAR (center), or CD4IL15RA (second from right) lentiviral supernatant were

incubated with MOLT4 cells at effector:target ratios of 1:1 or 2:1. After overnight co-culture, cells were stained with mouse-anti-human CD4 (APC) and CD56 (PerCp) antibodies and analyzed by flow cytometry (N=2). The upper right panel shows labeled MOLT4 cells alone. The percentage of target cells lysed is shown in the graph.

5           Figure 40. CD4IL15RACAR T cells demonstrate more potent anti-leukemic effects *in vivo* than CD4CAR. NSG mice were sublethally irradiated and intravenously (tail vein) injected the following day with luciferase-expressing MOLM13 cells to induce measurable tumor formation. After 3 days, the mice were intravenously injected with one course of  $8 \times 10^6$  CD4CAR, or CD4IL15RACAR T cells, or vector control T control cells. On days 3, 6, 9 and 11,  
10 mice were injected subcutaneously with RediJect D-Luciferin and subjected to IVIS imaging.

Figure 41. Percent tumor reduction in mice was measured and compared between the three groups based on the studies from Figure 40. Average light intensity measured for the CD4CAR and CD4IL15RACAR T injected mice was compared to that of vector control T injected mice, and correlated with remaining tumor burden. In each set of two, CD4CAR T is on  
15 the left and CD4IL15RA CAR T is on the right.

Figure 42. HEK 293 cells were transduced with either EF1-GFP or SFFV-GFP viral supernatant, using the volumes indicated, in DMEM with 10% FBS in a 6 well tissue culture plate. Culture media was changed the following morning. Forty-eight hours later, transduced cells were visualized on an EVOS fluorescent microscope using GFP at 10x.

20           Figure 43. HEK 293 cells transduced with either EF1-GFP or SFFV-GFP viral supernatant, using the volumes from the previous figure, were trypsinized, suspended in formalin, and subjected to flow cytometry analysis, using the FITC channel to determine the

percentage of GFP+ cells.

Figures 44 A-B. Activated cord blood buffy coat T cells transduced with either EF1-GFP or SFFV-GFP viral supernatant, with either low or high amounts of viral supernatant, were trypsinized, suspended in formalin, and subjected to flow cytometry analysis, using the FITC  
5 channel to determine the percentage of GFP+ cells, 7, 14, 21 and 28 days after transduction.

(A) Percent GFP+ T cells for cells transduced with either low or high amounts of supernatant.

(B) Percent of GFP+ T cells transduced with the high amount of EF1-GFP supernatant, relative to the percent GFP+ cells in the T cells transduced with the lower amount of SFFV-GFP supernatant. (50  $\mu$ L of SFFV-GFP and 1 mL of EF1-GFP supernatant was used). (N=2).

10 Figure 45. Ligand receptor interactions in malignant plasma cells. The APRIL ligand binds TAC1 or BCMA. The BAFF ligand binds TAC1, BCMA, or BAFF-R.

### DETAILED DESCRIPTION

The disclosure provides chimeric antigen receptor (CAR) compositions, methods of making and using thereof.

15 A chimeric antigen receptor (CAR) polypeptide includes a signal peptide, an antigen recognition domain, a hinge region, a transmembrane domain, at least one co-stimulatory domain, and a signaling domain.

First-generation CARs include CD3z as an intracellular signaling domain, whereas second-generation CARs include at least one single co-stimulatory domain derived from various  
20 proteins. Examples of co-stimulatory domains include, but are not limited to, CD28, CD2, 4-1BB (CD137, also referred to as “4-BB”), and OX-40 (CD124). Third generation CARs include two

co-stimulatory domains, such as, without limiting, CD28, 4-1BB, CD134 (OX-40), CD2 and/or CD137 (4-1BB).

As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound having amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can include a protein's or peptide's sequence. Polypeptides include any peptide or protein having two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides, and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

A “signal peptide” includes a peptide sequence that directs the transport and localization of the peptide and any attached polypeptide within a cell, e.g. to a certain cell organelle (such as the endoplasmic reticulum) and/or the cell surface.

The signal peptide is a peptide of any secreted or transmembrane protein that directs the transport of the polypeptide of the disclosure to the cell membrane and cell surface, and provides correct localization of the polypeptide of the present disclosure. In particular, the signal peptide of the present disclosure directs the polypeptide of the present disclosure to the cellular membrane, wherein the extracellular portion of the polypeptide is displayed on the cell surface,

the transmembrane portion spans the plasma membrane, and the active domain is in the cytoplasmic portion, or interior of the cell.

In one embodiment, the signal peptide is cleaved after passage through the endoplasmic reticulum (ER), i.e. is a cleavable signal peptide. In an embodiment, the signal peptide is human  
5 protein of type I, II, III, or IV. In an embodiment, the signal peptide includes an immunoglobulin heavy chain signal peptide.

The “antigen recognition domain” includes a polypeptide that is selective for or targets an antigen, receptor, peptide ligand, or protein ligand of the target; or a polypeptide of the target.

The antigen recognition domain may be obtained from any of the wide variety of  
10 extracellular domains or secreted proteins associated with ligand binding and/or signal transduction. The antigen recognition domain may include a portion of Ig heavy chain linked with a portion of Ig light chain, constituting a single chain fragment variable (scFv) that binds specifically to a target antigen. The antibody may be monoclonal or polyclonal antibody or may be of any type that binds specifically to the target antigen. In another embodiment, the antigen  
15 recognition domain can be a receptor or ligand. In particular embodiments, the target antigen is specific for a specific disease condition and the disease condition may be of any kind as long as it has a cell surface antigen, which may be recognized by at least one of the chimeric receptor construct present in the compound CAR architecture. In a specific embodiment, the chimeric receptor may be for any cancer for which a specific monoclonal or polyclonal antibody exists or  
20 is capable of being generated. In particular, cancers such as neuroblastoma, small cell lung cancer, melanoma, ovarian cancer, renal cell carcinoma, colon cancer, Hodgkin's lymphoma, and childhood acute lymphoblastic leukemia have antigens specific for the chimeric receptors.

The target specific antigen recognition domain preferably includes an antigen binding domain derived from an antibody against an antigen of the target, or a peptide binding an antigen of the target, or a peptide or protein binding an antibody that binds an antigen of the target, or a peptide or protein ligand (including but not limited to a growth factor, a cytokine, or a hormone) binding a receptor on the target, or a domain derived from a receptor (including but not limited to a growth factor receptor, a cytokine receptor or a hormone receptor) binding a peptide or protein ligand on the target.

In one embodiment, the antigen recognition domain includes the binding portion or variable region of a monoclonal or polyclonal antibody directed against (selective for) the target.

In another embodiment, the antigen recognition domain includes Camelid single domain antibody, or portions thereof. In one embodiment, Camelid single-domain antibodies include heavy-chain antibodies found in camelids, or VHH antibody. A VHH antibody of camelid (for example camel, dromedary, llama, and alpaca) refers to a variable fragment of a camelid single-chain antibody (See Nguyen et al, 2001; Muyldermans, 2001), and also includes an isolated VHH antibody of camelid, a recombinant VHH antibody of camelid, or a synthetic VHH antibody of camelid.

In another embodiment, the antigen recognition domain includes ligands that engage their cognate receptor. By way of example, APRIL is a ligand that binds the TAC1 receptor or the BCMA receptor. In accordance with an invention disclosed herein, the antigen recognition domain includes APRIL, or a fragment thereof. By way of further example, BAFF is a ligand that binds the BAFF-R receptor or the BCMA receptor. In accordance with an invention disclosed herein, the antigen recognition domain includes BAFF, or a fragment thereof. In another embodiment, the antigen recognition domain is humanized.

It is understood that the antigen recognition domain may include some variability within its sequence and still be selective for the targets disclosed herein. Therefore, it is contemplated that the polypeptide of the antigen recognition domain may be at least 95%, at least 90%, at least 80%, or at least 70% identical to the antigen recognition domain polypeptide disclosed herein and still be selective for the targets described herein and be within the scope of the disclosure.

The target includes interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BCMA, BAFF-R, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, CS1, CD45, ROR1, PSMA, MAGE A3, Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-2/neu, MAGE-3, MAGE-4, MAGE-5, MAGE- 6, alpha-fetoprotein, CA 19-9, CA 72-4, NY-ESO, FAP, ErbB, c-Met, MART-1, CD30, EGFRvIII, immunoglobulin kappa and lambda, CD38, CD52, CD3, CD4, CD8, CD5, CD7, CD2, and CD138

In another embodiment, the target includes any portion interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BCMA, BAFF-R, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, CS1, CD45, TACI, ROR1, PSMA, MAGE A3, Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-2/neu, MAGE-3, MAGE-4, MAGE-5, MAGE- 6, alpha-fetoprotein, CA 19-9, CA 72-4, NY-ESO, FAP, ErbB, c-Met, MART-1, CD30, EGFRvIII, immunoglobulin kappa and lambda, CD38, CD52, CD3, CD4, CD8, CD5, CD7, CD2, and CD138.

In one embodiment, the target includes surface exposed portions of interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BCMA, BAFF-R, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, CS1, CD45, TACI, ROR1, PSMA, MAGE A3,

Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-2/neu, MAGE-3, MAGE-4, MAGE-5, MAGE- 6, alpha-fetoprotein, CA 19-9, CA 72-4, NY-ESO, FAP, ErbB, c-Met, MART-1, CD30, EGFRvIII, immunoglobulin kappa and lambda, CD38, CD52, CD3, CD4, CD8, CD5, CD7, CD2, and CD138 polypeptides.

5           In another embodiment, the target antigens include viral or fungal antigens, such as E6 and E7 from the human papillomavirus (HPV) or EBV (Epstein Barr virus) antigens; portions thereof; or surface exposed regions thereof.

          In one embodiment, the TACI antigen recognition domain includes SEQ ID NO. 24.

          In one embodiment, the BCMA antigen recognition domain includes SEQ ID NO. 25.

10          In one embodiment, the CS1 antigen recognition domain includes SEQ ID NO. 26.

          In one embodiment, the BAFF-R antigen recognition domain includes SEQ ID NO. 27.

          In one embodiment, the CD33 antigen recognition domain includes SEQ ID NO. 28.

          In one embodiment, the CD123 antigen recognition domain includes SEQ ID NO. 29.

          In one embodiment, the CD19 antigen recognition domain includes SEQ ID NO. 30.

15          In one embodiment, the CD20 antigen recognition domain includes SEQ ID NO. 31. In another embodiment, the CD20 antigen recognition domain includes SEQ ID NO. 32.

          In one embodiment, the CD22 antigen recognition domain includes SEQ ID NO. 33.

          In on embodiment, the CD45 antigen recognition domain includes SEQ ID NO. 34

          The hinge region is a sequence positioned between for example, including, but not  
20   limited to, the chimeric antigen receptor, and at least one co-stimulatory domain and a signaling domain. The hinge sequence may be obtained including, for example, from any suitable



sequence from any genus, including human or a part thereof. Such hinge regions are known in the art. In one embodiment, the hinge region includes the hinge region of a human protein including CD-8 alpha, CD28, 4-1BB, OX40, CD3-zeta, T cell receptor  $\alpha$  or  $\beta$  chain, a CD3 zeta chain, CD28, CD3 $\epsilon$ , CD45, CD4, CD5, CD8, CD8a, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, ICOS, CD154, functional derivatives thereof, and combinations thereof.

In one embodiment the hinge region includes the CD8 a hinge region.

In some embodiments, the hinge region includes one selected from, but is not limited to, immunoglobulin (e.g. IgG1, IgG2, IgG3, IgG4, and IgD).

The transmembrane domain includes a hydrophobic polypeptide that spans the cellular membrane. In particular, the transmembrane domain spans from one side of a cell membrane (extracellular) through to the other side of the cell membrane (intracellular or cytoplasmic).

The transmembrane domain may be in the form of an alpha helix or a beta barrel, or combinations thereof. The transmembrane domain may include a polytopic protein, which has many transmembrane segments, each alpha-helical, beta sheets, or combinations thereof.

In one embodiment, the transmembrane domain that is naturally associated with one of the domains in the CAR is used. In another embodiment, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

For example, a transmembrane domain includes a transmembrane domain of a T-cell receptor  $\alpha$  or  $\beta$  chain, a CD3 zeta chain, CD28, CD3 $\epsilon$ , CD45, CD4, CD5, CD7, CD8, CD9,

CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD68, CD134, CD137, ICOS, CD41, CD154, functional derivatives thereof, and combinations thereof.

In one embodiment, the transmembrane domain is artificially designed so that more than 25%, more than 50% or more than 75% of the amino acid residues of the domain are hydrophobic residues such as leucine and valine. In one embodiment, a triplet of phenylalanine, tryptophan and valine is found at each end of the synthetic transmembrane domain.

In one embodiment, the transmembrane domain is the CD8 transmembrane domain. In another embodiment, the transmembrane domain is the CD28 transmembrane domain. Such transmembrane domains are known in the art.

The signaling domain and co-stimulatory domain include polypeptides that provide activation of an immune cell to stimulate or activate at least some aspect of the immune cell signaling pathway.

In an embodiment, the signaling domain includes the polypeptide of a functional signaling domain of CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa, FcR beta (Fc Epsilon Rib), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DNAX-activating protein 10 (DAP10), DNAX-activating protein 12 (DAP12), active fragments thereof, functional derivatives thereof, and combinations thereof. Such signaling domains are known in the art.

In an embodiment, the CAR polypeptide further includes one or more co-stimulatory domains. In an embodiment, the co-stimulatory domain is a functional signaling domain from a protein including OX40; CD27; CD28; CD30; CD40; PD-1; CD2; CD7; CD258; Natural killer Group 2 member C (NKG2C); Natural killer Group 2 member D (NKG2D), B7-H3; a ligand that binds to at least one of CD83, ICAM-1, LFA-1 (CD11a/CD18), ICOS, and 4-1BB (CD137);

CDS; ICAM-1; LFA-1 (CD1a/CD18); CD40; CD27; CD7; B7-H3; NKG2C; PD-1; ICOS; active fragments thereof; functional derivatives thereof; and combinations thereof.

As used herein, the at least one co-stimulatory domain and signaling domain may be collectively referred to as the intracellular domain. As used herein, the hinge region and the antigen recognition may be collectively referred to as the extracellular domain.

The present disclosure further provides a polynucleotide encoding the chimeric antigen receptor polypeptide described above.

The term “polynucleotide” as used herein is defined as a chain of nucleotides.

Polynucleotide includes DNA and RNA. Furthermore, nucleic acids are polymers of nucleotides.

Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric “nucleotides.” The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and polymerase chain reaction (PCR), and the like, and by synthetic means.

The polynucleotide encoding the CAR is easily prepared from an amino acid sequence of the specified CAR by any conventional method. A base sequence encoding an amino acid sequence can be obtained from the aforementioned NCBI RefSeq IDs or accession numbers of GenBank for an amino acid sequence of each domain, and the nucleic acid of the present disclosure can be prepared using a standard molecular biological and/or chemical procedure. For

example, based on the base sequence, a polynucleotide can be synthesized, and the polynucleotide of the present disclosure can be prepared by combining DNA fragments which are obtained from a cDNA library using a polymerase chain reaction (PCR).

In one embodiment, the polynucleotide disclosed herein is part of a gene, or an  
5 expression or cloning cassette.

The polynucleotide described above can be cloned into a vector. A “vector” is a composition of matter which includes an isolated polynucleotide and which can be used to deliver the isolated polynucleotide to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or  
10 amphiphilic compounds, plasmids, phagemid, cosmid, and viruses. Viruses include phages, phage derivatives. Thus, the term “vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral  
15 vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like. In one embodiment, vectors include cloning vectors, expression vectors, replication vectors, probe generation vectors, integration vectors, and sequencing vectors.

In an embodiment, the vector is a viral vector. In an embodiment, the viral vector is a retroviral vector or a lentiviral vector. In an embodiment, the engineered cell is virally  
20 transduced to express the polynucleotide sequence.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A

selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the patient either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art.

5 In one embodiment, lentivirus vectors are used.

Viral vector technology is well known in the art and is described, for example, in Sambrook et al, (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno- associated  
10 viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

Lentiviral vectors have been well known for their capability of transferring genes into  
15 human T cells with high efficiency but expression of the vector-encoded genes is dependent on the internal promoter that drives their expression. A strong promoter is particularly important for the third or fourth generation of CARs that bear additional co-stimulatory domains or genes encoding proliferative cytokines as increased CAR body size does not guarantee equal levels of expression. There are a wide range of promoters with different strength and cell-type specificity.  
20 Gene therapies using CAR T cells rely on the ability of T cells to express adequate CAR body and maintain expression over a long period of time. The EF-1 $\alpha$  promoter has been commonly selected for the CAR expression.

The present invention relates to an expression vector containing a strong promoter for high level gene expression in T cells or NK cells. In further embodiment, the inventor discloses a strong promoter useful for high level expression of CARs in T cells or NK cells. In particular embodiments, a strong promoter relates to the SFFV promoter, which is selectively introduced in an expression vector to obtain high levels of expression and maintain expression over a long period of time in T cells or NK cells. Expressed genes prefer CARs, T cell co-stimulatory factors and cytokines used for immunotherapy.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor - 1  $\alpha$  (EF- 1  $\alpha$ ). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the disclosure should not be limited to the use of constitutive promoters, inducible promoters are also contemplated as part of the disclosure. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence, which is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a

metalothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

Expression of chimeric antigen receptor polynucleotide may be achieved using, for example, expression vectors including, but not limited to, at least one of a SFFV (spleen-focus forming virus) (for example, SEQ ID NO. 23) or human elongation factor 1 $\alpha$  (EF) promoter, CAG (chicken beta-actin promoter with CMV enhancer) promoter human elongation factor 1 $\alpha$  (EF) promoter. Examples of less-strong/ lower-expressing promoters utilized may include, but is not limited to, the simian virus 40 (SV40) early promoter, cytomegalovirus (CMV) immediate-early promoter, Ubiquitin C (UBC) promoter, and the phosphoglycerate kinase 1 (PGK) promoter, or a part thereof. Inducible expression of chimeric antigen receptor may be achieved using, for example, a tetracycline responsive promoter, including, but not limited to, TRE3GV (Tet-response element, including all generations and preferably, the 3rd generation), inducible promoter (Clontech Laboratories, Mountain View, CA) or a part or a combination thereof.

In a preferred embodiment, the promoter is an SFFV promoter or a derivative thereof. It has been unexpectedly discovered that SFFV promoter provides stronger expression and greater persistence in the transduced cells in accordance with the present disclosure.

“Expression vector” refers to a vector including a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector includes sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide. The expression vector may be a

bicistronic or multicistronic expression vectors. Bicistronic or multicistronic expression vectors may include (1) multiple promoters fused to each of the open reading frames;(2) insertion of splicing signals between genes; fusion of genes whose expressions are driven by a single promoter;(3) insertion of proteolytic cleavage sites between genes (self-cleavage peptide); and  
5 (iv) insertion of internal ribosomal entry sites (IRESs) between genes.

In one embodiment, the disclosure provides an engineered cell having at least one chimeric antigen receptor polypeptide or polynucleotide.

An “engineered cell” means any cell of any organism that is modified, transformed, or manipulated by addition or modification of a gene, a DNA or RNA sequence, or protein or  
10 polypeptide. Isolated cells, host cells, and genetically engineered cells of the present disclosure include isolated immune cells, such as NK cells and T cells that contain the DNA or RNA sequences encoding a chimeric antigen receptor or chimeric antigen receptor complex and express the chimeric receptor on the cell surface. Isolated host cells and engineered cells may be used, for example, for enhancing an NK cell activity or a T lymphocyte activity, treatment of  
15 cancer, and treatment of infectious diseases.

In an embodiment, the engineered cell includes immunoregulatory cells. Immunoregulatory cells include T-cells, such as CD4 T-cells (Helper T-cells), CD8 T-cells (Cytotoxic T-cells, CTLs), and memory T cells or memory stem cell T cells. In another embodiment, T-cells include Natural Killer T-cells (NK T-cells).

20 In an embodiment, the engineered cell includes Natural Killer cells. Natural killer cells are well known in the art. In one embodiment, natural killer cells include cell lines, such as NK-



92 cells. Further examples of NK cell lines include NKG, YT, NK-YS, HANK-1, YTS cells, and NKL cells.

NK cells mediate anti-tumor effects without the risk of GvHD and are short-lived relative to T-cells. Accordingly, NK cells would be exhausted shortly after destroying cancer cells,  
5 decreasing the need for an inducible suicide gene on CAR constructs that would ablate the modified cells.

In accordance with the present disclosure, it was surprisingly found that NK cells provide a readily available cell to be engineered to contain and express the chimeric antigen receptor polypeptides disclosed herein.

10 Allogeneic or autologous NK cells induce a rapid immune response but disappear relatively rapidly from the circulation due to their limited lifespan. Thus, applicants surprisingly discovered that there is reduced concern of persisting side effects using CAR cell based therapy.

The disclosure includes a method of generating a cCAR. In some embodiments, the cCAR is generated using T-cells. In other embodiments, cCAR is using primary NK cells  
15 isolated from the peripheral blood or cord blood and NK-92 cells, such that they are administered “off-the-shelf” to any mammal with a disease or cancer.

According to one aspect of the present invention, NK cells can be expanded and transfected with CAR polynucleotides in accordance to the present invention. NK cells can be derived from cord blood, peripheral blood, iPS cells and embryonic stem cells. According to one  
20 aspect of the present invention, NK-92 cells may be expanded and transfected with CAR. NK-92 is a continuously growing cell line that has features and characteristics of natural killer (NK) cells (Arai, Meagher et al. 2008). NK-92 cell line is IL-2 dependent and has been proven to be

safe(Arai, Meagher et al. 2008) and feasible. CAR expressing NK-92 cells can be expanded in the serum free-medium with or without co-culturing with feeder cells. A pure population of NK-92 carrying the CAR of interest may be obtained by sorting.

In one embodiment, engineered cells include allogeneic T cells obtained from donors that are modified to inactivate components of TCR (T cell receptor) involved in MHC recognition. As a result, TCR deficient T cells would not cause graft versus host disease (GVHD).

In some embodiments, the engineered cell may be modified to prevent expression of cell surface antigens. For example, an engineered cell may be genetically modified to delete the native CD45 gene to prevent expression and cell surface display thereof.

In some embodiments, the engineered cell includes an inducible suicide gene (“safety switch”) or a combination of safety switches, which may be assembled on a vector, such as, without limiting, a retroviral vector, lentiviral vector, adenoviral vector or plasmid. Introduction of a “safety switch” greatly increases safety profile and limits on-target or off-tumor toxicities of the compound CARs. The “safety switch” may be an inducible suicide gene, such as, without limiting, caspase 9 gene, thymidine kinase, cytosine deaminase (CD) or cytochrome P450. Other safety switches for elimination of unwanted modified T cells involve expression of CD20 or CD19 or truncated epidermal growth factor receptor in T cells. All possible safety switches are have been contemplated and are embodied in the present invention.

In some embodiments, the suicide gene is integrated into the engineered cell genome.

In one embodiment, the present disclosure provides an engineered cell having a CD45 chimeric antigen receptor polynucleotide. In one embodiment, the CD45 CAR polypeptide includes SEQ ID NO. 13 and corresponding polynucleotide sequence SEQ ID NO. 14. In

another embodiment, the CD45 CAR polypeptide includes SEQ ID NO. 15, and corresponding polynucleotide sequence SEQ ID NO. 16. In another embodiment, the CD45 CAR polypeptide includes SEQ ID NO. 17, and corresponding polynucleotide sequence SEQ ID NO. 18.

### **Multiple CAR units**

5           The present disclosure provides an engineered cell having at least two distinct CAR polypeptides.

As used herein, compound CAR (cCAR) or multiple CAR refers to an engineered cell having at least two distinct chimeric antigen receptor polypeptides. As used herein, a “distinct chimeric antigen receptor polypeptide” has a unique antigen recognition domain, a signal  
10 peptide, a hinge region, a transmembrane domain, at least one costimulatory domain, and a signaling domain. Therefore, two unique chimeric antigen receptor polypeptides will have different antigen recognition domains. The signal peptide, hinge region, transmembrane domain, at least one costimulatory domain, and signaling domain may be the same or different between the two distinct chimeric antigen receptor polypeptides. As used herein, a chimeric antigen  
15 receptor (CAR) unit refers to a distinct chimeric antigen receptor polypeptide, or a polynucleotide encoding for the same.

As used herein, a unique antigen recognition domain is one that is specific for or targets a single target, or a single epitope of a target.

In some embodiments, the compound CAR targets the same antigen. For example, cCAR  
20 targets different epitopes or parts of a single antigen. In some embodiments, each of the CAR units present in the compound CAR targets different antigen specific to the same or different disease condition or side effects caused by a disease condition.

In some embodiments, the compound CAR targets two different antigens.

Creation of compound CARs bearing different CAR units can be quite challenging: (1) CAR-CAR interactions might have a deleterious effect and an appropriate CAR design is a key to offset this effect; (2) a compound CAR in a single construct could increase the length of the expression cassette, which may cause the reduction of the viral titer and level of protein expression; (3) an appropriate design to include various CAR body elements particularly to select a strategy to express multiple CARs in a single vector is required; (4) A strong promoter is particularly important for a compound CAR that bears additional units of CAR; (5) The hinge region in the CAR needs to be designed so that interaction of the hinge region between each CAR unit is avoided preferably; (6) two or more units of CARs expressing in a cell may cause toxic effects (CAR-CAR interaction). Applicants herein provide a novel and surprising CAR compositions and methods to overcome these hurdles.

In one embodiment, the present disclosure provides an engineered cell having multiple CAR units. This allows a single engineered cell to target multiple antigens. Targeting multiple surface markers or antigens simultaneously with a multiple CAR units prevents selection of resistant clones and reduces tumor recurrence. Multiple CAR T cell immunotherapies, with each individual component CAR comprising various domains and activation sites has not yet been developed for any malignancies.

In one aspect of the present invention, cCAR includes multiple CAR units. In some embodiments, cCAR includes at least two CAR units. In another embodiment, the cCAR includes at least three CAR units. In another embodiment, the cCAR includes at least four units.

In one embodiment, the present disclosure provides an engineered cell having at least two distinct chimeric antigen receptor polypeptides, each having a different antigen recognition domain.

In a preferred embodiment, the engineered cell having at least two distinct chimeric antigen receptor polypeptides is a primary NK cells isolated from the peripheral blood or cord blood and NK-92 cells, such that they are administered “off-the-shelf” to any mammal with a disease or cancer.

5           In one embodiment, the engineered cell includes (i.) a first chimeric antigen receptor polypeptide comprising a first antigen recognition domain, a first signal peptide, a first hinge region, a first transmembrane domain, a first co-stimulatory domain, and a first signaling domain; and (ii.) a second chimeric antigen receptor polypeptide comprising a second antigen recognition domain, a second signal peptide, a second hinge region, a second transmembrane domain, a second co-stimulatory domain, and a second signaling domain. The first antigen  
10           recognition domain is different from the second antigen recognition domain.

In a preferred embodiment, each engineered CAR unit polynucleotide have different nucleotide sequences in order to avoid homologous recombination.

In one embodiment, the target of the first antigen recognition domain is selected from the  
15           group consisting of interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BAFF-R, BCMA, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, and CS1; and the target of the second recognition domain is selected from the group consisting of interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BAFF-R,  
20           BCMA, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, and CS1.

In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a CD19 antigen recognition domain and second chimeric antigen receptor polypeptide having a CD20 recognition domain. In one embodiment, this engineered cell includes a polypeptide of SEQ ID NO. 3 and corresponding polynucleotide of SEQ ID NO. 4.

5 In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a CD19 antigen recognition domain and second chimeric antigen receptor polypeptide having a CD22 antigen recognition domain. In one embodiment, this engineered cell includes a polypeptide of SEQ ID NO. 5 and corresponding polynucleotide of SEQ ID NO. 6.

10 In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a CD19 antigen recognition domain and second chimeric antigen receptor polypeptide having a CD123 antigen recognition domain. In one embodiment, this engineered cell includes a polypeptide of SEQ ID NO. 7 and corresponding polynucleotide of SEQ ID NO. 8.

15 In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a CD33 antigen recognition domain and second chimeric antigen receptor polypeptide having a CD123 antigen recognition domain. In one embodiment, this engineered cell includes a polypeptide of SEQ ID NO. 9 and corresponding polynucleotide of SEQ ID NO. 10. In another embodiment, this engineered cell includes a polypeptide of SEQ ID NO. 11 and corresponding polynucleotide of SEQ ID NO. 12.

In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a BAFF-R antigen recognition domain and second chimeric antigen receptor polypeptide having a CS1 antigen recognition domain.

5 In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a CD269 antigen recognition domain and second chimeric antigen receptor polypeptide having a CS1 antigen recognition domain. In one embodiment, the engineered cell includes a polypeptide including SEQ ID NO. 19 and corresponding polynucleotide SEQ ID NO. 20. In one embodiment, the engineered cell includes a polypeptide including SEQ ID NO. 21 and corresponding polynucleotide SEQ ID NO. 22.

10 In one embodiment, the engineered cell includes a first chimeric antigen receptor polypeptide having a CD33 antigen recognition domain and second chimeric antigen receptor polypeptide having a CD123 antigen recognition domain.

In one embodiment, each CAR unit includes the same or different hinge region. In another embodiment, each CAR unit includes the same or different transmembrane region. In  
15 another embodiment, each CAR unit includes the same or different intracellular domain.

In one embodiment, each CAR unit includes the CD3 zeta chain signaling domain.

In one embodiment, each distinct CAR unit includes different co-stimulatory domains to avoid interaction. For example, the first chimeric antigen receptor polypeptide includes a 4-1BB co-stimulatory domain; and the second chimeric antigen receptor polypeptide includes a CD28  
20 co-stimulatory domain.

In another embodiment, the hinge region is designed to exclude amino acids that may cause undesired intra- or intermolecular interactions. For example, the hinge region may be

designed to exclude or minimize cysteine residues to prevent formation of disulfide bonds. In another embodiment, the hinge region may be designed to exclude or minimize hydrophobic residues to prevent unwanted hydrophobic interactions.

Compound CAR can perform killing independently or in combination. Multiple or  
5 compound CAR comprises same or different hinge region, same or different transmembrane, same or different co-stimulatory and same or different intracellular domains. Preferably, the hinge region is selected to avoid the interaction site.

The compound CAR of the present invention may target same or different tumor populations in T or NK cells. The first CAR, for example, may target the bulky tumor  
10 population and the next or the second CAR, for example, may eradicate cancer or leukemic stem cells, to avoid cancer relapses.

In accordance with the present invention it was surprisingly found that the compound CAR in a T or NK cells targeting different or same tumor populations combat tumor factors causing cancer cells resistant to the CAR killing activity, thereby producing down regulation of  
15 the target antigen from the cancer cell surface. It was also surprisingly found that this enables the cancer cell to “hide” from the CAR therapy referred to as “antigen escape” and tumor heterogeneity, by which different tumor cells can exhibit distinct surface antigen expression profiles.

#### **Engineered cell having CAR polypeptide and enhancer**

20 In another embodiment, the present disclosure provides an engineered cell having at least one chimeric antigen receptor polypeptide and an enhancer.

In one embodiment, the present disclosure provides an engineered cell having at least two distinct chimeric antigen receptor polypeptides and an enhancer.



As used herein, an enhancer includes a biological molecule that promotes or enhances the activity of the engineered cell having the chimeric antigen receptor polypeptide. Enhancers include cytokines. In another embodiment, enhancers include IL-2, IL-7, IL-12, IL-15, IL-21, PD-1, PD-L1, CSF1R, CTAL-4, TIM-3, and TGFR beta, receptors for the same, and functional  
5 fragments thereof.

Enhancers may be expressed by the engineered cell described herein and displayed on the surface of the engineered cell or the enhancer may be secreted into the surrounding extracellular space by the engineered cell. Methods of surface display and secretion are well known in the art. For example, the enhancer may be a fusion protein with a peptide that provides surface display  
10 or secretion into the extracellular space.

The effect of the enhancer may be complemented by additional factors such as enhancer receptors and functional fragments thereof. The additional factors may be co-expressed with the enhancer as a fusion protein or expressed as a separate peptide and secreted into the extracellular space.

15 In one embodiment, the enhancer is IL-15. In this instance, the additional factor is the IL-15 receptor, and functional fragments thereof. Functional fragments include the IL-15 receptor, IL-15RA, and the sushi domain of IL-15RA. An example of a suitable sushi domain includes SEQ ID NO. 35. In accordance with the present disclosure, any chimeric antigen receptor polypeptide disclosed herein includes the Human Interleukin 15 with human interleukin  
20 2 signal peptide SEQ ID NO. 36.

Interleukin (IL)-15 and its specific receptor chain, IL-15R $\alpha$  (IL-15-RA) play a key functional role in various effector cells, including NK and CD8 T cells. CD8+ T cells can be

modified to express autocrine growth factors including, but not limited to, IL-2, IL-7, IL21 or IL-15, to sustain survival following transfer in vivo. Without wishing to be bound by theory, it is believed that IL-15 could overcome the CD4 deficiency to induce primary and recall memory CD8T cells. Overexpression of IL-15-RA or an IL-15 IL-RA fusion on CD8 T cells significantly  
5 enhances its survival and proliferation in-vitro and in-vivo. In some embodiments, CD4CAR or any CAR can include expressing any one or more of moieties, IL-15, IL15RA and IL-15/IL-15R or IL15-RA/IL-15, or a part or a combination thereof, to enhance survival or proliferation of CAR T or NK, and to improve expansion of memory CAR CD8+ T cells.

The present disclosure relates to an engineered cell having a CAR as described herein and  
10 any one or more of moieties of IL-15, IL15RA and IL-15/IL-15R or IL15-RA/IL-15, or a part or a combination thereof, to enhance survival or persistent or proliferation of CAR T or NK for treating cancer in a patient.

In one embodiment, the engineered cell includes a CD4 chimeric antigen receptor polypeptide and IL-15RA (SEQ ID NO. 1), and corresponding polynucleotide (SEQ ID NO. 2).

## 15 **Methods of generating engineered cells**

Any of the polynucleotides disclosed herein may be introduced into an engineered cell by any method known in the art.

In one embodiment, CAR polynucleotides are delivered to the engineered cell by any viral vector as disclosed herein.

20 In one embodiment, to achieve enhanced safety profile or therapeutic index, the any of the engineered cells disclosed herein be constructed as a transient RNA-modified “biodegradable” version or derivatives, or a combination thereof. The RNA-modified CARs of

the present invention may be electroporated into T cells or NK cells. The expression of the compound CAR may be gradually diminished over few days.

In some embodiments of the present invention, any of the engineered cells disclosed herein may be constructed in a transposon system (also called a “Sleeping Beauty”), which  
5 integrates the CAR DNA into the host genome without a viral vector.

### **Methods of generating an engineered cell having multiple CAR units**

In another embodiment, the present disclosure provides a method making an engineered cell having at least two CAR units.

In some embodiments, multiple units of CAR are expressed in a T or NK cell using  
10 bicistronic or multicistronic expression vectors. There are several strategies can be employed to construct bicistronic or multicistronic vectors including, but not limited to, (1) multiple promoters fused to the CARs’ open reading frames;(2) insertion of splicing signals between units of CAR; fusion of CARs whose expressions are driven by a single promoter;(3) insertion of proteolytic cleavage sites between units of CAR (self-cleavage peptide); and (iv) insertion of  
15 internal ribosomal entry sites (IRESs).

In a preferred embodiment, multiple CAR units are expressed in a single open reading frame (ORF), thereby creating a single polypeptide having multiple CAR units. In this embodiment, an amino acid sequence or linker containing a high efficiency cleavage site is disposed between each CAR unit.

20 As used herein, high cleavage efficiency is defined as more than 50 %, more than 70 %, more than 80%, or more than 90% of the translated protein is cleaved. Cleavage efficiency may be measured by Western Blot analysis, as described by Kim 2011.

Furthermore, in a preferred embodiment, there are equal amounts of cleavage product, as shown on a Western Blot analysis.

Examples of high efficiency cleavage sites include porcine teschovirus-1 2A (P2A), FMDV 2A (abbreviated herein as F2A); equine rhinitis A virus (ERAV) 2A (E2A); and Thoseaasigna virus 2A (T2A), cytoplasmic polyhedrosis virus 2A (BmCPV2A) and flacherie Virus 2A (BmIFV2A), or a combination thereof. In a preferred embodiment, the high efficiency cleavage site is P2A. High efficiency cleavage sites are described in Kim JH, Lee S-R, Li L-H, Park H-J, Park J-H, Lee KY, et al. (2011) High Cleavage Efficiency of a 2A Peptide Derived from Porcine Teschovirus-1 in Human Cell Lines, Zebrafish and Mice. PLoS ONE 6(4): e18556, the contents of which are incorporated herein by reference.

In embodiments wherein multiple CAR units are expressed in a single open reading frame (ORF), expression is under the control of a strong promoter. Examples of strong promoters include the SFFV promoter, and derivatives thereof.

### **Engineered cell having CAR polypeptide and enhancer**

In another embodiment, the present disclosure provides a method making an engineered cell that expresses at least one CAR unit and an enhancer.

In some embodiments, at least one CAR unit and enhancer is expressed in a T or NK cell using bicistronic or multicistronic expression vectors. There are several strategies can be employed to construct bicistronic or multicistronic vectors including, but not limited to, (1) multiple promoters fused to the CARs' open reading frames;(2) insertion of splicing signals between units of CAR; fusion of CARs whose expressions are driven by a single promoter;(3)

insertion of proteolytic cleavage sites between units of CAR (self-cleavage peptide); and (iv) insertion of internal ribosomal entry sites (IRESs).

In a preferred embodiment, at least one CAR unit and an enhancer are expressed in a single open reading frame (ORF), thereby creating a single polypeptide having at least one CAR unit and an enhancer. In this embodiment, an amino acid sequence or linker containing a high efficiency cleavage site is disposed between each CAR unit and between a CAR unit and enhancer. In this embodiment, the ORF is under the control of a strong promoter. Examples of strong promoters include the SFFV promoter, and derivatives thereof.

Furthermore, in a preferred embodiment, there are equal amounts of cleavage product, as shown on a Western Blot analysis.

#### **Methods of treatment using the compositions disclosed herein**

In another embodiment, the present invention provides a method of targeting CD45 for conditioning prior to allogeneic transplantation in cancer treatment. CD45 is also known as leukocyte common antigen (LCA) and is a tyrosine phosphatase expressed on virtually all cells of hematopoietic origin except erythrocytes and platelets. Most hematologic malignancies express CD45. For instance, 85% to 90% acute lymphoid and myeloid leukemias express CD45. CD45 is not found in non-hematopoietic origin. In addition, CD45 is expressed at a high density of an average copy number of approximately 200,000 molecules per cells on malignant cells and leukocytes. CD45 presents an ideal target for a variety of hematologic malignancies. However, CAR T and NK cells also express CD45. Without inactivation of endogenous CD45, CAR T or NK cells armed with CARs targeting CD45 may result in self-killing.

The association of CD45 with TCR complexes is essential in regulation of T-cell activation in response to antigen. The inability of CD45-deficient T cells to present antigen is

due to reduced signaling through the T cell receptors (TCRs). TCRs are cell surface receptors that play an essential role in the activation of T cells in response to the presentation of antigen. The TCR is generally made from two chains, alpha and beta, which are associated with the transducing subunits, the CD3, to form the T-cell receptor complex present on the cell surface.

5           It was surprisingly found that multiple CARs (Compound CARs, cCAR) of the present invention combat a key mechanism by which cancer cells resist CAR activity, i.e., the downregulation or heterogeneous expression of the target antigen from the cancer cell surface. This mechanism allows the cancer cell to “hide” from the CAR therapy, a phenomenon referred to as ‘antigen escape’. The present disclosure pre-empts cancer antigen escape by recognizing a  
10 combination of two or more antigens to rapidly eliminate the tumor.

The invention provides a method of simultaneous targeting of multi-antigens using a cCAR resulting in improved tumor control by minimizing the possibility of tumor selection on the basis of target antigen loss or down-regulation.

The disclosed invention includes compound (multiple or compound) cCAR in a T or NK  
15 cell targeting different or same surface antigens present in tumor cells. The compound chimeric antigen receptors of present invention comprise at least multiple chimeric receptor constructs linked by a linker and target same or different antigens. For example, each of the CAR construct present in the compound CAR (cCAR) construct includes an antigen recognition domain, an extracellular domain, a transmembrane domain and/or a cytoplasmic domain. The extracellular  
20 domain and transmembrane domain can be derived from any desired source for such domains. The multiple CAR constructs are linked by a linker. The expression of the compound CAR construct is driven by a promoter. The linker may be a peptide or a part of a protein, which is self-cleaved after a protein or peptide is generated (also called as a self-cleaving peptide).

In one embodiment, the compound CARs of the present invention target Myelodysplastic Syndrome and acute myeloid leukemia (AML) population. Myelodysplastic Syndrome (MDS) remains an incurable hematopoietic stem cell malignancy that occurs most frequently among the elderly, with about 14,000 new cases each year in the USA. About 30–40% of MDS cases progress to AML. The incidence of MDS continues to increase as our population ages. Although MDS and AML have been studied intensely, no satisfactory treatments have been developed.

The compositions and methods of this invention can be used to generate a population of T lymphocyte or NK cells that deliver both primary and co-stimulatory signals for use in immunotherapy in the treatment of cancer, in particular, the treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, brain cancer, sarcoma, leukemia and lymphoma.

Immunotherapeutics generally rely on the use of immune effector cells and molecules to target and destroy cancer cells. The effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells, NK cells and NK-92 cells. The compositions and methods described in the present invention may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth. The compositions and methods described in the present invention may be utilized in other disease conditions that rely on immune responses such as inflammation, immune diseases, and infectious diseases.

In some embodiments, the compound CAR of the present invention may act as a bridge to bone marrow transplant, by achieving complete remission for patients who have minimal residual disease and are no longer responding to chemotherapy. In other embodiments, the

compound CAR eliminates leukemic cells followed by bone marrow stem cell rescue to support leukopenia.

In some embodiments, the compound CAR of the present disclosure can combat a key mechanism by which cancer cells resist CAR activity by the down-regulation of the target antigen. In another embodiment, the invented compound CAR can also combat the heterogeneity of cancer cells, which creates significant challenges in a regular CAR T/NK cell therapy. In a further embodiment, the disclosed compound CAR is designed that the first CAR targets the bulky tumor population and another eradicates cancer or leukemic stem cells to avoid cancer relapses.

In one embodiment, the present disclosure provides a method of destroying cells having a CD33 antigen or a CD123 antigen, or both by contacting said cells with an engineered cell having at least one of chimeric antigen receptor polypeptide having a CD33 antigen recognition domain and chimeric antigen receptor polypeptide having a CD23 antigen recognition domain. The engineered cell may be a T or NK cell.

Cells having at least one of the CD33 antigen and the CD123 antigen include acute myeloid leukemia, precursor acute lymphoblastic leukemia, chronic myeloproliferative neoplasms, chronic myeloid leukemia, myelodysplasia syndromes, blastic plasmacytoid dendritic neoplasms (BPDCN), Hodgkin's lymphoma, mastocytosis, and hairy cell leukemia cells.

In another embodiment, the present disclosure provides a method of providing myeloblastic conditioning regimens for hematopoietic stem cell transplantation. In this embodiment, a T or NK engineered cell having a CD33 unit and a CD123 unit is administered to a patient in need thereof.



In further embodiments, the present disclosure provides a method of eradicating or killing leukemic stem cells (LSCs) or bulk leukemic cells expressing CD123 or CD33, or both. In this embodiment, a T or NK engineered cell having a CD33 unit and a CD123 unit is administered to a patient in need thereof.

5           In further embodiments, the compound CAR in a T or NK cell may be used to eradicate or kill CD34+ CD38- leukemic stem cells or bulk leukemic cells expressing CD123 or CD33 or both.

          In some embodiments, a compound CAR targets cells expressing CD19 or CD20 antigens or both. In another embodiment, a compound CAR targets cells expressing CD19 or  
10   CD22 antigens or both. The targeted cells may be cancer cells, such as, without limiting, B-cell lymphomas or leukemias. In further embodiments, the target antigens can include at least one of this group, but not limited to, ROR1, PSMA, MAGE A3, Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-2/neu, MAGE-3, MAGE-4, MAGE-5, MAGE- 6, alpha-fetoprotein, CA 19-9, CA 72-4, NY-ESO, FAP, ErbB, c-Met, MART-1, CD30, EGFRvIII, immunoglobulin kappa and  
15   lambda, CD38, CD52, CD3, CD4, CD8, CD5, CD7, CD2, and CD138. The target antigens can also include viral or fungal antigens, such as E6 and E7 from the human papillomavirus (HPV) or EBV (Epstein Barr virus) antigens.

          In some embodiments, the compound CAR targets cells expressing CD19 or CD123 antigen or both. The targeted cells are cancer cells, such as, without limiting, B-cell lymphomas  
20   or leukemias.

          In further embodiments, the compound CAR targets cells expressing CS1 and/or B-cell maturation antigens (BCMA) or both. In another embodiment, the targeting cells are malignant plasma cells, such as, without limiting, multiple myeloma.

In some embodiments, the compound CAR targets cells expressing multiple antigens including, but not limited to, CS1, BCMA, CD267, BAFF-R, CD38, CD138, CD52, CD19, CD20, interleukin 6 receptor and NY-ESO-1 antigens. In another embodiment, the targeting cells are malignant plasma cells such as, without limiting, multiple myeloma.

5           In some embodiments, the compound CAR targets cells expressing multiple antigens including but not limited to, alpha fetoprotein (AFP) and Glypican-3 (GPC3). In another embodiment, the targeting cells are hepatocellular carcinoma, fibrolamellar carcinoma, hepatoblastoma, undifferentiated embryonal sarcoma and mesenchymal hamartoma of liver, lung-squamous cell carcinoma, testicular nonseminomatous germ cell tumors, liposarcoma, 10 ovarian and extragonadal yolk sac tumors, ovarian choriocarcinoma, teratomas, ovarian clear cell carcinoma, and placental site trophoblastic tumor.

In accordance with the present invention, the T or NK cell comprising compound CARs targeting different or same antigens offset tumor escape and enables simultaneous targeting of tumor cells.

15           The T or NK host cells comprising compound CAR disclosed herein is embodied in the present disclosure. The nucleotide and polypeptide constructs, sequences, host cells, vectors of the compound CAR is considered to be part of the present disclosure and is embodied herein.

In some embodiments, the compound CAR is administrated in combination with any chemotherapy agents currently being developed or available in the market. In some 20 embodiments, the compound CAR is administrated as a first line treatment for diseases including, but not limited to, hematologic malignancies, cancers, non-hematologic malignances, inflammatory diseases, infectious diseases such as HIV and HTLV and others. In one embodiment, T cells expressing the compound CAR are co-administrated with NK cells

expressing the same or different compound CAR as an adaptive immunotherapy. Compound CAR NK cells provide rapid, innate activity targeting cells while compound T cells provide relative long-lasting adaptive immune activity.

In one embodiment, the cells expressing a compound CAR are administrated as a bridge  
5 to bone marrow stem transplantation for mammals, e.g. patients who are resistant to chemotherapies and are not qualified for bone marrow stem cell transplantation.

In some embodiments, the compound CAR co-expresses a transgene and releases a transgenic product, such as IL-12 in the targeted tumor lesion and further modulates the tumor microenvironment.

10 In one embodiment, cells expressing a compound CAR are administrated to a mammal for bone marrow myeloid ablation as a part of the treatment to a disease.

In a specific embodiment, the cells expressing a compound CAR can be T cells or NK cells, administrated to a mammal, e.g. human. The presented disclosure includes a method of treating a mammal having a disorder or disease by administration of a compound CAR. The  
15 targeted cells may be cancer cells such as, or cells affected by any other disease condition, such as infectious diseases, inflammation, and autoimmune disorders.

The present invention is intended to include the use of fragments, mutants, or variants (e.g., modified forms) of the compound CAR or antigens that retain the ability to induce stimulation and proliferation of T/NK cells. A “form of the protein” is intended to mean a protein  
20 that shares a significant homology with at least one CAR or antigen and is capable of effecting stimulation and proliferation of T/NK cells. The terms “biologically active” or “biologically active form of the protein,” as used herein, are meant to include forms of the proteins or variants that are capable of effecting anti-tumor activity of the cells.

The compositions and methods of this invention can be used to generate a population of T/NK cells that deliver both primary and co-stimulatory signals for use in immunotherapy in the treatment of cancer, in particular the treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. The compositions and methods described in the present invention may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth.

In some embodiments, the invention discloses a method of depletion B cells, immature B cells, memory B cells, plasmablasts, long lived plasma cells, or plasma cells in patients with an autoimmune disease by administering to patients with CAR or compound CAR T cells or NK cells. CAR targeted cells are B or plasma cells expressing one or two or all of antigens, BCMA, TACI and BAFF-R. The autoimmune diseases include systemic scleroderma, multiple sclerosis, psoriasis, dermatitis, inflammatory bowel diseases (such as Crohn's disease and ulcerative colitis), systemic lupus erythematosus, vasculitis, rheumatoid arthritis, Sjorgen's syndrome, polymyositis, granulomatosis and vasculitis, Addison's disease, antigen-antibody complex mediated diseases and anti-glomerular basement membrane disease.

Multiple extracellular cell markers are now being studied for value as tumor-associated antigens and thus potential targets for CAR T/NK cell therapy. However, expression of these antigens on healthy tissue leading to on-target, off-tumor adverse events remains a major safety concern in addition to off-target toxicities. Furthermore, a major limitation of CAR T/NK cell therapy is in the possibility of selecting for antigen escape variants when targeting molecules non-essential to tumorigenesis. Thus, malignant cells that persist with little or no expression of the target antigens may evade CAR T/NK cells, despite their high-affinity action.

In accordance with the present invention, natural killer (NK) cells represent alternative cytotoxic effectors for CAR driven killing. Unlike T-cells, NK cells do not need pre-activation and constitutively exhibit cytolytic functions. Further expression of cCARs in NK cells allow NK cells to effectively kill cancers, particularly cancer cells that are resistant to NK cell treatment.

Further, NK cells are known to mediate anti-cancer effects without the risk of inducing graft-versus-host disease (GvHD).

Studies have shown an aberrant overexpression of CD123 on CD34+ CD38- AML cells, while the normal bone marrow counterpart CD34+ CD38- does not express CD123(Jordan, Upchurch et al. 2000). This population of CD123+, CD34+CD38- has been considered as LSCs as these cells are able to initiate and maintain the leukemic process into immunodeficient mice.

The number of CD34+ /CD38- /CD123+ LSCs can be used to predict the clinical outcome for AML patients. The CD34+ /CD38- /CD123+ cells, greater than 15% in AML patients, are associated with a lack of complete remission and unfavorable cytogenetic profiles. In addition, the presence of more than 1% of CD34+ /CD38- /CD123+ cells could also have a negative impact on disease-free survival and overall survival.

At the present, therapies for MDS and AML have focused on the leukemic blast cells because they are very abundant and clearly represent the most immediate problem for patients. Importantly, leukemic stem cells (LSCs) are quite different from most of the other leukemia cells (“blast” cells), and they constitute a rare subpopulation. While killing blast cells can provide short-term relief, LSCs, if not destroyed, will always re-grow, causing the patient to relapse. It is imperative that LSCs be destroyed in order to achieve durable cures for MDS disease. Unfortunately, standard drug regimens are not effective against MDS or AML LSCs. Therefore,

it is critical to develop of new therapies that can specifically target both the leukemic stem cell population and the bulky leukemic population. The compound CAR disclosed in the present invention target both of these populations and is embodied herein.

In accordance to the present invention, it was surprisingly found that NK cells provide an off-the-shelf product that may be used as an allogeneic product for treatment. Thus, according to the present invention, cCAR cell therapy needs to be performed on a patient-specific basis as required by the current state of art. The applicants of the present invention have discovered a novel immunotherapy, where the patient's lymphocytes or tumor infiltrated lymphocytes need not be isolated for an effective CAR cell based therapy.

Allogeneic or autologous NK cells are expected to induce a rapid immune response but disappear relatively rapidly from the circulation due to their limited lifespan. Thus, applicants surprisingly discovered that there is reduced concern of persisting side effects using cCAR cell based therapy.

According to one aspect of the present invention, NK cells can be expanded and transfected with cCAR in accordance to the present invention. NK cells can be derived from cord blood, peripheral blood, iPS cells and embryonic stem cells. According to one aspect of the present invention, NK-92 cells may be expanded and transfected with cCAR. NK-92 is a continuously growing cell line that has features and characteristics of natural killer (NK) cells. NK-92 cell line is IL-2 dependent and has been proven to be safe and feasible. cCAR expressing NK-92 cells can be expanded in the serum free-medium with or without co-culturing with feeder cells. A pure population of NK-92 carrying the cCAR of interest may be obtained by sorting.

Identification of appropriate surface target antigens is a prerequisite for developing CAR T/NK cells in adaptive immune therapy.

In one aspect of the present invention, CD123 antigen is one of the targets for cCAR therapy. CD123, the alpha chain of the interleukin 3 receptor, is overexpressed on a variety of hematologic malignancies, including acute myeloid leukemia (AML), B-cell acute lymphoblastic leukemia (B-ALL), hairy cell leukemia, and blastic plasmocytoid dendritic neoplasms. CD123 is  
5 absent or minimally expressed on normal hematopoietic stem cells. More importantly, CD123 is expressed on a subset of leukemic cells related to leukemic stem cells (LSCs), the ablation of which is essential in preventing disease refractoriness and relapse.

In one aspect of the present invention, CD 33 antigen is one of the targets for cCAR therapy. CD33 is a transmembrane receptor expressed on 90% of malignant cells in acute  
10 myeloid leukemia. Thus, according to the present invention, CD123 and CD33 target antigens are particularly attractive from a safety standpoint.

In accordance with the present invention, the compound CD33CD123 CARs may be highly effective for therapeutic treatment of chronic myeloid leukemia (CML) population. In chronic myeloid leukemia (CML), there is a rare subset of cells that are CD34+CD38-. This  
15 population is considered as comprised of LSCs. Increased number of LSCs is associated with the progression of the disease. A small-molecule Bcr-Abl tyrosine kinase inhibitor (TKI) is shown to significantly improve the overall survival in CP-CML patients. However, LSCs are thought to be resistant to TKI therapy. A novel therapy targeting CML resistant LSCs is urgently needed for treatment of CML and the novel therapy is embodied in the compound CD33CD123  
20 CAR disclosed in the present invention. CD123 expression is high in the CD34+CD38- population. In accordance with the present invention, the compound CD33CD123 CARs is highly effective for therapeutic treatment of this population.

In one embodiment of the present invention, leukemic cells expressing both CD123 and CD33 in the cCAR is used as a therapeutic treatment. CD33 is expressed on cells of myeloid lineage, myeloid leukemic blasts, and mature monocytes but not normal pluripotent hematopoietic stem cells (Griffin, Linch et al. 1984). CD33 is widely expressed in leukemic cells  
5 in CML, myeloproliferative neoplasms, and MDS.

As a significant number of patient with acute myeloid leukemia (AML) are refractory to standard chemotherapy regimens or experience disease relapse following treatment (Burnett 2012), the development of CAR T cell immunotherapy for AML has the potential to address a great clinical need. In the majority of these patients, leukemic cells express both CD123 and  
10 CD33, giving broad clinical applicability to the compound CD33CD123 CAR disclosed herein. Thus, the present invention discloses a novel multiple cCAR T/NK cell construct comprising multiple CAR targeting multiple leukemia-associated antigens, thereby offsetting antigen escape mechanism, targeting leukemia cells, including leukemic stem cells, by synergistic effects of co-stimulatory domain activation and thereby providing a more potent, safe and effective therapy.

15 The present invention further discloses compound CAR construct, with enhanced potency of anti-tumor activity against cells co-expressing target antigens, and yet retains sensitivity to tumor cells only expressing one antigen. In addition, each CAR of the compound CAR includes one or two co-stimulatory domains and potent killing capability in presence of the specific target.

In pre-clinical studies on dual specificity, trans-signaling CARs targeting solid tumors  
20 including breast cancer and epithelial ovarian cancer, a CD3 $\zeta$  intracellular signaling domain is separated from co-stimulatory domains from second generation of CARs. In other words, one CAR contains the first generation of CAR without any co-stimulatory domain, and another lacks a CD3 zeta intracellular domain. Therefore, the presence of both target antigens is required for T



cell activation and potent killing. Thus, they were proposed as a way to decrease off-tumor toxicity caused by healthy tissue expression of one of the two target antigens, increasing target specificity, but at the expense of sensitivity. In one embodiment, the compound CAR is a compound CD123CD19 CAR. It has been shown that more than 90% of B-ALLs express CD123  
5 in a subset of population. Like AML and MDS, it has been considered that a rare LSC population exists in B-ALL. Therefore, targeting both leukemic stem cell and bulky leukemic populations in accordance to the present invention, can be applied to B-ALLs. CD123 and CD19 surface antigens expressed in the B-ALLs may be targets as CD19 is widely expressed in different stages of B-cell lymphoid populations, in accordance with the present invention.

10 Multiple myeloma (MM) is the second most common hematologic malignancy in the US and is derived from clonal plasma cells accumulated in the bone marrow or extramedullary sites. MM is an incurable disease with a median survival of approximately 4.5 years (Kumar, Rajkumar et al. 2008). Anti-Myeloma CARs in Pre-clinical Development have been developed and CAR targets include CD38, CS1, B cell maturation Antigen (BCMA) and CD38. However,  
15 heterogeneity of surface antigen expression commonly occurs in malignant plasma cells (Ruiz-Arguelles and San Miguel 1994), which makes it a difficult target for CARs. Malignant plasma cells also express low levels of CD19. Previously it has been shown that myeloma stem cells also express some B-cell markers including CD19. Targeting this population could be effective in the treatment of myeloma in conjunction with standard and other myeloma CAR therapies.

20 Multiple myeloma (MM) is a haematological malignancy with a clonal expansion of plasma cells. Despite important advances in the treatment, myeloma remains an incurable disease; thus novel therapeutic approaches are urgently needed.

CS1 (also called as CD319 or SLAMF7) is a protein encoded by the SLAMF7 gene. The surface antigen CS1 is a robust marker for normal plasma cells and myeloma cells (malignant plasma cells).

5 Tumour necrosis factor receptor superfamily, member 17 (TNFRSF17), also referred to as B-cell maturation antigen (BCMA) or CD269 is almost exclusively expressed at the terminal stages of plasma cells and malignant plasma cells. Its expression is absent other tissues, indicating the potential as a target for CAR T or NK cells.

10 Malignant plasma cells display variable degrees of antigenic heterogeneity for CD269 and CS1. A single CAR unit product targeting either CD269 or CS1 could target the majority of the cells in a bulk tumor resulting in an initial robust anti-tumor response. Subsequently residual rare non-targeted cells are expanded and cause a disease relapse. While multiple myeloma is particularly heterogeneous, this phenomena could certainly apply to other leukemias or tumors. A recent clinical trial at NIH using BCMA CAR T cells showed a promising result with a complete response in some patients with multiple myeloma. However, these patients relapsed  
15 after 17 weeks, which may be due to the antigen escape. The antigen escape is also seen in CD19 CAR and NY-ESO1 CAR T cell treatments. Thus, there is an urgent need for more effective CAR T cell treatment in order to prevent the relapse.

In one aspect of the present invention, BCMA and CS1 are the targets for BCMACS1 CAR therapy.

20 In some embodiments, a compound CAR targets cells expressing BCMA or CS1 antigens or both. The targeted cells may be cancer cells, such as, without limiting, lymphomas, or leukemias or plasma cell neoplasms. In further embodiments, plasma cell neoplasms is selected from plasma cell leukemia, multiple myeloma, plasmacytoma, heavy chain diseases,

amyloidosis, waldestrom's macroglobulinema, heavy chain diseases, solitary bone plamacytoma, monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma.

BAFF (B-cell-activation factor) and APRIL (a proliferation-induced ligand) are two TNF  
5 homologs that bind specifically TACI (also called as TNFRSF1 3B or CD267) and BCMA with high affinity. BAFF (also known as BLyS) binds BAFF-R and functionally involves in the enhancement of survival and proliferation of later stage of B cells. BAFF has been shown to involve some autoimmune disorders. APRIL plays an important role in the enhancement of antibody class switching. Both BAFF and APRIL have been implicated as growth and survival  
10 factors for malignant plasma cells.

Ligand-receptor interactions in the malignant plasma cells are described in Figure 45.

In some embodiments, a compound CAR targets cells expressing TACI or CS1 antigens or both. In another embodiment, a compound CAR targets cells expressing TACI or CS1 antigens or both. The targeted cells may be cancer cells, such as, without limiting, lymphomas,  
15 or leukemias or plasma cell neoplasms. In further embodiments, plasma cell neoplasms is selected from plasma cell leukemia, multiple myeloma, plasmacytoma, heavy chain diseases, amyloidosis, waldestrom's macroglobulinema, heavy chain diseases, solitary bone plamacytoma, monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma. The target cells may also be one or two or multiple different cell types of B cells,  
20 immature B cells, naïve B cells, centroblasts, centrocytes, memory B cells, plasmablasts, long lived plasma cells, plasma cells. These cells involve autoimmune diseases include systemic scleroderma, multiple sclerosis, psoriasis, dermatitis, inflammatory bowel diseases (such as Crohn's disease and ulcerative colitis), systemic lupus erythematosus, vasculitis, rheumatoid

arthritis, Sjorgen's syndrome, polymyositis, granulomatosis and vasculitis, Addison's disease, antigen-antibody complex mediated diseases, and anti-glomerular basement membrane disease.

In some embodiments, a compound CAR targets cells expressing BAFF-R or CS1 antigens or both. In another embodiment, a compound CAR targets cells expressing BAFF-R or  
5 CS1 antigens or both. The targeted cells may be cancer cells, such as, without limiting, lymphomas, or leukemias or plasma cell neoplasms. In further embodiments, plasma cell neoplasms is selected from plasma cell leukemia, multiple myeloma, plasmacytoma, heavy chain diseases, amyloidosis, waldestrom's macroglobulinemia, heavy chain diseases, solitary bone  
10 plamacytoma, monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma.

In some embodiments, a compound CAR (cCAR) targets cells expressing one or two or all of BAFF-R, BCMA, TACI and CS1 antigens.

In some embodiments, a unit of CAR in a cCAR can comprise: 1) a scFv against either  
15 BAFF-R, BCMA, TACI and CS1; 2) a hinge region; 3) co-stimulatory domain (s) and intracellular signaling domain.

In some embodiments, a unit of CAR in a cCAR can comprise: 1) BCMA or TACI or BAFF-R binding domain, or APRIL binding domain; 2) a hinge region; 3) co-stimulatory domain (s) and intracellular signaling domain.

20 In a further embodiment, BCMA or TAC1 or BAFF-R binding domain can be a part of or entire APRIL and BAFF molecules.

In some embodiments, a unit of CAR in a cCAR can comprise: 1) a scFv against BCMA or CS1; 2) a hinge region; 3) co-stimulatory domain (s) and intracellular signaling domain.

In further embodiments, cCAR can comprise one or two or multiple units of CAR. Each unit CAR could bear same or different hinge region and co-stimulatory domain.

In further embodiments, the target antigens can include at least one of this group, but not limited to, ROR1, PSMA, MAGE A3, Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-  
5 2/neu, MAGE-3, MAGE-4, MAGE-5, MAGE- 6, alpha-fetoprotein, CA 19-9, CA 72-4, NY-ESO, FAP, ErbB, c-Met, MART-1, CD30, EGFRvIII, immunoglobulin kappa and lambda, CD38, CD52, CD3, CD4, CD8, CD5, CD7, CD2, and CD138. The target antigens can also include viral or fungal antigens, such as E6 and E7 from the human papillomavirus (HPV) or EBV (Epstein Barr virus) antigens.

10 In some embodiments, a cCAR targets a cell expressing either CD19 or CD20 antigens or both of them. In another embodiment, a cCAR targets a cell expressing either CD19 or CD22 antigens or both of them. The targeting cells are cancer cells such as B-cell lymphomas or leukemias.

Acute graft-versus-host disease (GVHD) remains the most important cause of morbidity  
15 and mortality after allogeneic hematopoietic stem cell transplantation. In the effector phase of GVHD, T cell receptor (TCR), a heterodimer of alpha and beta chains, is expressed on the surface of T cells, TCR recognizes some antigens on the HLA molecule on host cells, enhances T cell proliferation, and releases cytotoxic agents that cause the damage on host cells. TCR gene inactivation is efficient at preventing potential graft-versus-host reaction. The inactivation of  
20 TCRs can result in the prevention of the TCR recognition of alloantigen and thus GVHD.

The role of CD45 on NK cells is quite different from that of T cells. NK cells from CD45-deficient mice have normal cytotoxic activity against the prototypic tumor cell line, Yac-1. In

addition, CD45-deficient NK cells proliferate normally and respond to IL15 and IL-21.

Therefore, CD45 disruption or deletion would not affect the NK cell killing and proliferation.

The present disclosure includes methods of permanent deletion of CD45 in a T or NK cell with subsequent stable introduction of CD45-specific CARs. As a result, the engineered T cells

5 display the desired properties of redirected specificity for CD45 without causing self-killing and response to presentation of antigen. In a further embodiment, the engineered T cells may have efficacy as an off-the-shelf therapy for treating malignancies or other diseases.

The present disclosure relates to a method where T-cells are engineered to allow proliferation when TCR signaling is reduced or lost through the inactivation or deletion of endogenous CD45.

10 The reduction or loss of TCR signaling could result in the prevention of GVHD.

In a further embodiment, T cells reducing or losing the TCR signaling by the inactivation of CD45 could be used as an “off the shelf “ therapeutic product.

The present disclosure includes methods of modified T or NK cells, which comprises: (a) modifying T or NK cells by inactivating CD45; (b) expanding these modified cells; (c) sorting  
15 modified T or NK cells, which do not express CD45; (d) introducing CD45CAR.

In embodiments, the CD45CAR gene encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one of an antigen recognition domain, a hinge region, a transmembrane domain, and T cell activation domains, and the antigen recognition domain is redirected against CD45 surface antigen present on a cell. The antigen recognition domain includes a monoclonal  
20 antibody or a polyclonal antibody directed against CD45 antigen. The antigen recognition domain includes the binding portion or a variable region of a monoclonal or a polyclonal antibody.

In some embodiments, the modified T cells are obtained from allogeneic donors and used as an 'off-the-shelf product'.

Targeting CD45 using CAR T or NK cells may cause self-killing as T and NK cells express this surface antigen. To overcome this drawback, the inventor proposes to inactivate  
5 CD45 gene using engineered CRISPR/Cas9 system, zinc finger nuclease (ZFNs) and TALE nucleases (TALENs) and meganucleases. The loss of CD45 in T or NK cells is further transduced with CARs targeting neoplasms expressing CD45.

The disclosure includes methods for eliminating or reducing abnormal or malignant cells in bone marrow, blood and organs. In some embodiments, malignant cells expressing CD45 are  
10 present in patients with acute leukemia, chronic leukemia, B and T cell lymphomas, myeloid leukemia, Acute lymphoblastic lymphoma or leukemia, primary effusion lymphoma, Reticulohistiocytoma, transient myeloproliferative disorder of Down's syndrome, lymphocyte predominant Hodgkin's lymphoma, myeloid leukemia or sarcoma, dendrocytoma, histiocytic sarcoma, Giant cell tumor of tendon sheath, interdigitating dendritic cell sarcoma, post-  
15 transplant lymphoproliferative disorders, etc.

In some embodiments, CD45CAR cells can be used to make space in the bone marrow for bone marrow stem cell transplant by removing hematopoietic cells, at the same time removing leukemic/lymphoma cells or immunologic cells capable of graft rejection. In a further embodiment, CD45CAR cells may be used for pre-treatment of patients before their  
20 undergoing a bone marrow transplant to receive stem cells. In a further embodiment, CD45CAR can be used as myeloblastic conditioning regimens for hematopoietic stem cell transplantation.

In some embodiment, CD45CAR cells are utilized for treating or preventing a residual disease after stem cell transplant and/or chemotherapy.

In some embodiments, the CD45CAR is part of an expressing gene or a cassette. In a preferred embodiment, the expressing gene or the cassette may include an accessory gene or a tag or a part thereof, in addition to the CD45CAR. The accessory gene may be an inducible suicide gene or a part thereof, including, but not limited to, caspase 9 gene, thymidine kinase, cytosine deaminase (CD) or cytochrome P450. The “suicide gene” ablation approach improves safety of the gene therapy and kills cells only when activated by a specific compound or a molecule. In some embodiments, the suicide gene is inducible and is activated using a specific chemical inducer of dimerization (CID).

In some embodiments, safety switch can include the accessory tags are a c-myc tag, CD20, CD52 (Campath), truncated EGFR gene (EGFRt) or a part or a combination thereof. The accessory tag may be used as a nonimmunogenic selection tool or for tracking markers.

In some embodiments, safety switch can include a 24-residue peptide that corresponds to residues 254–277 of the RSV F glycoprotein A2 strain (NSELLSLINDMPITNDQKKLMSNN).

In some embodiments, safety switch can include the amino acid sequence of TNF  $\alpha$  bound by monoclonal anti-TNF  $\alpha$  drugs.

Administration of any of the engineered cells described herein may be supplemented with the co-administration of a CAR enhancing agent. Examples of CAR enhancing agents include immunomodulatory drugs that enhance CAR activities, such as, but not limited to agents that target immune-checkpoint pathways, inhibitors of colony stimulating factor-1 receptor (CSF1R) for better therapeutic outcomes. Agents that target immune-checkpoint pathways include small molecules, proteins, or antibodies that bind inhibitory immune receptors CTLA-4, PD-1, and PD-L1, and result in CTLA-4 and PD-1/PD-L1 blockades. As used herein, enhancing agent includes enhancer as described above.



As used herein, “patient” includes mammals. The mammal referred to herein can be any mammal. As used herein, the term “mammal” refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). Preferably, the mammal is a human. A patient includes subject.

In certain embodiments, the patient is a human 0 to 6 months old, 6 to 12 months old, 1 to 5 years old, 5 to 10 years old, 5 to 12 years old, 10 to 15 years old, 15 to 20 years old, 13 to 19 years old, 20 to 25 years old, 25 to 30 years old, 20 to 65 years old, 30 to 35 years old, 35 to 40 years old, 40 to 45 years old, 45 to 50 years old, 50 to 55 years old, 55 to 60 years old, 60 to 65 years old, 65 to 70 years old, 70 to 75 years old, 75 to 80 years old, 80 to 85 years old, 85 to 90 years old, 90 to 95 years old or 95 to 100 years old.

The terms “effective amount” and “therapeutically effective amount” of an engineered cell as used herein mean a sufficient amount of the engineered cell to provide the desired therapeutic or physiological or effect or outcome. Such, an effect or outcome includes reduction or amelioration of the symptoms of cellular disease. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what an appropriate “effective amount” is. The exact amount required will vary from patient to patient, depending on the species, age and general condition of the patient, mode of administration and the like. Thus, it may not be possible to specify an exact “effective amount”. However, an appropriate “effective

amount” in any individual case may be determined by one of ordinary skill in the art using only routine experimentation. Generally, the engineered cell or engineered cells is/are given in an amount and under conditions sufficient to reduce proliferation of target cells.

Following administration of the delivery system for treating, inhibiting, or preventing a cancer, the efficacy of the therapeutic engineered cell can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a therapeutic engineered cell delivered in conjunction with the chemo-adjuvant is efficacious in treating or inhibiting a cancer in a patient by observing that the therapeutic engineered cell reduces the cancer cell load or prevents a further increase in cancer cell load. Cancer cell loads can be measured by methods that are known in the art, for example, using polymerase chain reaction assays to detect the presence of certain cancer cell nucleic acids or identification of certain cancer cell markers in the blood using, for example, an antibody assay to detect the presence of the markers in a sample (e.g., but not limited to, blood) from a subject or patient, or by measuring the level of circulating cancer cell antibody levels in the patient.

Throughout this specification, quantities are defined by ranges, and by lower and upper boundaries of ranges. Each lower boundary can be combined with each upper boundary to define a range. The lower and upper boundaries should each be taken as a separate element.

Reference throughout this specification to “one embodiment,” “an embodiment,” “one example,” or “an example” means that a particular feature, structure or characteristic described in connection with the embodiment or example is included in at least one embodiment of the present embodiments. Thus, appearances of the phrases “in one embodiment,” “in an embodiment,” “one example,” or “an example” in various places throughout this specification are not necessarily all referring to the same embodiment or example. Furthermore, the particular

features, structures or characteristics may be combined in any suitable combinations and/or sub-combinations in one or more embodiments or examples. In addition, it is appreciated that the figures provided herewith are for explanation purposes to persons ordinarily skilled in the art and that the drawings are not necessarily drawn to scale.

5           As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having,” or any other variation thereof, are intended to cover a non-exclusive inclusion. For example, a process, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such process, article, or apparatus.

10           Further, unless expressly stated to the contrary, “or” refers to an inclusive “or” and not to an exclusive “or”. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

          Additionally, any examples or illustrations given herein are not to be regarded in any way  
15 as restrictions on, limits to, or express definitions of any term or terms with which they are utilized. Instead, these examples or illustrations are to be regarded as being described with respect to one particular embodiment and as being illustrative only. Those of ordinary skill in the art will appreciate that any term or terms with which these examples or illustrations are utilized will encompass other embodiments which may or may not be given therewith or  
20 elsewhere in the specification and all such embodiments are intended to be included within the scope of that term or terms. Language designating such nonlimiting examples and illustrations includes, but is not limited to: “for example,” “for instance,” “e.g.,” and “in one embodiment.”

In this specification, groups of various parameters containing multiple members are described. Within a group of parameters, each member may be combined with any one or more of the other members to make additional sub-groups. For example, if the members of a group are a, b, c, d, and e, additional sub-groups specifically contemplated include any one, two, three, or four of the members, e.g., a and c; a, d, and e; b, c, d, and e; etc.

As used herein, a XXXX antigen recognition domain is a polypeptide that is selective for XXXX. Therefore, XXXX is the target. For example, a CD38 antigen recognition domain is a polypeptide that is specific for CD38.

As used herein, CDXCAR refers to a chimeric antigen receptor having a CDX antigen recognition domain.

The present disclosure may be better understood with reference to the examples, set forth below. 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 the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure.

## EXAMPLES

### Generation of compound CAR (cCAR)

The construction of the CD33CD123 cCAR follows the schematic in Figure 1A. It includes SFFV (spleen focus-forming virus) promoter that drives the expression of the functional compound CAR (cCAR) bearing two different units of CARs. The antigen receptor head, a scFv (single-chain variable fragment) nucleotide sequence of the anti-CD33 and anti-CD123. A P2A peptide derived from picornavirus is utilized due to the highly efficient mechanism of its self-cleaving dynamics for bicistronic genetic constructs. The self-cleaving P2A peptide serves to

link the two independent units of CARs, CD33CAR, and CD123CAR together during expression. The advantages of this approach over an internal ribosomal entry site (IRES), which is commonly used in the literature, include its small size and high cleavage efficiency between two unit proteins upstream and downstream of the 2A peptide. In addition, the use of self-cleaving P2A peptide can avoid a problem of differences in expression levels between gene  
5 before and after IRES when IRES is applied.

The modular unit, CD33CAR includes the CD33 scFv domain, a CD8a hinge region, a CD8a transmembrane domain, 4-BB co-stimulatory domain and an intracellular domain of CD3 zeta chain. The second modular CAR, CD123CAR bears the same hinge, transmembrane and  
10 intracellular signaling domains as CD33CAR but different scFv, and co-stimulatory domains. The CD33 CAR recognizes its corresponding antigen and the CD123 CAR binds to its corresponding antigen. The hinge region was designed such that sequences where disulfide interactions are avoided. Different co-stimulatory domains, 4-BB and CD28 were used. The CD33CD123 compound CAR was subcloned into a lentiviral plasmid.

#### 15 **Generation of a high-efficiency compound CAR (cCAR)**

Compound CAR lentivirus was generated by transfection of HEK-293 FT cells with Lipofectamine 2000 according to manufacturer's directions, except with 2x the vector DNA due to a large size of insert, in order to increase titer as shown in Figure 2. After about 12-16 hours incubation, media containing Lipofectamine was removed and replaced with DMEM containing  
20 10% FBS, 20 mM HEPES, 1 mM sodium pyruvate and 1 mM sodium butyrate. After about 24 hours, the supernatant was harvested and refrigerated, and replaced with fresh media. After about another 24 hours, this was collected and combined with the previous supernatant, and filtered through a 0.45  $\mu$ M filter disc. Supernatant was split into aliquots, flash frozen with liquid

nitrogen and stored at -80°C. HEK-293 FT cells were harvested, stored frozen, and lysed for subsequent electrophoresis and Western blotting.

PB (peripheral blood) or CB (human umbilical cord blood) buffy coat cells were activated 2 days with anti-CD3 antibody and IL-2. cCAR lentiviral supernatant was spinoculated  
5 onto retronectin-coated multiwell plates. Activated T cells were transduced in multiple wells with lentiviral supernatant at a low concentration of about  $0.3 \times 10^6$  cells/mL to increase transduction efficiency (Figure 2).

Following the first overnight transduction, cells were added directly to a second virus-coated plate for a second transduction without washing, unless the cells did not look healthy.

10 Following the second overnight transduction, cells were washed, combined and incubated in tissue culture treated plates. CAR T cells were allowed to expand for up to about 5 days prior to co-culture killing assays. After about 3 days of incubation, cells were incubated with goat anti-mouse F(Ab')<sub>2</sub> or goat IgG (isotype) antibodies conjugated with biotin, washed and followed by incubation with streptavidin-PE and conjugated anti-human CD3. After washing and suspension  
15 in 2% formalin, cells were then analyzed by flow cytometry to determine percent transduction efficiency.

### **Characterization of the CD33CD123 cCAR**

Transfected CD33CD123 cCAR HEK293T cells were subjected to Western blot analysis in order to confirm the compound construct. Immunoblot with an anti-CD3ζ monoclonal  
20 antibody showed bands of predicted size for the compoundCAR CD3ζ fusion protein (Figure 1B). Importantly, two distinct bands of similar intensity were observed on the blot signaling the successful high cleavage action of the P2A peptide as expected. No CD3ζ expression was seen

for the GFP control vector as expected. The surface expression of scFv was also tested on HEK 293 cells (Figure 1C) and primary T cells (Figure 1C).

The compound CD33CD23CAR lentivirus was tested for transduction efficiency in the HEK293 cell line and analyzed by flow cytometry (Beckman Coulter) (Figure 1C). Flow  
5 cytometry showed that about 67% of HEK cells expressed CD33CD123 CARs. Human peripheral blood (PB) is often used for autologous T cell therapy. Human PB buffy coat cells were activated with anti-CD3 antibody and IL-2, and transduced with either CD4CAR or control (GFP) lentiviruses. After transduction, flow cytometry analysis showed that about 22% of T-cells expressed the CD33CD123CAR (Figure 1C).

## 10 RESULTS

### **CD33CD123 cCAR T-cells derived from Umbilical Cord Blood (UCB) and Peripheral Blood (PB) specifically kill CD33-expressing tumor cells**

CD33CD123 cCAR T cells or GFP T cells (control) were incubated with target cells at  
15 ratios ranging from 0.5:1 from 50:1, preferably, at about 2:1, 5:1, 10:1, 20:1, 50:1, at about 100,000, 200,000, 500,000, about 1 million, or 2 million effector cells to about 50,000, 100,000, 200,000 target cells, respectively) in about 1-2 mL T cell culture media, without IL-2 for about 24h. Target cells were leukemic cell lines and leukemia cells from a patient with leukemia. After about 24 hours of co-culture, cells were stained with mouse anti-human CD33, CD123, CD34  
20 and CD3 antibodies.

CD33CD123 cCAR T cells expressing the CD33CAR and CD123 CAR were generated and tested for anti-leukemic functions using the HL60 and KG-1a cell lines. The HL60 cell line is a promyelocytic leukemia cell line highly enriched for CD33. About 100% of its cell population is CD33+ with a small subset (<10%) of it being dim CD123+. In culture, this cell  
25 line was tested to determine the effectiveness of the CD33CD123 CAR with an emphasis on

targeting CD33-expressing leukemic cells. Additionally, due to the strong expression of CD33 in HL60, it is CD33CD123 cCAR action may be profound. Indeed, during 24h co-culture conditions with various ratios of effector to target cells, the CD33CD123 cCAR exhibited significant leukemic cell killing properties (Figure 3). CB-derived CD33CD123 CAR T-cells were first tested for their ability to kill HL60 cells. At about 24h incubation and low effector:target (E:T) ratios ranging from about 0.5:1 to 50:1, preferably, 1:1 to about 5:1, more preferably about 2:1 to 4:1, CD33CD123 CAR cells eliminated about 55% of the CD33 expressing HL60 cells when compared to GFP control. At a ratio of about 5:1, the killing action rose to about 82%.

CD33CD123 CAR derived from peripheral blood mononuclear cells (PBMCs) were co-cultured with the myelogenous leukemia cell line KG1a, which also expresses about 100% CD33 at moderate levels compared to HL60 and 50-80% CD123. KG1a is, therefore, a relatively dual target cell population that is double positive for the antigens targeted by the CD33CD123 CAR. At about 24 hours of incubation and low effector:target (E:T) ratios ranging from about 0.5:1 to 50:1 were used. While at a low E:T ratio of about 2:1, the CD33CD123 CAR exhibited modest anti-leukemic activity about 26%, an increase in E:T ratio to 10:1 resulted in a killing of KG1a of about 62% compared to GFP control (Figure 4), signaling that the intensity of the CD33 marker may be an indicator for the efficacy of killing with HL60 presenting strongly and harnessing more CAR action than KG1a. These experiments provide evidence for the function of the whole CD33CD123 CAR against its relevant antigen presenting cell populations.

Additional compound CAR, CD33CD123-BB cCAR has been generated. This compound CAR comprises two independent units of CARs, CD33 and CD123. The first CAR comprises scFv binding to CD33 and the second CAR bears a different scFv recognizing CD123. Both



CARs contain the same hinge region, transmembrane, co-stimulatory and intracellular domains. CD33CD123-BB cCAR lentiviruses were produced and their killing ability was tested in KG-1a cells. As shown in Figure 5, there was substantial killing at a ratio of about 10:1 but it is less potent than that of CD33CD123 cCAR.

5 **CD33CD123 cCAR possesses activity against patient samples expressing CD33 and/or CD123**

In addition to cell line experiments, studies were also conducted on patient samples in order to test the function of each individual CAR unit. An aggressive acute myeloid leukemia  
10 (AML), AML-9 was used for testing efficacy of the CD33CD123 cCAR. Due to the heterogeneity of the patient cell population, which includes multiple cell types in the AML-9 sample, leukemic blasts were gated with CD34 and CD33, as they were positive for these two markers. The depletion of this CD33+CD34+ population of leukemic cells was observed to be 48% over the GFP control at a ratio of CAR T cell:target cell (Figure 6).

15 Leukemic cells that were CD123 positive and CD33 negative were also tested. For this purpose, human B cell acute lymphoblastic leukemia (B-ALL) sample, Sp-BM-B6 was chosen. All leukemic blasts in this sample were CD34+CD33 -, and more than about 50% positive for CD123. Depletion of the CD34+ leukemic cell population by CD33CD123 cCAR T cells was about 86% as compared to that of the GFP control (Figure 7). Based on the cell line and human  
20 sample studies, our data strongly suggest that the compound CD33CD123 CAR is able to target leukemic cells expressing CD33 or CD123 or both.

**CD33CD123 cCAR NK cells targeting leukemia cells expressing CD33 or CD23 or both**

Natural killer (NK) cells are CD56+ CD3- and can efficiently kill infected and tumor cells like CD8+ T cells. Unlike CD8+ T cells, NK cells launch cytotoxicity against tumors  
25 without the requirement of activation to kill cells. NK cells are safer effector cells, as they may

avoid the potentially lethal complications of cytokine storms. However, the use of either CD33 or CD123 or both CAR NK cells in killing leukemias is entirely unexplored.

### **Production of CD33CD123 cCAR NK cells**

NK-92 cells were transduced with CD33CD123 CAR lentiviral supernatant in two consecutive overnight transductions with a change of retronectin- and virus-coated plates in between. The transduced cells were expanded for 3 or 4 days and then analyzed by flow cytometry for CAR expression. Cells were harvested and incubated with goat anti-mouse F(Ab')<sub>2</sub> at about 1:250 for about 30 minutes. Cells were washed, suspended and stained with streptavidin-PE for about 30 minutes. Cells were washed and suspended in 2% formalin, and analyzed by flow cytometry. NK-92 cells expressing CD33CD123 cCAR were then labeled as above and sorted on FACS Aria, with the top 0.2% of F(Ab')<sub>2</sub>-expressing cells collected and cultured. Subsequent labeling of sorted, expanded cells showed about 89% of NK-92 cell positive for anti-mouse F(Ab')<sub>2</sub> (Figure 8).

### **CD33CD123 cCAR NK cells efficiently lyse or eliminate leukemic cells**

First, we tested the function of CD33CD123 cCAR NK-92 cells by assessing their ability to kill a HL-60 cancerous cell line in co-culture. Virtually all HL-60 cells highly express CD33 but CD123 expression in this cell line is only less than 10% (weak). Therefore, it is likely that the killing ability of CD33CD123cCAR is dependent on the ability for cCAR to properly targeting CD33.

CD33CD123 cCAR NK-92 cells were co-cultured with the HL-60 cells for about 24 hours in NK cell media without IL-2. After the incubation, the CD33CD123 cCAR NK-92 cells were labeled and compared to a control of non-CAR, GFP NK-92 cells. Dramatic killing of HL-

60 cells by CD33CD123 cCAR NK-92 cells was observed as compared to the control, GFP NK-92 cells. Moreover, the killing ability of CD33CD123 cCAR NK-92 cells was dose-dependent, with a about 10 to 1 ratio of about 100% compared to the control (Figure 9 and 11).

A second co-culture experiment using the myeloid leukemia cell line was performed using KG1a, which expresses CD33 in all cells but at a moderate level compared to that of HL-60. The CD123 antigen is expressed in about 50-80% of KG1a cells. The experimental design was similar to the first experiment of the HL-60 killing assay described above, with the same incubation time, effector:cancer cell ratios and GFP NK-92 cell controls. Results show a remarkable killing of KG1a cells by CD33CD123 cCAR NK-92 cells in a dose-dependent manner as compared to the GFP NK-92 cell control. At a ratio of effector: target of 10:1, killing of KG1a cells by CD33CD123 cCAR NK-92 cells was about 85% as compared to that of GFP control (Figure 10 and 11).

Analysis of KG1a cells showed two different populations, CD33+CD123- and CD33+CD123+. Figure 11 showed a dose dependent increase in cell killing seen in both populations. Surprisingly, the double positive population showed a higher efficient killing for each increased ratio, suggesting a possible synergistic effect of two modular CARs of CD33 and CD123 (Figure 12).

#### **Generation of CD19CD20, CD19CD22, CD19CD138 cCARs**

The three cCARs have been generated (Figure 13) using the similar strategy to that of the CD33CD123 cCAR described above.

#### **Generation of cCAR including BCMA CS1 cCAR and BCMA CD19 cCAR for treatment of multiple myeloma**

Pre-clinical studies have been developed for cCARs to target surface antigens including CD38, CS1, CD138, B cell maturation antigen (BCMA) and CD38. CD19 CAR has also

demonstrated some efficacy for the treatment of multiple myeloma in a phase I clinical trial. However, given that the heterogeneity of surface antigen expression commonly occurs in malignant plasma cells (Ruiz-Arguelles and San Miguel 1994), it is unlikely that a single target is sufficient to eliminate this disease. BCMA CS1 cCAR, BCMA CD19 cCAR, BCMA CD38 cCAR and BCMA CD138 cCAR were generated and the experimental design was similar to that of CD33CD123 cCAR as described above.

### **Generation of cCAR including BCMA CS1 cCAR (BC1cCAR) for treatment of multiple myeloma**

#### **Generation and characterization of BCMA-CS1 cCAR (BC1cCAR) construct**

BC1cCAR's modular design consists of an anti-CD269 (BCMA, B-cell maturation antigen) single-chain variable fragment (scFv) region fused to an anti-CD319 (CS1) scFv by a self-cleaving P2A peptide, CD8-derived hinge (H) and transmembrane (TM) regions, and tandem 4-1BB co-activation domains linked to the CD3 $\zeta$  signaling domain (Figure 14A). A strong spleen focus forming virus promoter (SFFV) and a CD8 leader sequence were used for efficient expression of the BC1cCAR)CAR molecule on the T-cell surface. Two unit CARs use same co-stimulatory domain, 4-1BB. Transfected BC1cCAR HEK293T cells were subjected to Western blot analysis in order to confirm the compound construct. Immunoblot with an anti-CD3 $\zeta$  monoclonal antibody showed bands of predicted size for the compound CAR CD3 $\zeta$  fusion protein (Figure 14E). Importantly, two distinct bands of similar intensity were observed on the blot signaling the successful high cleavage action of the P2A peptide as expected. No CD3 $\zeta$  expression was seen for the GFP control vector as expected.

### Generation of BC1cCAR (cCAR) T-cells

T-cells isolated from umbilical cord blood (UCB) buffy coats were transduced with BC1cCAR lentivirus after 2 days of activation. Two unit CARs used the same co-stimulatory domain, 4-1BB. BC1cCAR's transduction efficiency was determined to be about 15% as  
5 determined by flow cytometry (Figure 14B). BC1cCAR T-cells were first tested on a CML (chronic myeloid leukemia) cell line negative for the myeloma markers, BCMA and CS1. As expected, there was no lysis from either control T-cells or BC1cCAR T-cells against wild-type K562 (Figure 14C). BCMA-K562 (Kochenderfer, NIH) were K562 cells transduced with BCMA expressing cDNA to express BCMA at >80% of the cell population. BC1cCAR T-cells were co-  
10 cultured with this cell line at E:T ratios of 2:1 and 5:1 and show over 30% lysis as compared to control (undetectable)(Figure 14C). These results are compatible with other cultures performed on antigen-transduced cell lines for other CARs, such as CS1CAR T-cells.

However, when BCMA-CS1-2G (a cCAR) used a different co-stimulatory domain, either 4-BB or CD28 for each unit, rare surface CAR expression was detected, which indicate  
15 that an appropriate selection of a co-stimulatory domain may be important for ensuring the surface CAR expression on T cells (Figure 14D). Although protein was detected in HEK cells by Western blotting (Fig. 14E), we were unable to detect surface expression in activated T cells transduced with CD269-CS1-2G lentiviral supernatant. This may be due to an inability to export the expressed protein to the cell membrane. In future, we may need to optimize the sequence of  
20 this construct to allow for greater cell surface expression.

### BC1cCAR T-cells specifically lyse BCMA<sup>+</sup> and CS1<sup>+</sup> cell lines

To assess the cytotoxicity ability of BC1cCAR T-cells, we conducted co-culture assays with myeloma cell lines: MM1S (BCMA<sup>+</sup> CS1<sup>+</sup>), RPMI-8226 (BCMA<sup>+</sup> CS1<sup>-</sup>), and U266

(BCMA<sup>+</sup> CS1<sup>dim</sup>). The ability of the BC1cCAR T-cells to lyse the target cells was quantified by flow cytometry analysis, and target cells were stained with Cytotracker dye (CMTMR). In 24 hour co-cultures, the BC1cCAR exhibited virtually complete lysis of MM1S cells, with over 90% depletion of target cells at an E:T ratio of 2:1 and over 95% depletion at an E:T of 5:1 (Figure 15). In RPMI-8226 cells, BC1cCAR lysed over 70% of BCMA<sup>+</sup> target cells at an E:T ratio of 2:1, and over 75% at an E:T of 5:1 (Figure 16). In 24 hour co-culture with U266 target cells, BC1cCAR lysed 80% of BCMA<sup>+</sup> U266 cells at an E:T ratio of 2:1, reaching saturation (Figure 17).

#### **BC1cCAR T-cells specifically target BCMA<sup>+</sup> and CS1<sup>+</sup> populations in primary patient myeloma samples**

Flow cytometry analysis of the MM10-G patient sample reveals distinct and consistent BCMA<sup>+</sup> and CS1<sup>+</sup> population subsets (Figure 18). MM7-G sample shows a complete BCMA<sup>+</sup> CS1<sup>+</sup> phenotype while MM11-G exhibits a noisy BCMA<sup>dim</sup> CS1<sup>dim</sup> phenotype likely attributable to its property of being a bone-marrow aspirate. After 24 hours, BC1cCAR T-cells show robust ablation of the MM7-G primary patient sample, with over 75% lysis at an E:T ratio of 5:1, increasing to over 85% at 10:1 (Figure 19). Against the MM11-G (Figure 20), BC1cCAR T-cells were able to lyse over 45% of BCMA<sup>+</sup> CS1<sup>+</sup> population at an E:T of 10:1.

BC1cCAR show targeted and specific lysis ability, by significantly ablating both the BCMA<sup>+</sup> CS1<sup>+</sup> and the BCMA<sup>-</sup> CS1<sup>+</sup> population subsets in MM10-G co-cultures over 24 hours. At an E:T ratio of 2:1, BC1cCAR T-cells ablate over 60% of the BCMA<sup>+</sup> CS1<sup>+</sup> population, and 70% of the CS1<sup>+</sup> only population. At an E:T ratio of 5:1, the ablation of CS1<sup>+</sup> only population increases to 80% (Figure 18).

**BC1cCAR T-cells exhibit significant control and reduction of tumor *in vivo***

In order to evaluate the *in vivo* anti-tumor activity of BC1cCAR T cells, we developed a xenogeneic mouse model using NSG mice sublethally irradiated and intravenously injected with luciferase-expressing MM.1S cells, a multiple myeloma cell line, to induce measurable leukemic formation. Three days following tumor cell injection, mice were intravenously injected with 8 x 10<sup>6</sup> BC1cCAR T cells or vector control cells in a single dose. On days 3, 6, and 8, mice were injected subcutaneously with RediJect D-Luciferin (Perkin Elmer) and subjected to IVIS imaging to measure tumor burden (Figure 21). Average light intensity measured for the BC1cCAR T cells injected mice was compared to that of vector control injected mice in order to determine the percentage of tumor cells in treated versus control mice (Figure 21 and 22). Unpaired T test analysis revealed an extremely significant difference (P=0.0001) between the two groups by day 8 with less light intensity and thus less tumor burden in the BC1cCAR T cells injected group compared to control (p <0.0001). On day 1, and every other day afterwards, tumor size area was measured and the average tumor size between the two groups was compared (Figure 21). In summary, these *in vivo* data indicate that CD269-CS1-BBCAR T cells significantly reduce tumor burden in MM.1S-injected NSG mice when compared to vector control NK control cells.

**CD45 CAR therapy**

Three pairs of sgRNA are designed with CHOPCHOP to target the gene of interest. Gene-specific sgRNAs are then cloned into the lentiviral vector (Lenti U6-sgRNA-SFFV-Cas9-puro-wpre) expressing a human Cas9 and puromycin resistance genes linked with an E2A self-cleaving linker. The U6-sgRNA cassette is in front of the Cas9 element. The expression of

sgRNA and Cas9puro is driven by the U6 promoter and SFFV promoter, respectively (Figure 23).

The following gene-specific sgRNA sequences were used and constructed,

In a non-limiting embodiment of the invention, exemplary gene-specific sgRNAs have been

5 designed and constructed as set forth below:

CD45 sgRNA construct::

Lenti-U6-sgCD45a-SFFV-Cas9-puro GTGGTGTGAGTAGGTAA

Lenti-U6-sgCD45b-SFFV-Cas9-puro GAGTTTTGCATTGGCGG

Lenti-U6-sgCD45c-SFFV-Cas9-puro GAGGGTGGTTGTCAATG

10 Figure 24 shows steps of generation of CD45 CAR T or NK cell targeting hematologic malignancies.

#### **CRISPR/Cas nucleases target to CD45 on NK cells**

Lentiviruses carried gene-specific sgRNAs were used to transduce NK-92 cells. The loss of CD45 expression on NK-92 cells was determined by flow cytometry analysis. The CD45

15 negative population of NK-92 cells was sorted and expanded (Figure 25). The sorted and expanded CD45 negative NK-92 cells were used to generate CD45CAR NK cells. The resulting CD45CAR NK cells were used to test their ability of killing CD45+ cells.

#### **Functional characterization of CD45 inactivated NK-92 cells (NK<sup>45i</sup>-92) after CRISPR/Cas nucleases target**

20 We demonstrated that, following CRISPR/Cas nuclease inactivation of CD45, the growth of NK<sup>45i</sup>-92 cells was similar to that of the wild NK-92 cells (Figure 26). Inactivation of CD45 did not significantly affect the cell proliferation of NK-92. In addition, we showed that the lysis ability of NK<sup>45i</sup>-92 cells was compatible to that of wild type, NK-92 when cells were co-cultured with leukemic cells, CCRF (Fig 27).



To demonstrate that CD45<sup>-</sup>inactivated NK-92 was compatible with CAR lysis, NK<sup>45i</sup>-92 cells and their wild type, NK-92 were transduced with lentiviruses expressing CD5CAR or GFP. The resulting CD5CAR NK<sup>45i</sup>-92 cells and GFP NK<sup>45i</sup>-92 were sorted by FACS, and used to compare their ability of killing targeted cells. CD5CAR NK<sup>45i</sup>-92 cells displayed the ability of robustly killing CD5 target leukemic cells at ratios (E:T), 2:1 and 5:1 when they were co-cultured with CCRF-CEM cells. We showed that there was a similar efficacy of elimination of CCRF-CEM cells in vitro between CD5CAR NK<sup>45i</sup>-92 and CD5 CAR NK-92 cells (Figure 28). This suggests that the loss of CD45 expression does not diminish the anti-tumor activity of CAR NK-cells.

#### 10 **Generation of CD45CAR construct**

We next investigate that CD45CAR in NK<sup>45i</sup>-92 cells response to the CD45 antigen in leukemic cells. We generated CD45CAR. CD45CAR consists of an anti-CD45 single-chain variable fragment (scFv) region, CD8-derived hinge (H) and transmembrane (TM) regions, and tandem CD28 and 4-1BB co-activation domains linked to the CD3ζ signaling domain (Figure 29A). A strong spleen focus forming virus promoter (SFFV) and a CD8 leader sequence were used. CD45CAR protein was characterized by Western blot of HEK293-FT cells transfected with CD45CAR lentiviral plasmid with appropriate vector control. Additionally, anti-CD3zeta monoclonal antibody immunoblots revealed bands of predicted size for the CD45CAR protein with no bands observed in vector control (Figure 29B).

#### 20 **CD45CAR NK<sup>45i</sup>-92 NK cells**

Following fluorescence-activated cell sorting (FACS) to enrich for NK<sup>45i</sup>-92 cells, CD45CAR NK-92 transduction efficiency was determined to be 87%, as determined by flow cytometry (Figure 30) after sorting. After FACS collection of NK<sup>45i</sup>-92 cells, CD45CAR expression levels remained consistently stable for at least 10 passages.

### **CD45CAR NK<sup>45i</sup>-92 cells specifically lyse CD45+ leukemic cells.**

To assess CD45CAR NK<sup>45i</sup>-92 anti-leukemic activity, we conducted co-culture assays using T-ALL cell lines, CCRF-CEM and Jurkat, and NK cell line and NK-92 cells since they all express CD45 (Figures 31, 32 and 33). We demonstrated that CD45CAR NK<sup>45i</sup>-92 cells consistently displayed robust lysis of leukemic cells. Following 6-hour incubation at a low effective to target cell (E:T ratio 5:1), CD45CAR NK<sup>45i</sup>-92 cells effectively lysed more than 60% of CCRF-CEM cells (Figure 31). After 6-hour co-culture, CD45CAR NK<sup>45i</sup>-92 cells were also able to eliminate about 60% of Jurkat cells at a ratio of E:T, 2:1 or 5:1 (Figure 32). After 6 hours of co-culture, CD45CAR NK<sup>45i</sup>-92 cells efficiently lysed 20% CD45 positive NK-92 cells at an E:T ratio of 2:1, with close to 60% lysis at an E:T of 5:1 (Figure 33A-33C).

To further analyze the CD45 target for hematologic malignancies, we also generated additional two CARs: CD45-28 and CD45-BB, and the lentiviruses expressing CD45-28 or CD45-BB CAR were used to transduce NK45i-92 cells. CD45-28 and CD45-BB CARs contain a new anti-CD45 scFv, which is different from that of CD45CAR described above. CD45-28 CAR uses a CD28 co-stimulatory domain while the CD45-BB bears a 4-BB co-stimulatory domain. Both CARs use the CD8-derived hinge (H), transmembrane (TM) regions and CD3 $\zeta$  signaling domain. CD45CARs displayed robust lysis of B acute lymphoblastic cell line, REH. CD45CAR NK45i-92 cells lysed about 76% REH cells. CD45b-BB CAR NK45i-92 cells and CD45b-28 CAR NK45i-92 cells showed about 79% and 100% lysis of REH cells, respectively compared to control GFP NK-92 cells (Fig. 33D-G). CD45b-28 CAR NK45i-92 cells exhibited the highest ability of lysis of REH cells.

**IL15 and its receptor in enhancing CAR T and NK cell functions**

Recent studies have demonstrated that T cell persistence correlates well with CAR T cell therapeutic efficacy. Recent trials demonstrate that potent and persistent antitumor activity can be generated by an infused small number of CAR T cells indicative that quality rather than  
5 quantity of infused products is more important in contributing to the anti-tumor activity.

Interleukin (IL)-15 is a cytokine that promotes the development and hemostasis of lymphocytes. Increased levels of IL-15 promote T-cell proliferation and enhance T cell effector response. Data from recent studies have shown that IL-15 is crucial for the generation and maintenance of memory CD8 T-cells, one of the key factors associated with anti-tumor activity. IL-15 binds the  
10 IL-15 receptor alpha chain (also called IL15RA or RA) contributing to IL-15-mediated effects such as T-cell survival, proliferation and memory T cell generation.

IL-15RA binds the  $\beta\gamma$  complex in the surface of T cells and IL15 signals by binding with this IL-15RA/  $\beta\gamma$  complex on the cell surface of T cells and other types of cells.

Recent data have shown that while transfection of IL-15 alone does not significantly  
15 influence T-cell function, transfection of IL-15/IL-15RA allows T cells to survive and proliferate autonomously.

The efficacy of administered IL-15 alone may be limited by the availability of free IL-15RA and its short half-life. Administration of soluble IL-15/RA complexes greatly enhanced IL-15 half-life and bioavailability in vivo. Therefore, treatment of mice with this complex, but not  
20 with IL-15 alone results in robust proliferation and maintenance of memory CD8 T cells and NK cells. Recent studies have shown that a portion of the extracellular region of IL-15RA called sushi domain is required for its binding of IL15 (WEI et al., J. Immunol., vol.167(1), p:277-282, 2001). The IL-15/RA fusion protein or IL-15/sushi fusion protein containing the linker is more

potent than IL-15 and soluble IL-15RA alone. The combination of IL-15/RA or IL-15/sushi can maximize IL-15 activity. However, it is unclear if a design incorporating both CAR and IL-15/RA or IL15/sushi in the same construct maintains its desired biological properties in T or NK cells as insert sequence length is able to affect transfection efficiency and gene expression levels.

5           The present disclosure provides an engineered cell having both CAR and IL15/RA or IL15/sushi in a single construct. In some embodiments, the disclosure includes methods to generate higher virus titer and use a stronger promoter to drive both CAR and IL15/RA or IL-15/sushi.

10           In some embodiments, the present disclosure provides an engineered cell having: (1) a CAR targeting an antigen including, but not limited to, CD4, CD2, CD3, CD7, CD5, CD45, CD20, CD19, CD33, CD123, CS1, and B-cell mature antigen (BCMA); and (2) IL-15; (3) IL15RA (RA) or sushi. In further embodiments, CAR comprises chimeric antigen receptor, one or more of co-stimulatory endodomains, such as CD28, CD2, 4-1BB and OX40 and intracellular domain of CD3 zeta chain. In further embodiments, a strong promoter can be, but is not limited to, SFFV. CARs, IL-15/RA or sushi and inducible suicide gene ("safety switch"), or a  
15           combination can be assembled on a vector, such as a lentiviral vector, adenoviral vector and retroviral vector or a plasmid. The introduction of "safety switch" could significantly increase safety profile, and limit on-target or off-tumor toxicities of CARs.

#### **Characterization of CD4IL15RA-CAR**

20           The CD4IL15RA-CAR has been generated and it contains the third generation of CD4CAR linked to IL15RA (Figure 34). A combination of CAR, (third generation), sushi/IL-15 is assembled on an expression vector and their expression is driven by the SFFV promoter (Figure 34). CAR with sushi/IL-15 is linked with the P2A cleaving sequence. The sushi/IL-15 portion is

composed of IL-2 signal peptide fused to sushi domain and linked to IL-5 via a 26-amino acid poly-proline linker (Figure 34).

To verify the CD4IL15RA construct, HEK293FT cells were transfected with lentiviral plasmids for either GFP (control) or CD4IL15RA. Approximately 60 hours after transfection,

5 both HEK-293FT cells and supernatant were collected. Cells were lysed in RIPA buffer containing protease inhibitor cocktail and electrophoresed. The gel was transferred to Immobilon FL blotting membrane, blocked, and probed with mouse anti-human CD3z antibody at 1:500.

After washes, membrane was probed with goat anti-mouse HRP conjugate, washed, and exposed to film following treatment with HyGlo HRP substrate. The CD4IL15RA-CAR was successfully

10 expressed in HEK 293 cells (Lane 2, Figure 35a, as shown next to recombinant IL-15 protein in Lane 3 (arrow). The CD4IL15RA-CAR lentiviral supernatant was further examined by the

transduction of fresh HEK-293 cells (Figure 35a). HEK-293 cells were transduced with either GFP or CD4IL15RA-CAR viral supernatant from transfected HEK-293FT cells. Polybrene was added to 4 uL/mL. Media was changed after 16 hours and replaced with media containing no

15 viral supernatant or polybrene. Three days after transduction, cells were harvested and stained with goat-anti-mouse F(Ab')<sub>2</sub> antibody at 1:250 for 30 minutes. Cells were washed and stained with streptavidin-PE conjugate at 1:500, washed, suspended in 2% formalin, and analyzed by flow cytometry. Figure 34b shows that HEK-293 cells that were transduced with the

CD4IL15RA-CAR lentivirus were 80% positive for F(Ab)<sub>2</sub>-PE (circled, Figure 35b), while  
20 transduction with GFP control lentivirus was minimal for F(Ab)<sub>2</sub>-PE (Figure 35b,left).

### **Production of CD4IL15RA-CAR NK cells**

NK-92 cells were transduced with CD4IL15RA-CAR lentiviral supernatant. After 5 days incubation, cells were harvested and incubated with goat anti-mouse F(Ab')<sub>2</sub> at 1:250 for 30

minutes. Cells were washed, suspended and stained with streptavidin-PE for 30 minutes. Cells were washed and suspended in 2% formalin, and analyzed by flow cytometry, resulting in nearly 70% of the transduced cells expressing CD4IL15RA-CAR (circled, Figure 36). Further experimental tests for CD4IL15RA-CAR will include leukemia/lymphoma killing assays in vitro and vivo, and comparison of target killing and proliferation rates with cells transduced with CD4CAR. The inventor also used the same strategy described above to generate CD19IL15RA-CAR.

#### **Production of CD4IL15RA-CAR T cells**

Human umbilical cord buffy coat cells were transduced with CD4IL15RA-CAR lentiviral supernatant. After 5 days incubation, cells were harvested and incubated with goat anti-mouse F(Ab')<sub>2</sub> at 1:250 for 30 minutes. Cells were washed, suspended and stained with streptavidin-PE for 30 minutes. Cells were washed and suspended in 2% formalin, and analyzed by flow cytometry, resulting in 63% of the transduced cells expressing CD4IL15RA-CAR (circled, Figure 37). Further experimental tests for CD4IL15RA-CAR will include leukemia/lymphoma killing assays in vitro and vivo, and comparison of target killing and proliferation rates with cells transduced with CD4CAR.

#### **CD4IL15RACAR NK cells were tested for anti-leukemic activity relative to CD4CAR NK cells in vitro by co-culturing them with the following CD4 positive cell lines: Karpas 299 and MOLT4.**

The Karpas 299 cell line was derived from a patient with anaplastic large T cell lymphoma. The MOLT4 cell line expressing CD4 was established from the peripheral blood of a 19-year-old patient with acute lymphoblastic leukemia (T-ALL). During 4-hour co-culture experiments, CD4IL15RA CAR NK cells showed profound killing (95%) of Karpas 299 cells at a 5:1 ratio of effector:target, at an even higher rate than that of CD4CAR NK cells (82%; Figure

38). Similarly, when co-cultured 1:1 with MOLT4 cells, CD4IL15RA CAR NK cells lysed target cells at a higher rate (84% to 65%) than CD4CAR NK cells in an overnight assay (Figure 39).

These results show that CD4IL15 CAR NK cells can ablate tumor cells at least as well as CD4CAR NK cells.

**5 CD4CAR and CD4IL15RA CAR T cells exhibit more potent anti-tumor activity *in vivo* than CD4CAR**

In order to evaluate the *in vivo* anti-tumor activity of CD4CAR and CD4IL15RACAR T cells, and to determine the possible increase in persistence of the CD4IL15RA CAR T cells  
10 relative to the CD4CAR T cells, we developed a xenogeneic mouse model using NSG mice sublethally irradiated and intravenously injected with luciferase-expressing MOLM13 cells, an acute myeloid leukemia cell line (M5) that is 100% CD4, to induce measurable tumor formation. Three days following tumor cell injection, 6 mice each were intravenously injected with a course of  $8 \times 10^6$  CD4CAR, CD4IL15RACAR T cells or vector control T cells. On days 3, 6, 9 and 11,  
15 mice were injected subcutaneously with RediJect D-Luciferin (Perkin Elmer) and subjected to IVIS imaging to measure tumor burden (Figure 40). CD4CAR T cell-treated mice had a 52% lower tumor burden relative to control on Day 6, whereas CD4IL15RA CAR T cell-treated mice had a 74% lower tumor burden (Figure 41). On Day 11, nearly all tumor cells had been lysed in both of these groups. Unpaired T test analysis revealed an very significant difference ( $P=0.0045$ )  
20 between control and the two groups by day 9 with less light intensity and thus less tumor burden in the CD4CAR and CD4IL15RACAR T cells treated group compared to control.

Promoter testing using the GFP reporter

HEK293FT cells were transfected with lentiviral plasmids expressing GFP under the SFFV, EF1 or CAG promoters. Approximately 60 hours after transfection, supernatant was  
25 collected from each. Relative viral titer was determined by first transducing HEK293 cells with

supernatant from each of the 3 promoters. HEK-293 cells were transduced with GFP viral supernatant from each of the 3 transfected HEK-293FT cells. Polybrene was added to 4 uL/mL. Media was changed after 16 hours and replaced with media containing no viral supernatant or polybrene. Three days after transduction, cells were harvested and washed, suspended in 2% formalin, and analyzed by flow cytometry for GFP expression (FITC). GFP expression was seen in each sample, but was highest for the cells transduced with virus made using the SFFV promoter.

Activated human umbilical cord buffy coat cells were transduced with GFP lentiviral supernatant (amount based on the results of the HEK293 transduction efficiency) from each of the promoters. After 5 days incubation, cells were harvested, washed and suspended in 2% formalin, and analyzed by flow cytometry for GFP expression. 43% of cells expressed GFP at high levels ( $>10^3$ ) while GFP-expression for cells transduced with virus using promoters EF1 (15%) and CAG (3%) were considerably lower. Five days later, cells analyzed the same way showed nearly the same percentages for each (46%, 15% and 3%, respectively; Figure 23). These results indicate that SFFV promoter leads to stronger expression than EF1 or CAG promoters, and that the expression remains high for at least 10 days post-transduction. Further experimental tests will include longer incubation times for transduced cells beyond the 10-day window.

Methods of generating the CAR gene including at least one of a T antigen recognition moiety (at least one of CD4, CD8, CD3, CD5, CD7, and CD2, or a part or a combination thereof), a hinge region and T-cell activation domains is provided.

Methods of generating multiple units of CARs (cCAR) targeting antigen (s) including at least one of CD33, CD123, CD19, CD20, CD22, CD269, CS1, CD38, CD52, ROR1, PSMA,



CD138, and GPC3, or a part or a combination of a hinge region and T- cell activation domains is provided. All references cited and/or disclosed herein are hereby incorporated by reference in their entirety.

The provided methods also include: 1) generating of the CAR T or NK cells targeting  
5 leukemias and lymphomas expressing CD45 and avoiding self-killing; 2) generation of "armored" CAR T or NK cells designed to both overcome the inhibitory tumor microenvironment and exhibit enhanced anti-tumor activity and long-term persistence.

The present invention is not limited to the embodiments described and exemplified above, but is capable of variation and modification within the scope of the appended claims. Various

10 publications, including patents, published applications, technical articles and scholarly articles are cited throughout the specification. Each cited publication is incorporated by reference herein, in its entirety and for all purposes. Various terms relating to aspects of the invention are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art, unless otherwise indicated. Other specifically defined terms are to be construed in a  
15 manner consistent with the definition provided herein.

**Functional titer of viral vector particles in supernatants** (*The % GFP cells as determined by flow cytometry allows for proxy viral titer adjustments as higher titer virus infiltrates more cells, leading to higher %GFP cell populations*).

To determine functional titer of viral vector particles in each of our supernatants, HEK  
20 293 cells were transduced with either EF1-GFP or SFFV-GFP viral supernatant, with either 30  $\mu$ L (low), 125  $\mu$ L (medium), or 500  $\mu$ L (high) per well of a 12 well tissue-culture treated plate. Culture media was changed the following morning to DMEM plus 10% FBS.

Transduced cells were then trypsinized, washed, and suspended in formalin and subjected to flow analysis. The percentage of GFP+ cells in each of the conditions was determined by flow cytometry using the FITC channel (Figure 43). In each case, the percentage of GFP+ was higher in cells transduced with SFFV-GFP than the cells transduced with the corresponding volume of EF1-GFP viral supernatant (50% to 18% for low, 80% to 40% for medium, and 82% to 70% for high). From this, we determined that using the highest volume of EF1-promoter virus was comparable to using the lowest volume of SFFV-promoter virus in terms of titer, and would allow for comparison of relative promoter strengths for the following transduction experiments

Transduced cells were also visualized on an EVOS fluorescent microscope using GFP at 20x at the same exposure conditions for each well (Figure 42). Cells transduced with SFFV-GFP viral supernatant were dramatically brighter than cells transduced with EF1-GFP. Furthermore, comparing the image of the EF1-promoter under high viral volume loads with the image of the SFFV-promoter using low viral volume loads show similar fluorescent intensity. This suggests that the SFFV promoter is a stronger driver of gene expression.

**Comparison of surface expression and persistence of different promoters in primary T-cells** (*The % GFP cells as determined by flow cytometry for T-cell transductions show expected differences in GFP cell populations as expected from the prior experiments on HEK293 cells*)

To determine promoter transduction efficiency and persistence of surface expression in primary T cells, activated cord blood buffy coat T cells were transduced with either 50  $\mu$ L of SFFV-GFP or 1 mL of EF1-GFP EF1-GFP viral supernatant, in 12-well tissue culture-treated plates pre-coated with retronectin (Clontech). Following two overnight transductions, cells were cultured on T cell media with 300 IU/mL IL-2 (Peprotech) and maintained at  $1.0-4.0 \times 10^6$ /mL. Cells were washed, suspended in formalin, and subjected to flow cytometry analysis, using the

FITC channel to determine the percentage of GFP+ cells, on 7, 14, 21 and 28 days after transduction. The percentage of GFP+ cells was consistently higher for T cells transduced with SFFV-GFP compared to EF1-GFP-transduced T cells (Figure 44A), even as the percentage of total GFP+ cells decreased over this period. A further comparison showed that T cells transduced with the higher (1 mL) amount of EF1-GFP supernatant actually decreased in percentage relative to the percent of GFP+ cells transduced with the lower amount (50  $\mu$ L, or 20-fold less) of SFFV-GFP, between Day 7 and Day 28, from over 60% to under 40% (Figure 44B). This suggests that transduction using the SFFV promoter led to greater persistence of transduced cells.

**BCMA or TACI or BAFF-R CAR NK cells or T-cells targeting cells expressing at least one of BCMA or TACI or BAFF-R CAR antigen**

To assess the cytotoxicity ability of CAR targeting at least one of BCMA or TACI or BAFF-R NK cells or T cells, co-culture assays are conducted with cell lines or primary human cells expressing at least one of BCMA or TACI or BAFF-R. The ability of the aforementioned CAR NK cells or T cells to lyse the target cells was quantified by flow cytometry analysis, and target cells were stained with Cytotracker dye (CMTMR). Lysis is observed at 24 hour long cultures.

**BAFF or APRIL CAR NK or T cells targeting cells expressing at least one of BCMA or TACI or BAFF-R antigen.**

The chimeric antigen receptor in the CAR is the ligand for BCMA or TACI or BAFF-R.

To assess the cytotoxicity ability of CAR targeting at least one of BCMA or TACI or BAFF-R NK or T cells, co-culture assays are conducted with cell lines or human primary cells expressing at least one of BCMA or TACI or BAFF-R. The ability of the aforementioned CAR NK or T cells to lyse the target cells was quantified by flow cytometry analysis, and target cells were stained with Cytotracker dye (CMTMR). Lysis is observed at 24 hour-long cultures.

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**INCORPORATION OF SEQUENCE LISTING**

Incorporated herein by reference in its entirety is the Sequence Listing for the application. The Sequence Listing is disclosed on a computer-readable ASCII text file titled, “sequence\_listing.txt”, created on June 24, 2016. The sequence-listing.txt file is 140KB in size.

# CLAIMS

1. An engineered cell comprising a polynucleotide construct that encodes for single polypeptide, wherein the single polypeptide comprises a first chimeric antigen receptor polypeptide (CAR), a second chimeric antigen receptor polypeptide (CAR), and a first cleavage site disposed between the first CAR and second CAR, and a single promoter that drives expression of the single polypeptide which results in expression of both the first and second CARs, wherein:

(i) the first CAR comprises a first signal peptide, a first antigen recognition domain, a first hinge region, a first transmembrane domain, a first co-stimulatory domain, and a first signaling domain that, form a first fusion protein; and

(ii) the second CAR comprises a second signal peptide, a second antigen recognition domain, a second hinge region, a second transmembrane domain, a second co-stimulatory domain, and a second signaling domain that, form a second fusion protein;

and wherein the first antigen recognition domain and the second antigen recognition domain are different, and each bind to a different target,

wherein the first and second co-stimulatory domains are intracellular, and

wherein the interaction sites between said hinge and transmembrane regions in the first and second fusion proteins are excluded or disrupted, and

wherein the target of the first antigen recognition domain comprises CD123, the target of the second antigen recognition domain comprises CD33, the first co-stimulatory domain comprises CD28, the second co-stimulatory domain comprises 4-1BB, the first cleavage site comprises P2A, and the engineered cell is a T cell.

2. An engineered polypeptide comprising a CAR comprising an antigen recognition domain selective for a target selected from the group consisting of: CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD33, CD38, CD45, CD52, CD123, CD138, CD267, CD269, and CS1, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR

beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

3. The engineered polypeptide according to claim 2, wherein the CAR and enhancer are on a single polypeptide molecule.

4. The engineered polypeptide of claim 3, further comprising a high efficiency cleavage site selected from the group consisting of: P2A, T2A, E2A, and F2A, and wherein the high efficiency cleavage site the is disposed between the CAR and the enhancer.

5. The engineered polypeptide according to any one of claims 2 to 4, wherein the CAR comprises an antigen recognition domain selective for a target selected from the group consisting of: CD2, CD4, and CD19, and the enhancer comprises IL-15 or IL-15RA.

6. A method of treating chronic myeloid leukemia comprising administering to a patient in need thereof the engineered polypeptide according to claim 2, wherein the target of the first antigen recognition domain comprises CD33; and the target of the second antigen recognition domain comprises CD123.

7. Use of the engineered polypeptide according to claim 2 in the manufacture of a medicament for treating chronic myeloid leukemia, wherein the target of the first antigen recognition domain comprises CD33; and the target of the second antigen recognition domain comprises CD123.

8. A method of treating B-cell acute lymphoblastic leukemia (B-ALL) comprising administering to a patient in need thereof the engineered polypeptide according to claim 2, wherein the target of the first antigen recognition domain comprises CD19; and the target of the second antigen recognition domain comprises CD123.



9. Use of the engineered polypeptide according to claim 2 in the manufacture of a medicament for treating B-cell acute lymphoblastic leukemia (B-ALL), wherein the target of the first antigen recognition domain comprises CD19; and the target of the second antigen recognition domain comprises CD123.

10. A method of treating multiple myeloma comprising administering to a patient in need thereof the engineered polypeptide according to claim 2, wherein the target of the first antigen recognition domain is selected from the group consisting of CD38, CS1, CD269, and CD19; and the target of the second antigen recognition domain is selected from the group consisting of CD38, CS1, CD269 and CD19.

11. Use of the engineered polypeptide according to claim 2 in the manufacture of a medicament for treating multiple myeloma, wherein the target of the first antigen recognition domain is selected from the group consisting of: CD38, CS1, CD269 and CD19; and the target of the second antigen recognition domain is selected from the group consisting of CD38, CS1, CD269 and CD19.

12. An engineered polypeptide comprising a CAR comprising an antigen recognition domain selective for a target selected from the group consisting of: CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD33, CD38, CD45, CD52, CD123, CD138, CD267, CD269, and CS1, an enhancer wherein the enhancer is IL-21 and an enhancer receptor comprising IL-15RA, or a functional fragment thereof.

13. An engineered polypeptide comprising a CAR comprising an antigen recognition domain selective for a target selected from the group consisting of: CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD22, CD33, CD38, CD45, CD52, CD138, CD267, CD269, and CS1, an enhancer

wherein the enhancer is IL-15 and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

14. An engineered polypeptide comprising a CAR comprising an antigen recognition domain selective for a target selected from the group consisting of: CD2, CD3, CD4, CD5, CD7, CD38, CD45, CD52, CD267, CD269, and CS1, an enhancer wherein the enhancer is IL-15 and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

15. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD19, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

16. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD20, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

17. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD4, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

18. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD5, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4,

TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

19. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD7, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

20. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD33, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

21. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD45, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

22. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD269, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

23. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD38, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

24. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD2, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

25. An engineered polypeptide comprising a CAR comprising an antigen recognition domain of CD22, an enhancer selected from the group consisting of: PD- 1, PD-L1, CSFIR, CTAL-4, TIM-3, TGFR beta, IL- 2, IL-7, IL-12, IL-15, IL-21 functional fragments thereof, or combinations thereof, and an enhancer receptor comprising IL- 15RA, or a functional fragment thereof.

**iCell Gene Therapeutics, LLC**

**Patent Attorneys for the Applicant/Nominated Person**

**SPRUSON & FERGUSON**

FIG. 1

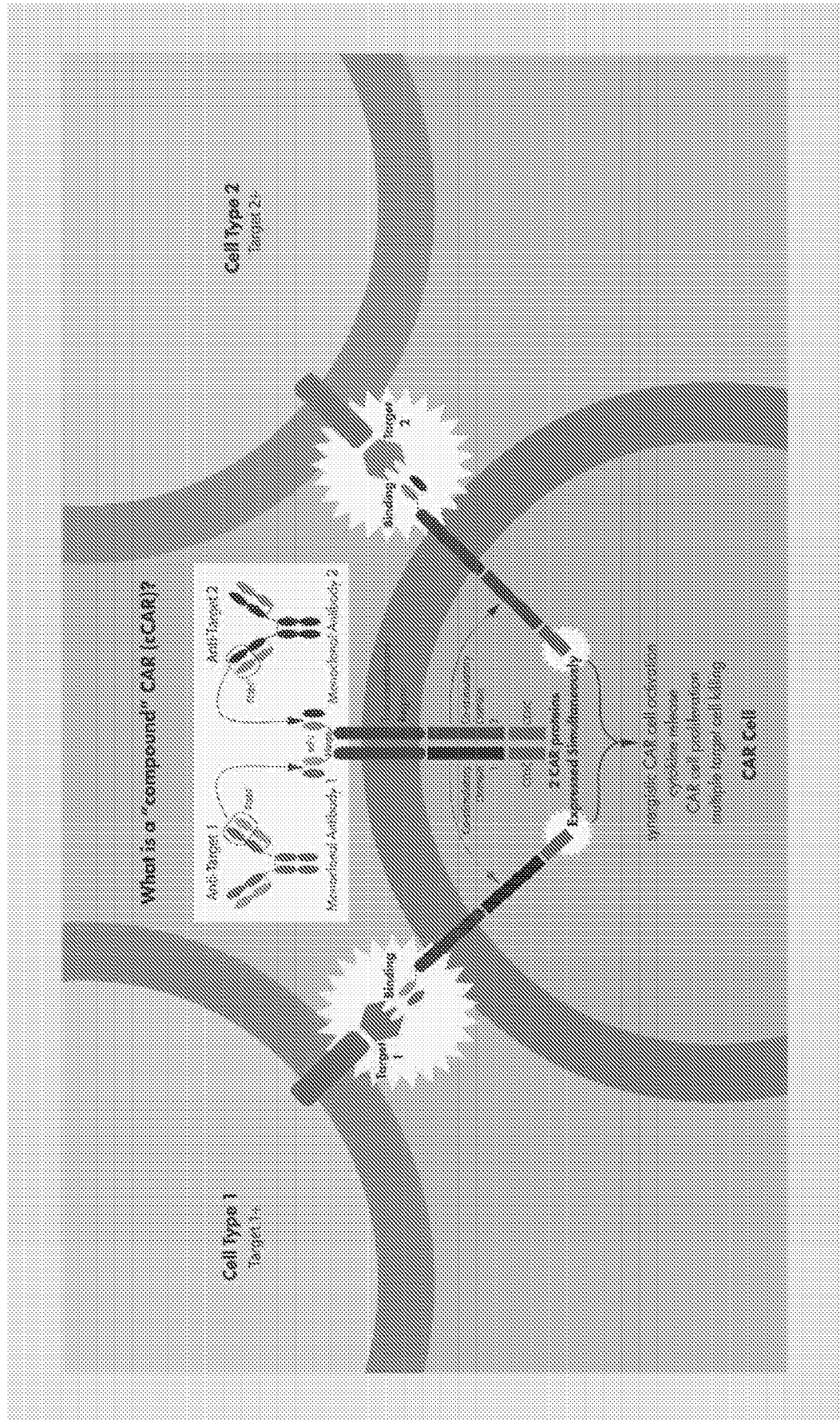


FIG. 2A

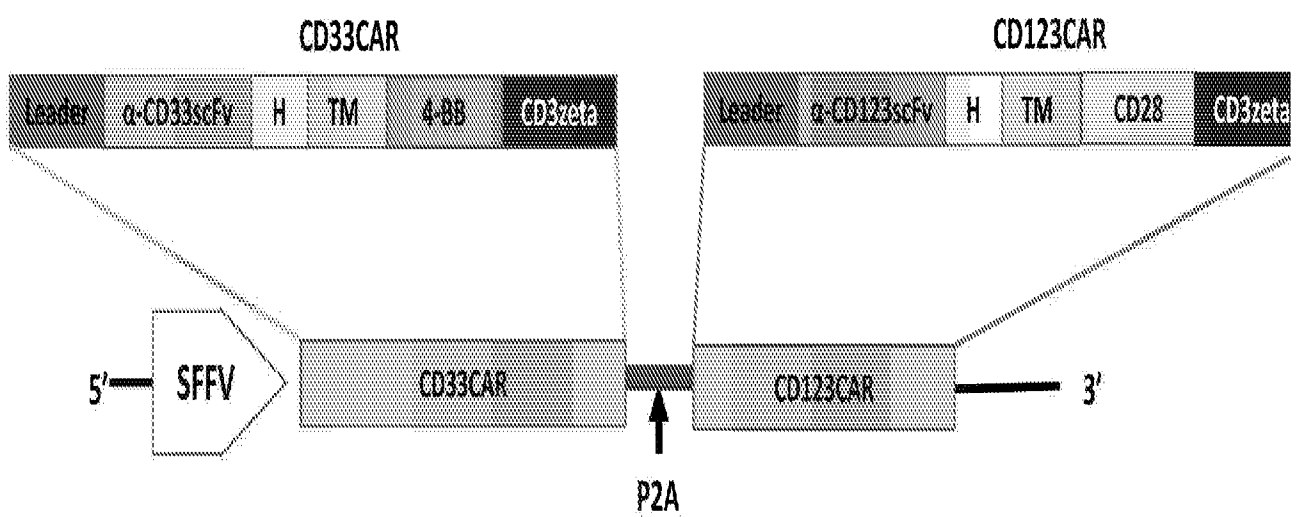


FIG. 2B

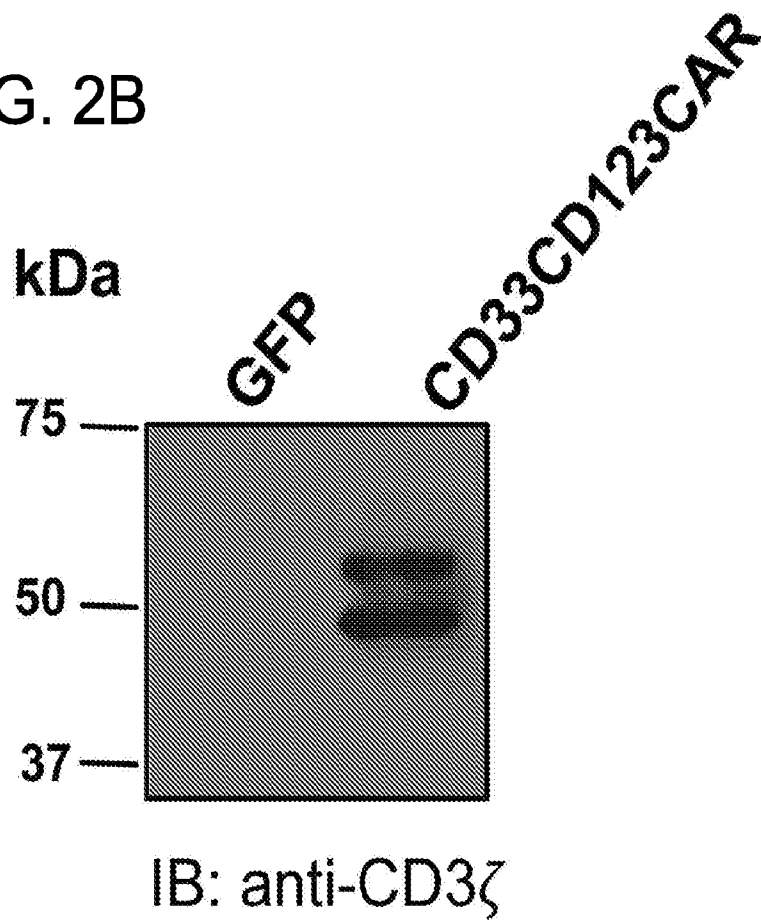


FIG. 2C

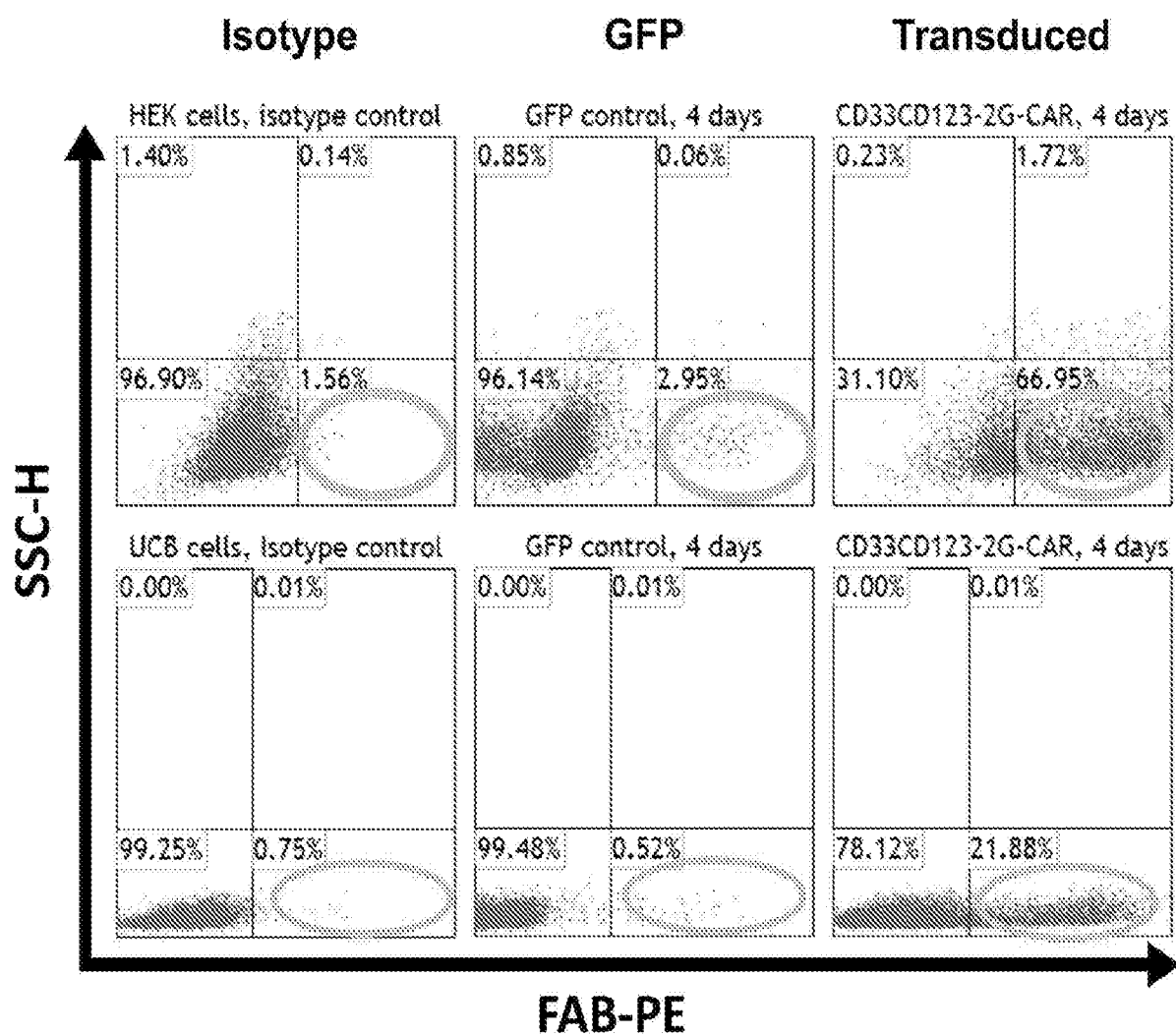




FIG. 3

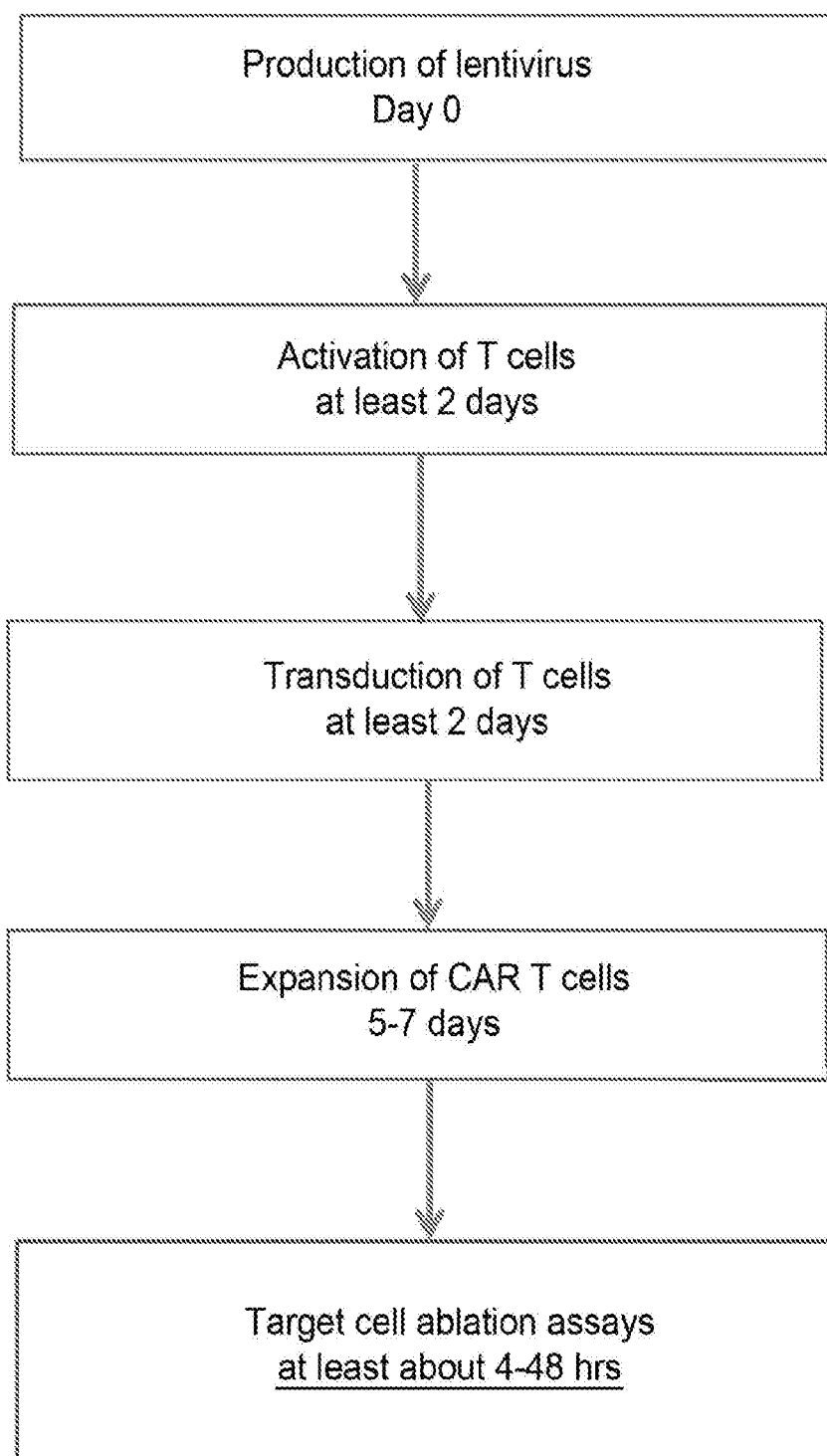


FIG. 4

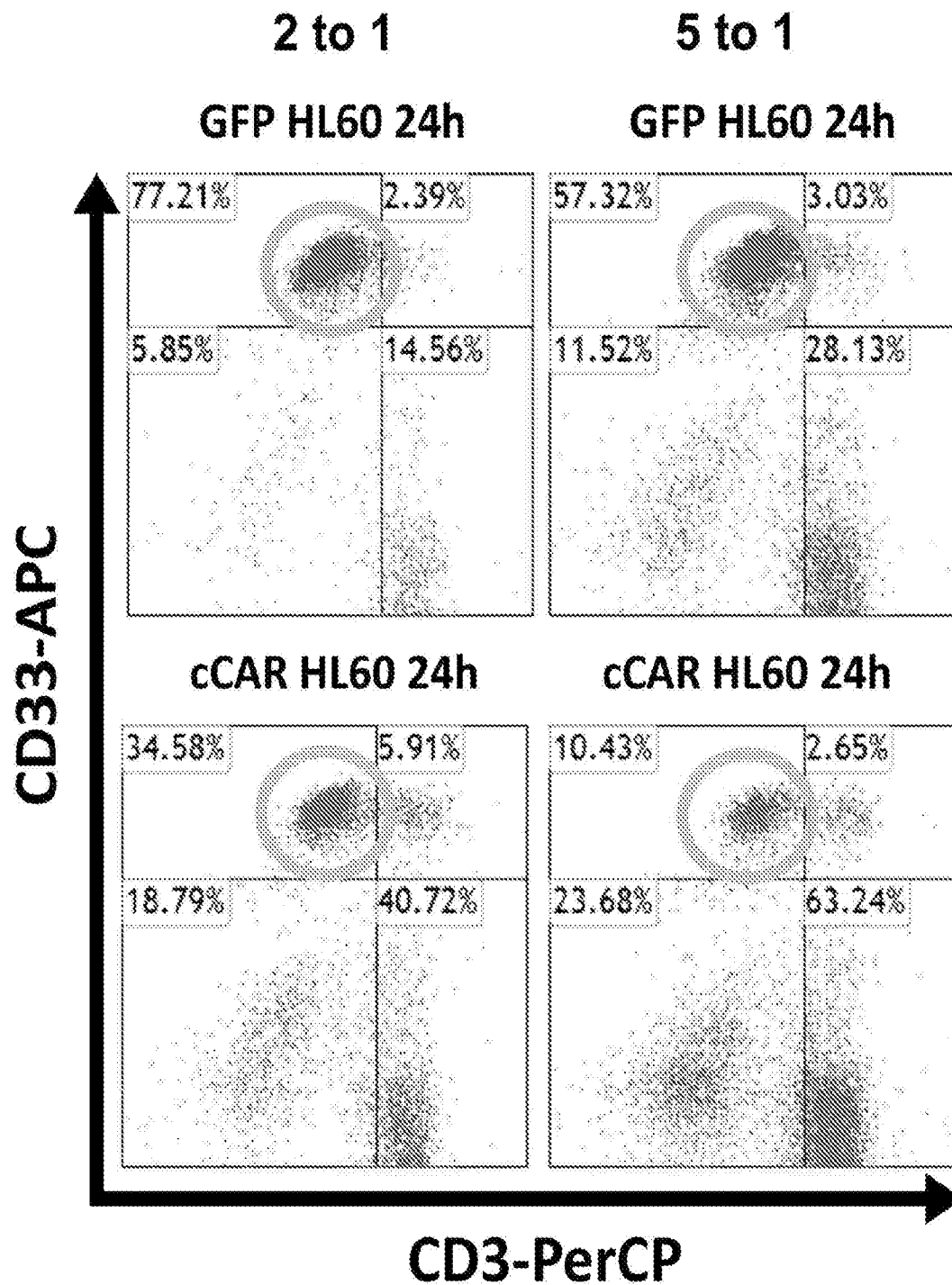


FIG. 5

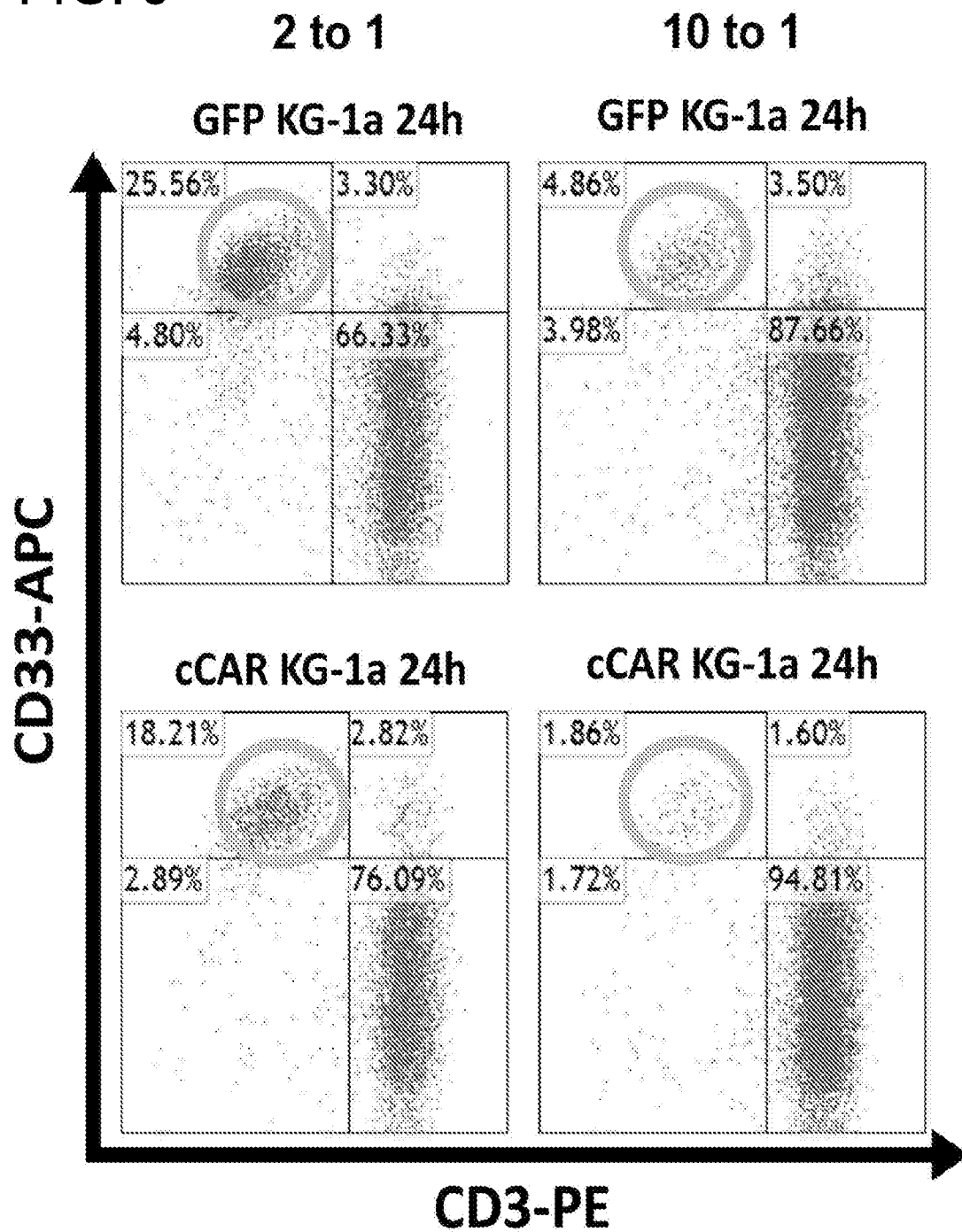


FIG. 6

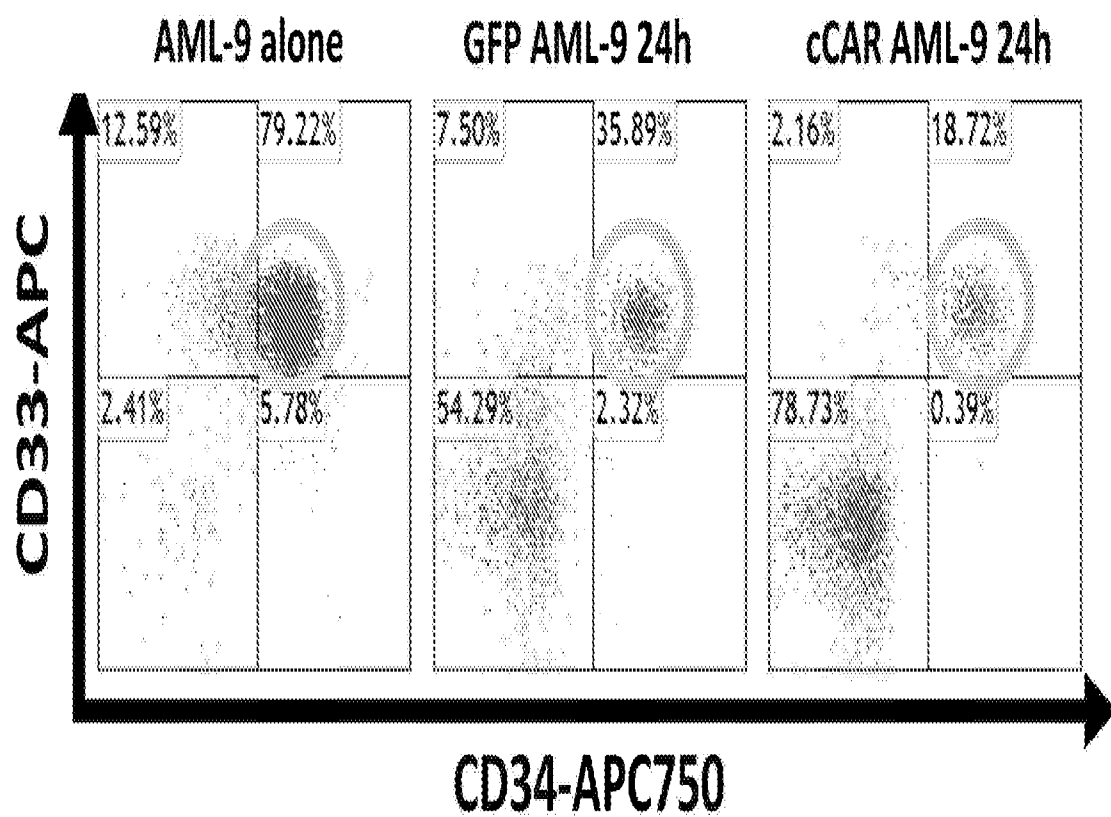


FIG. 7

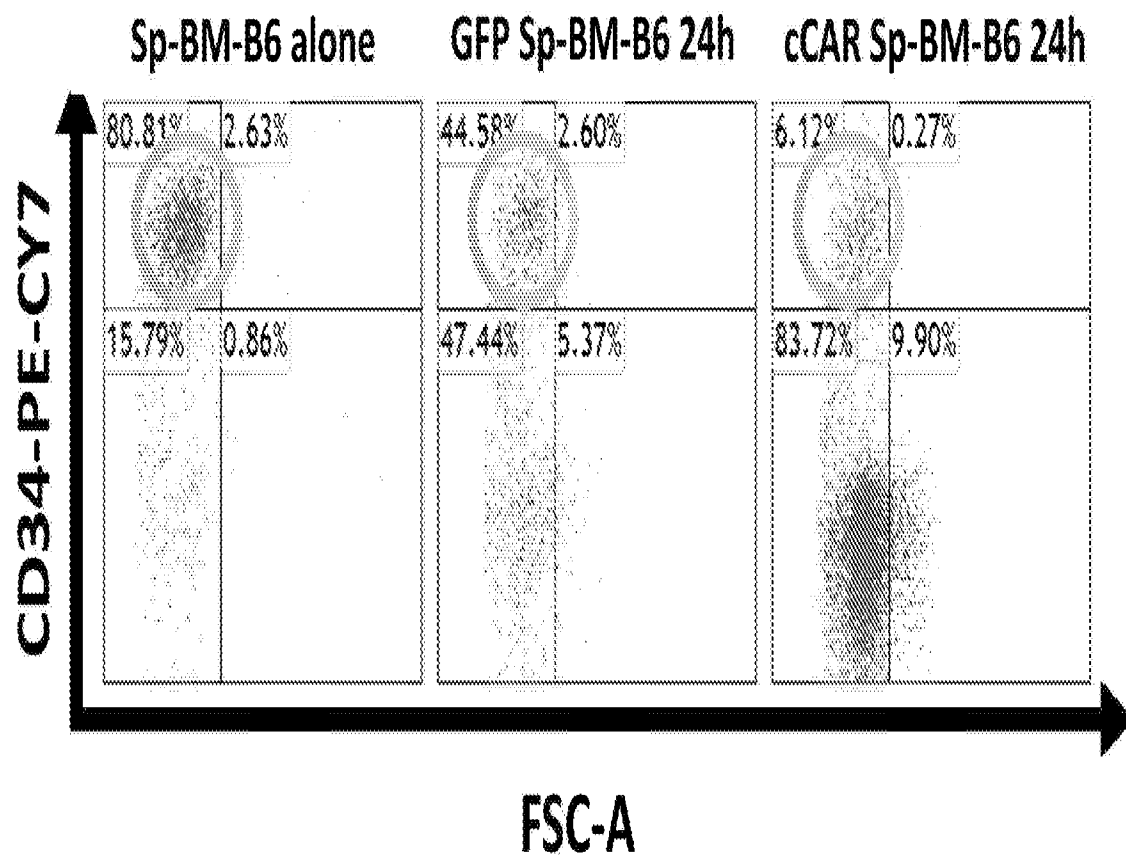


FIG. 8

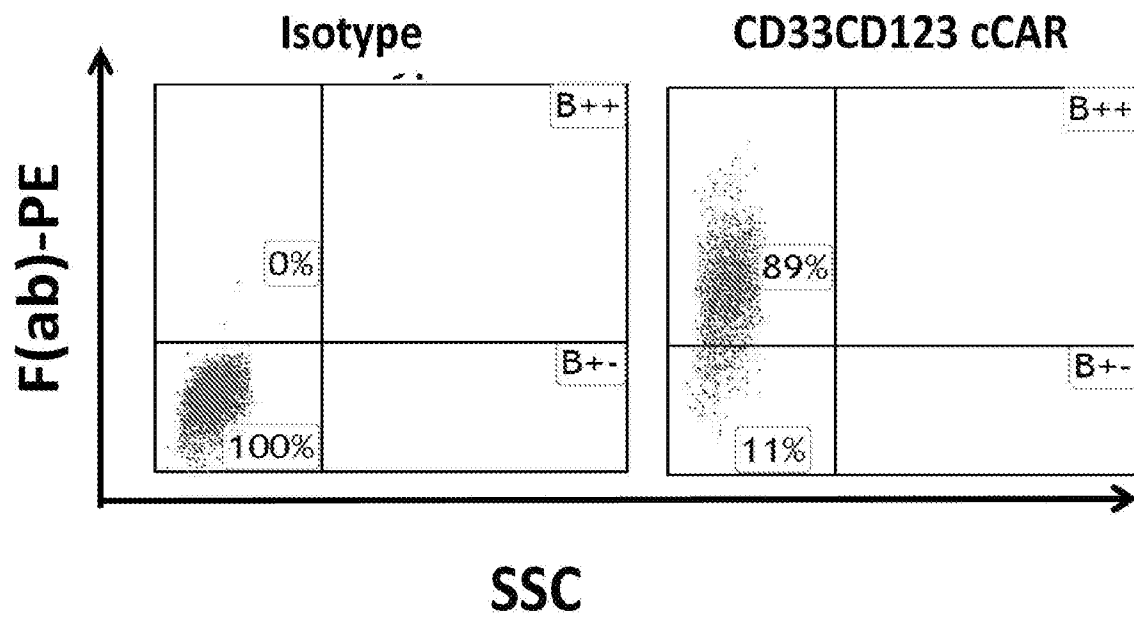


FIG. 9

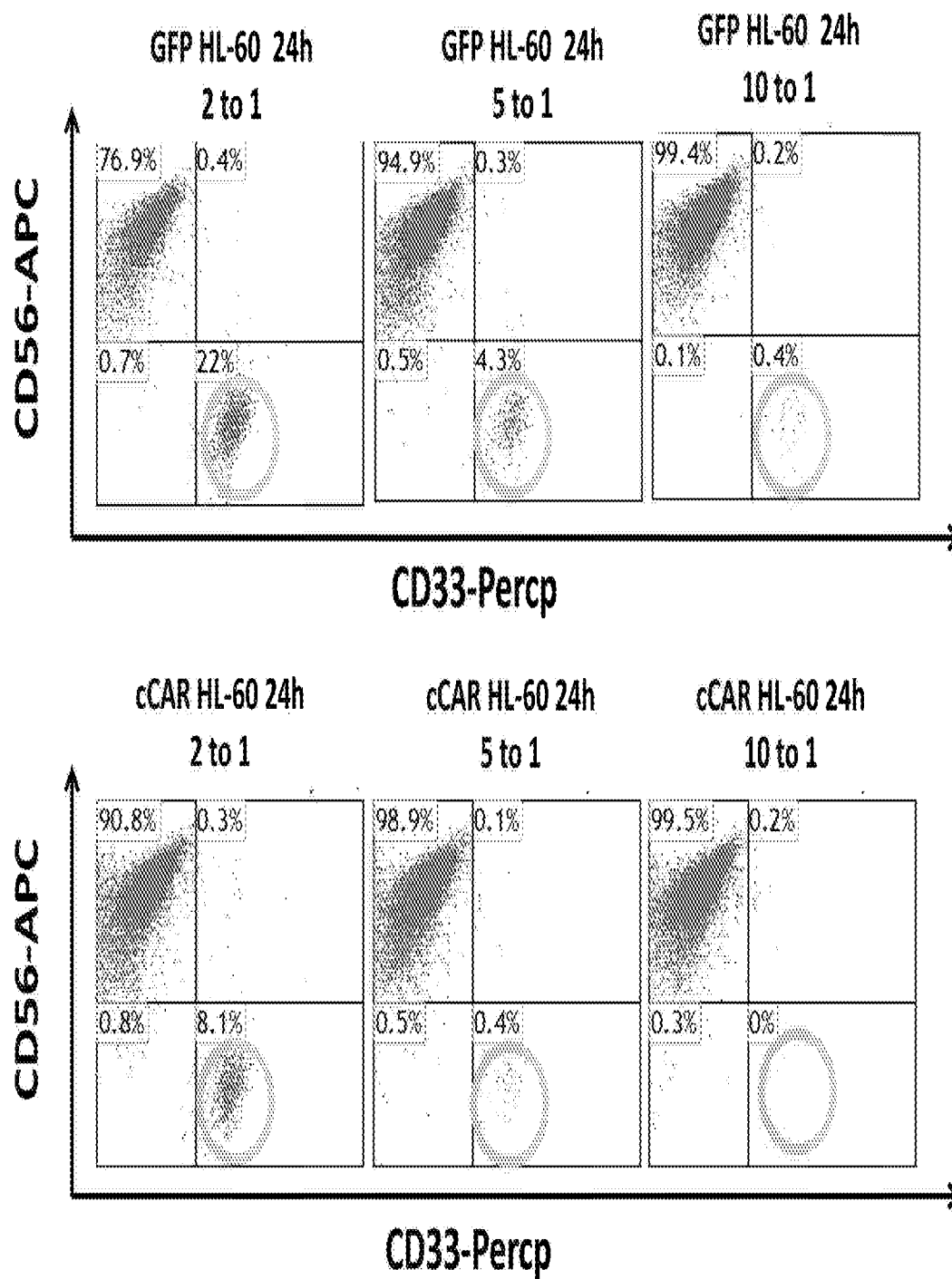


FIG. 10

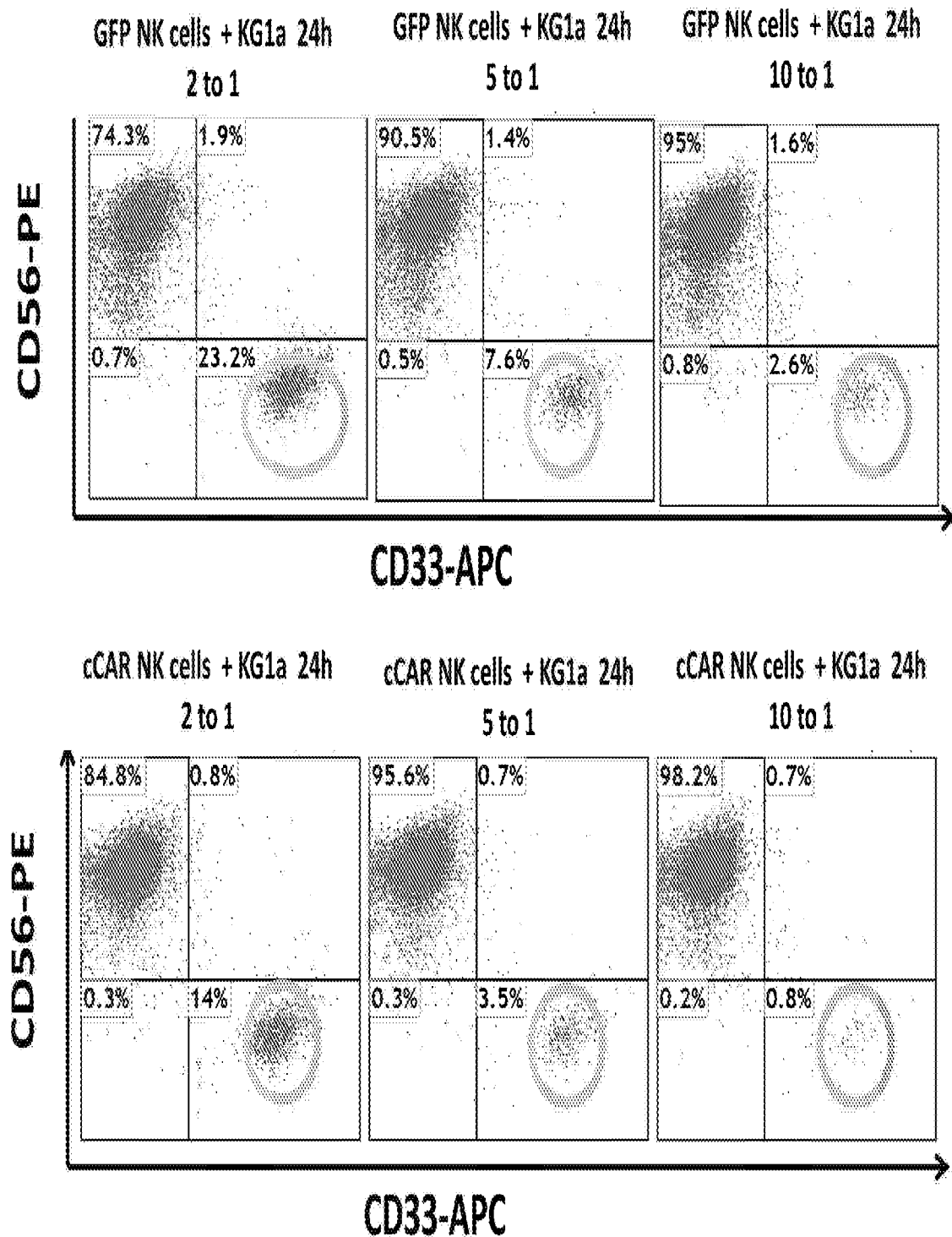




FIG. 11

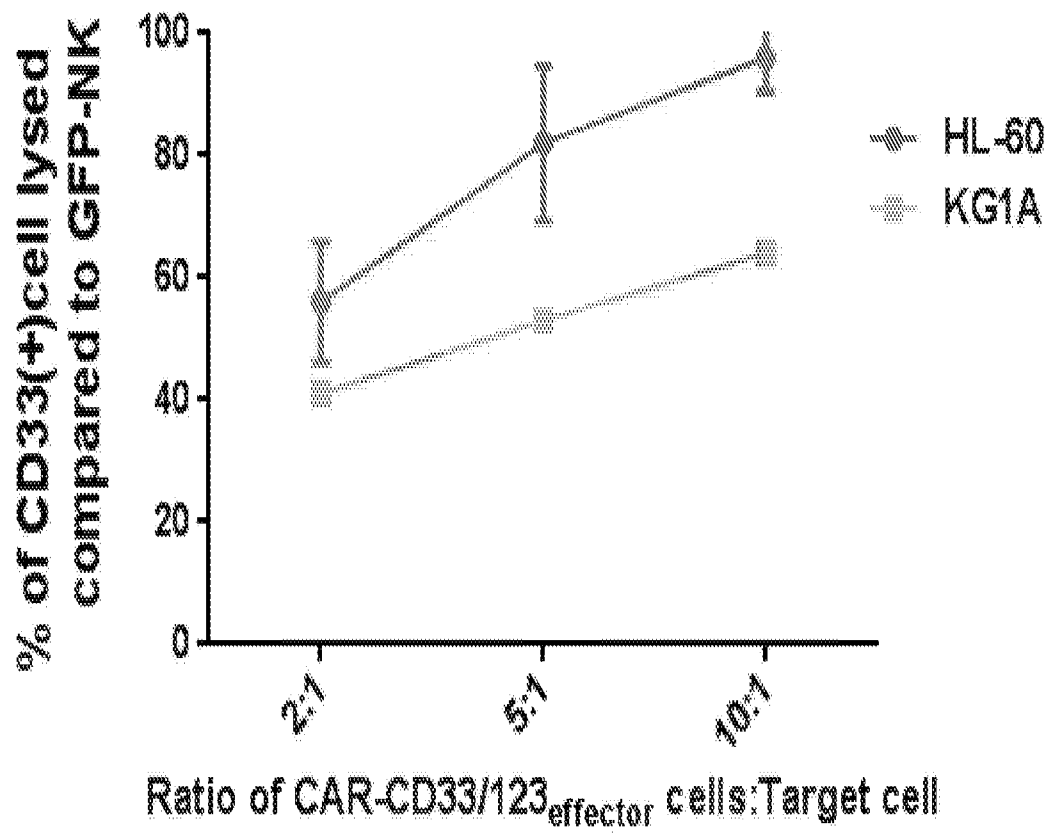


FIG. 12

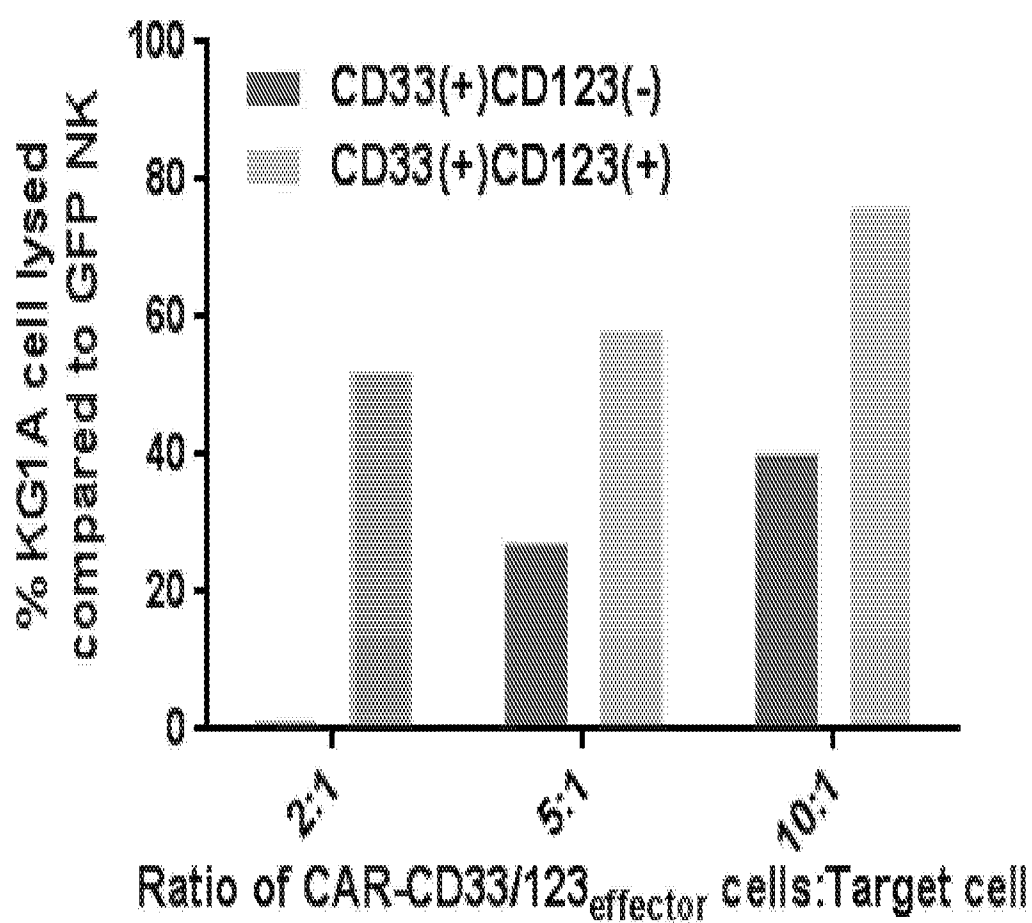


FIG. 13

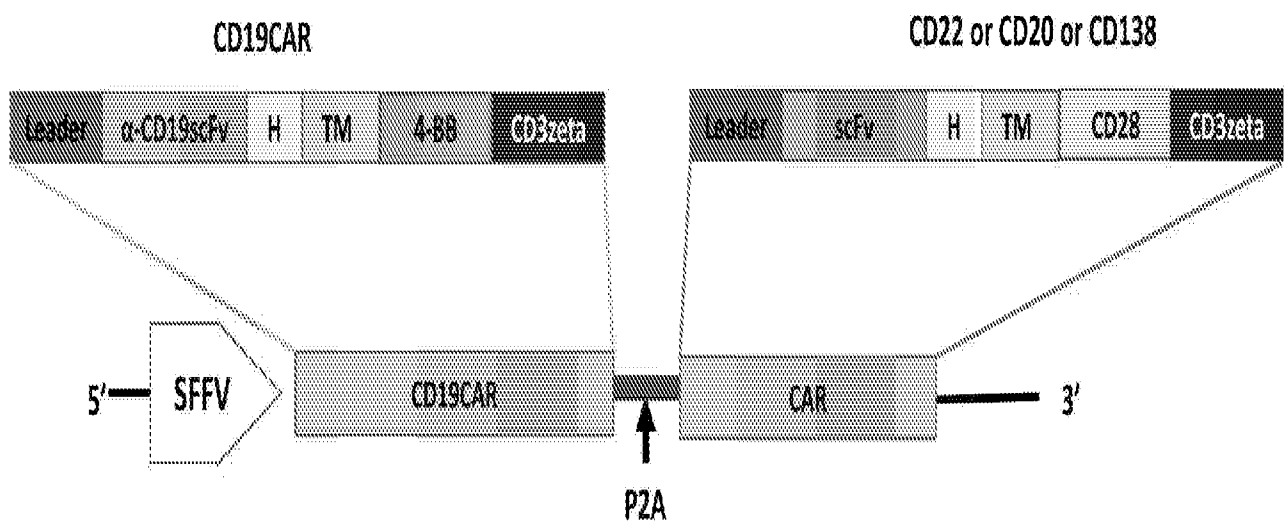


FIG. 14A

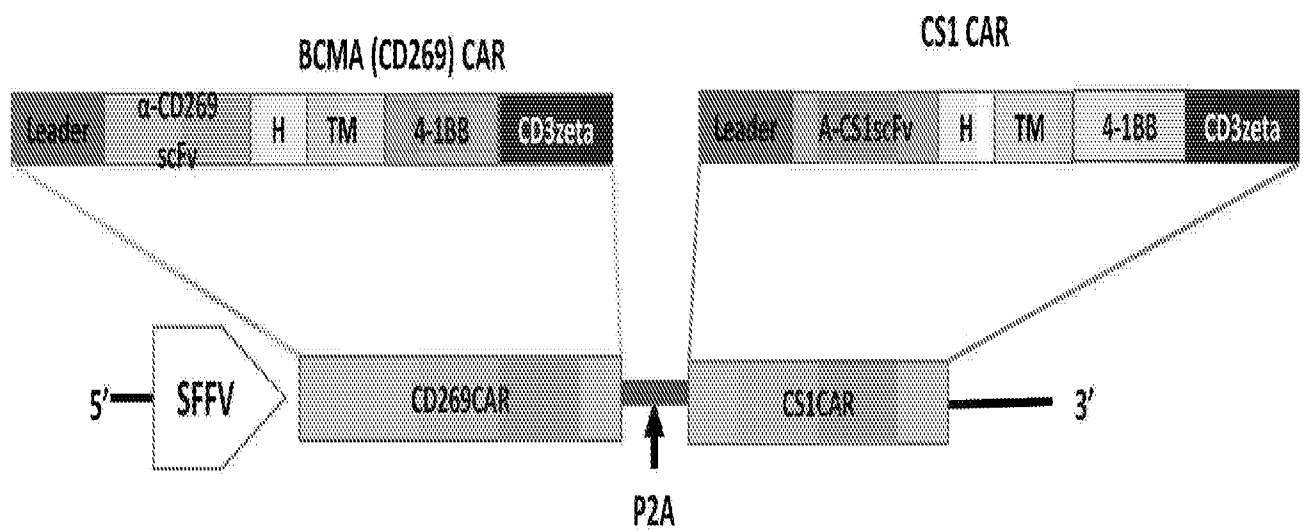


FIG. 14B

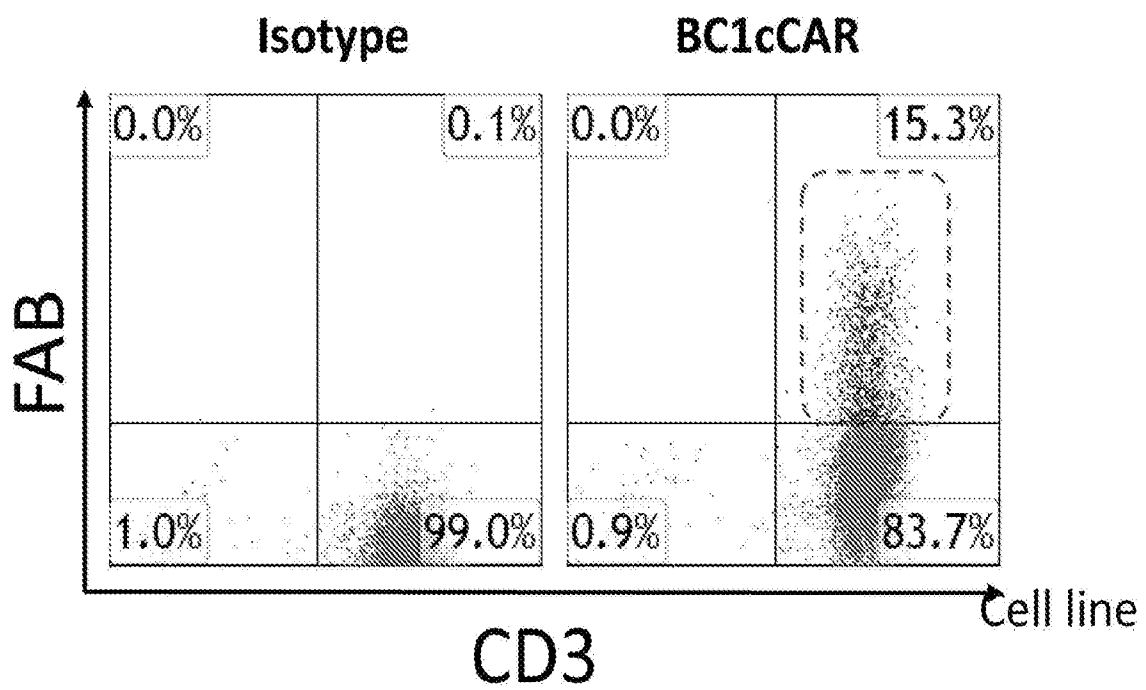


FIG. 14C

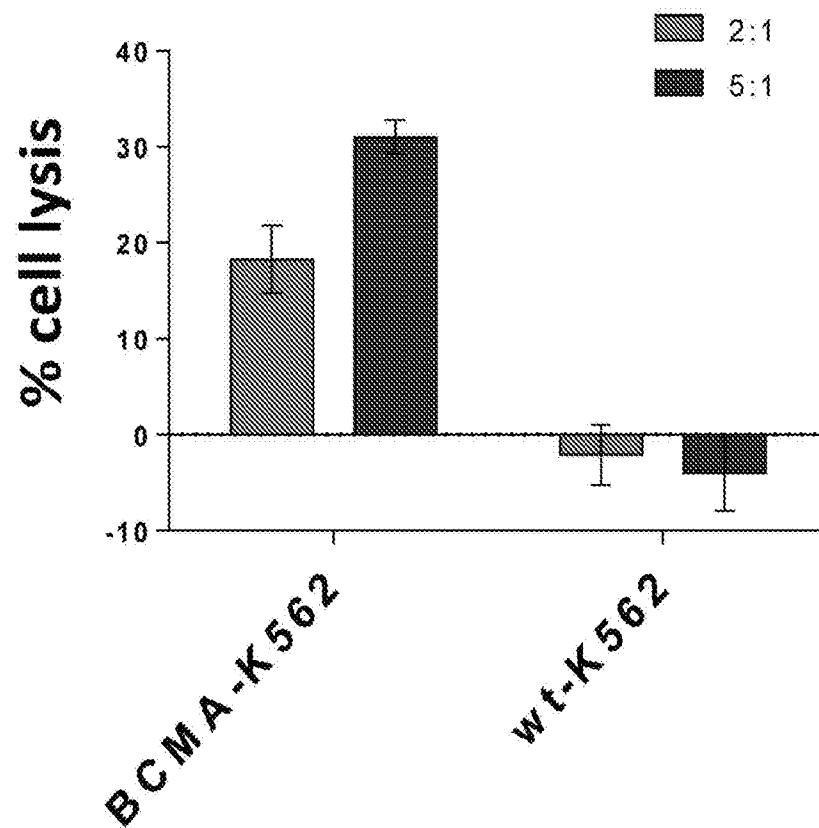


FIG. 14D

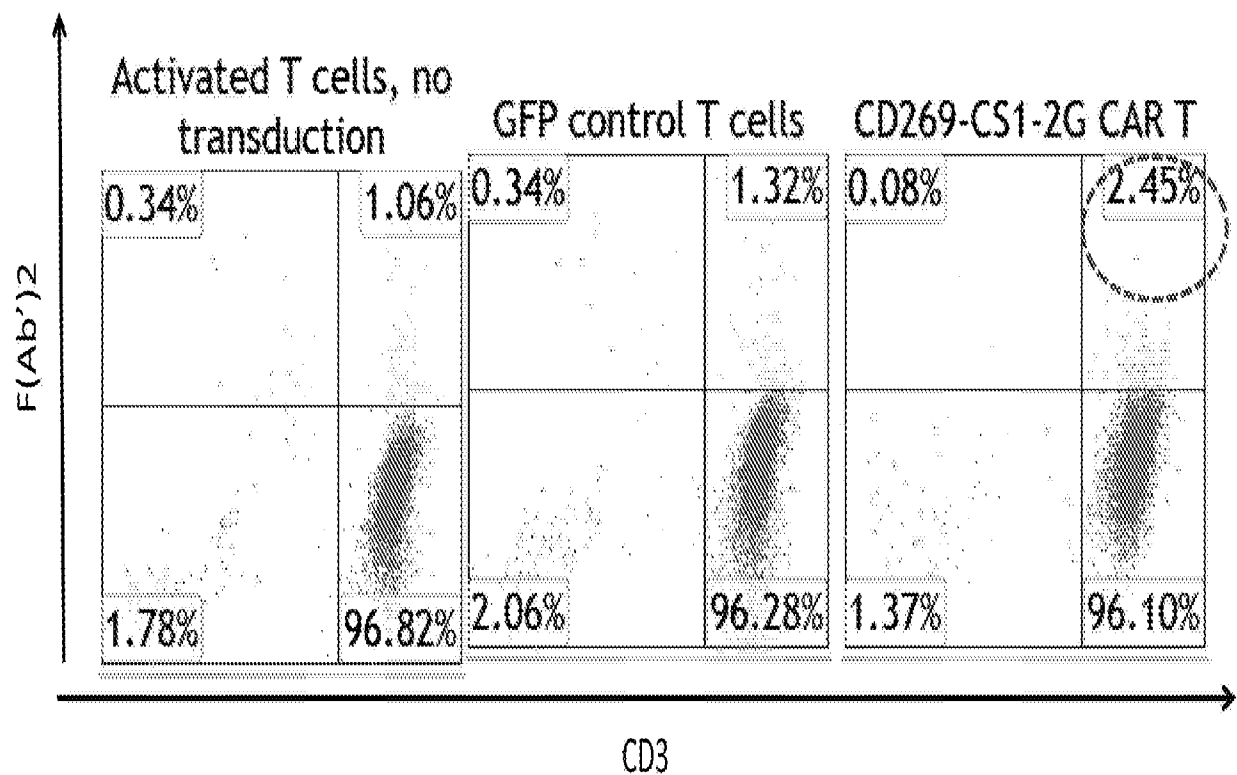


FIG. 14E

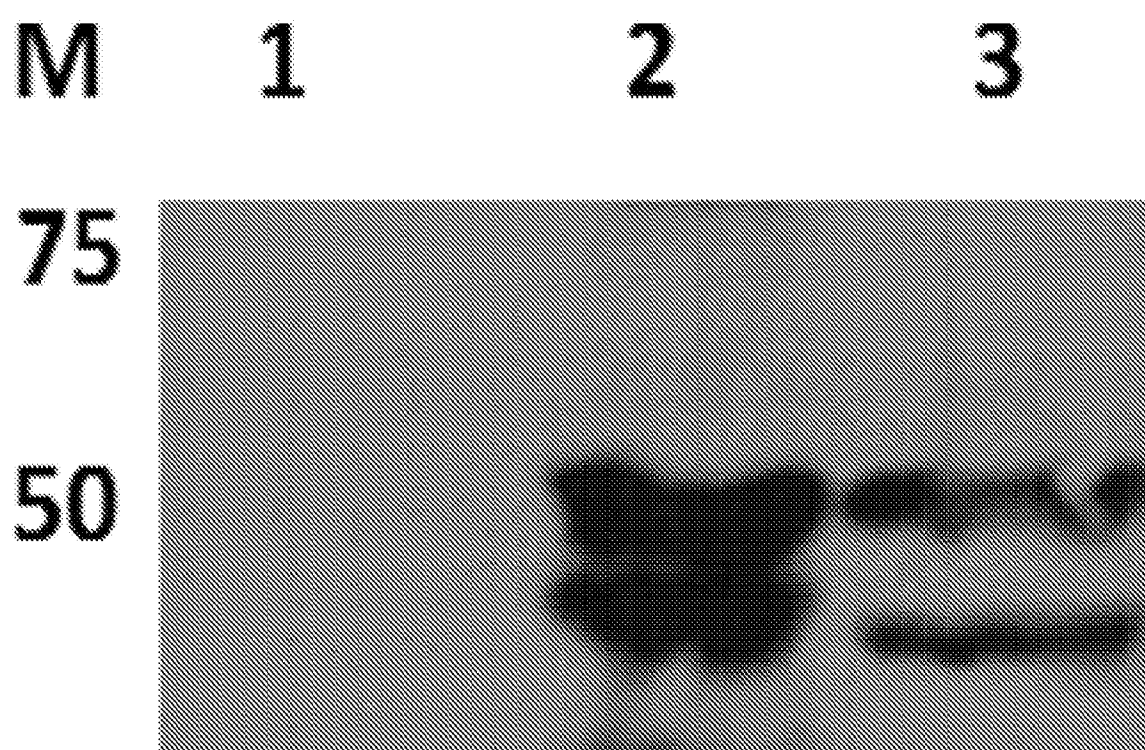




FIG. 15A

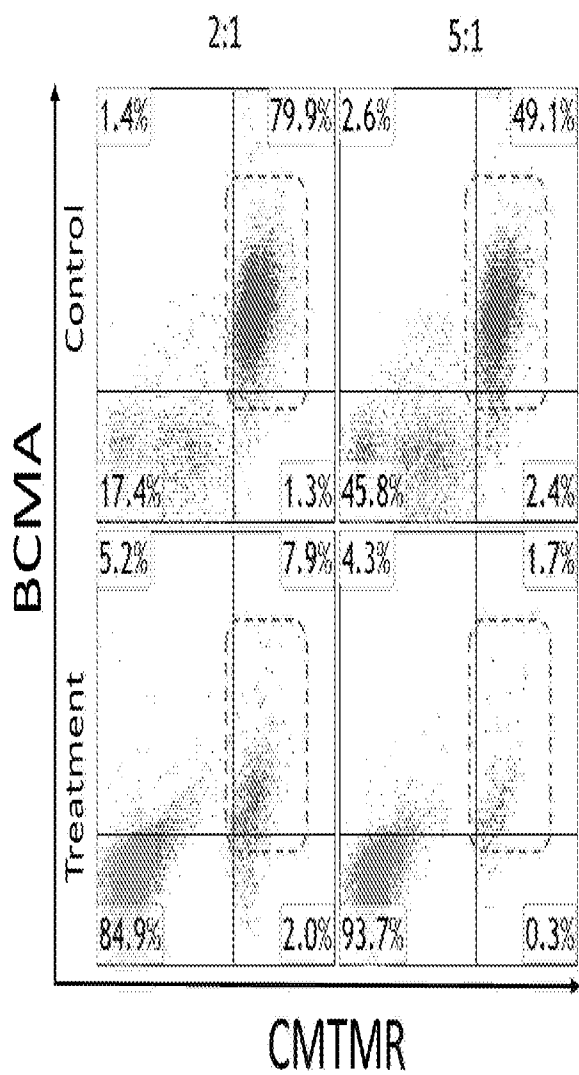


FIG. 15B

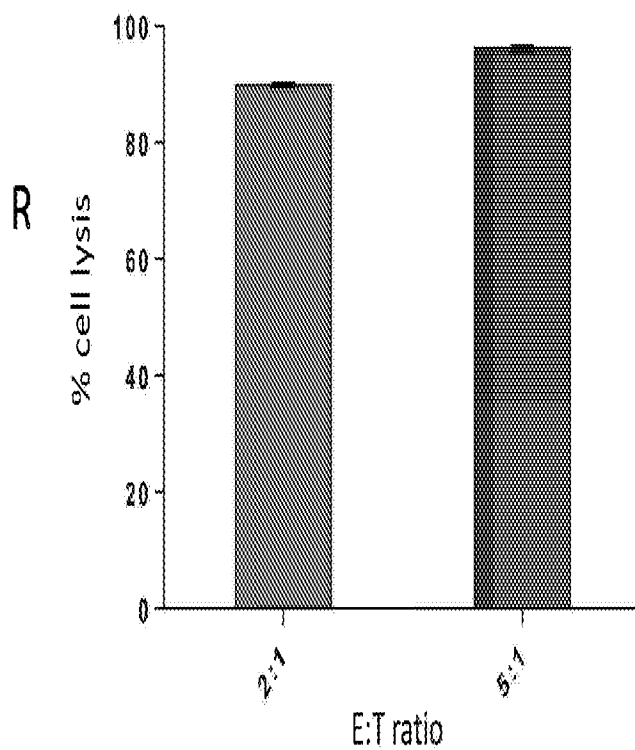


FIG. 16A

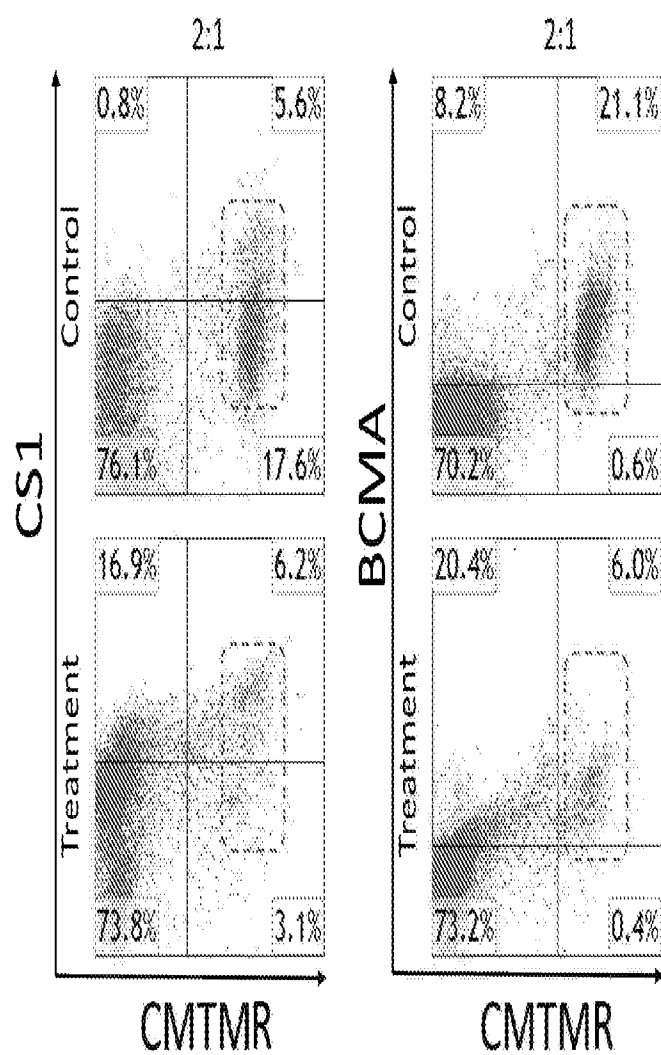


FIG. 16B

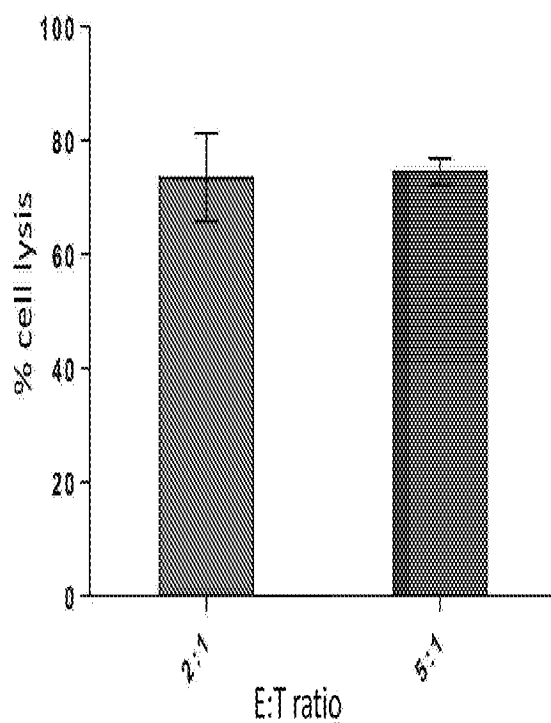


FIG. 17A

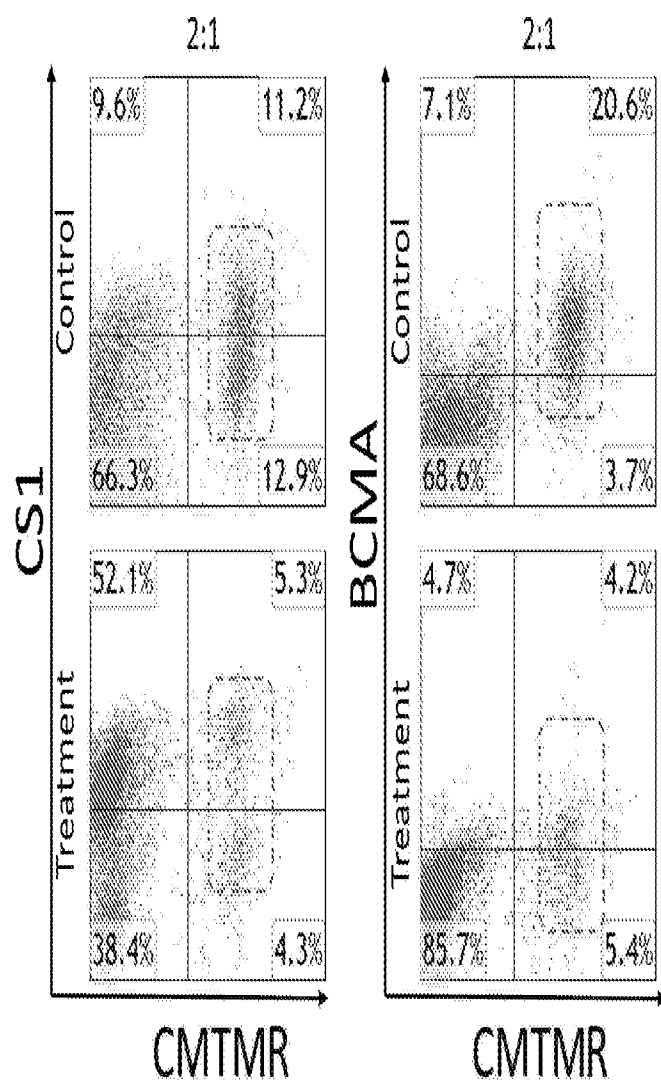


FIG. 17B

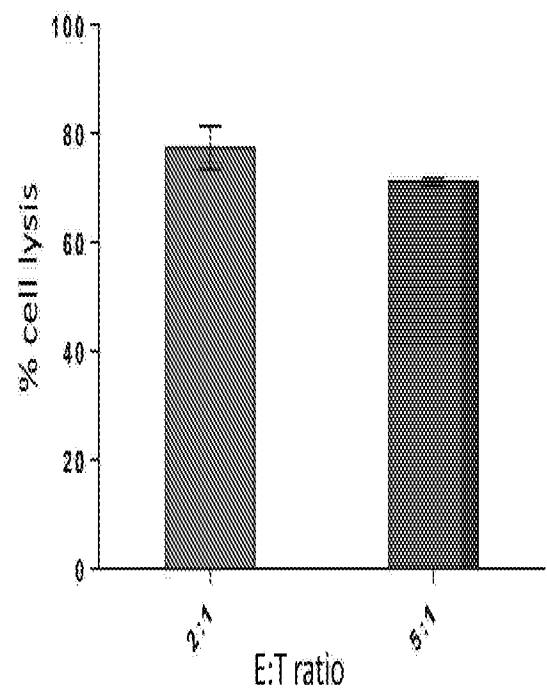


FIG. 18A

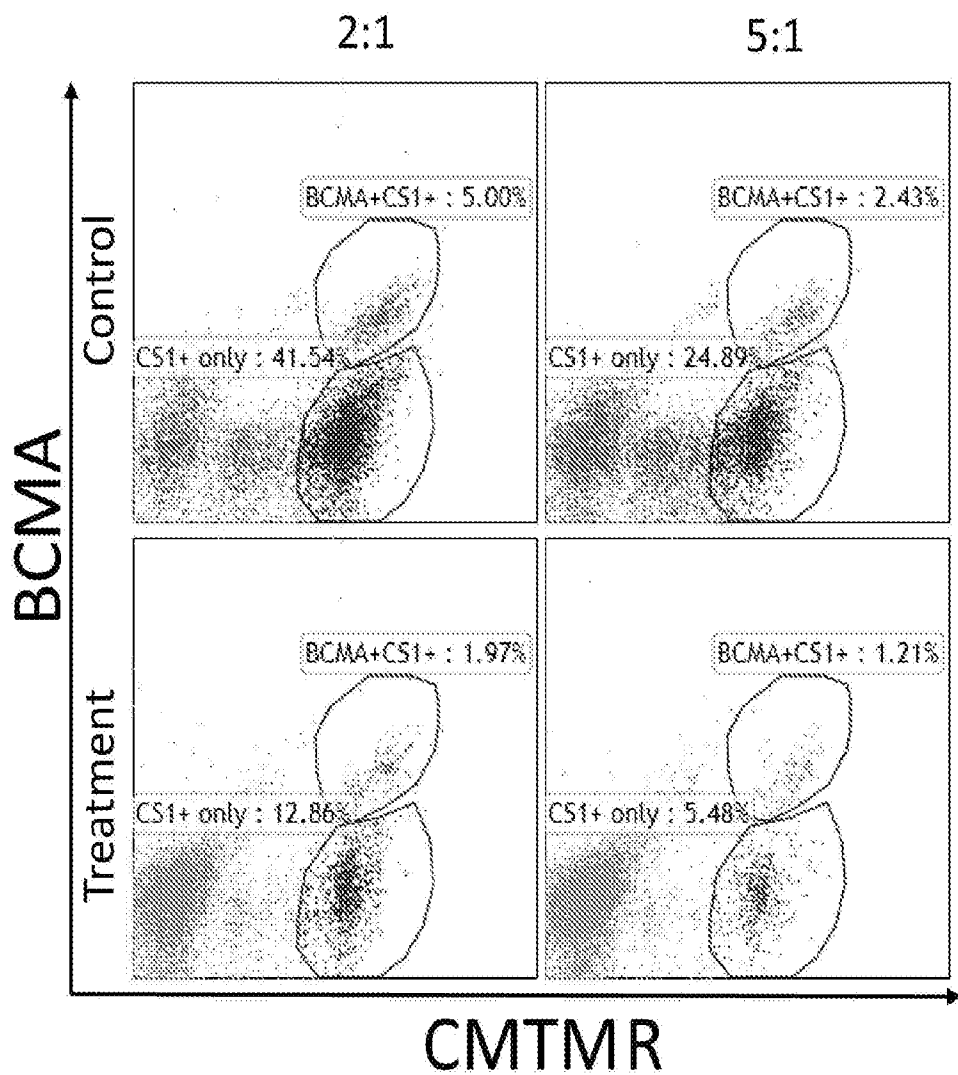


FIG. 18B

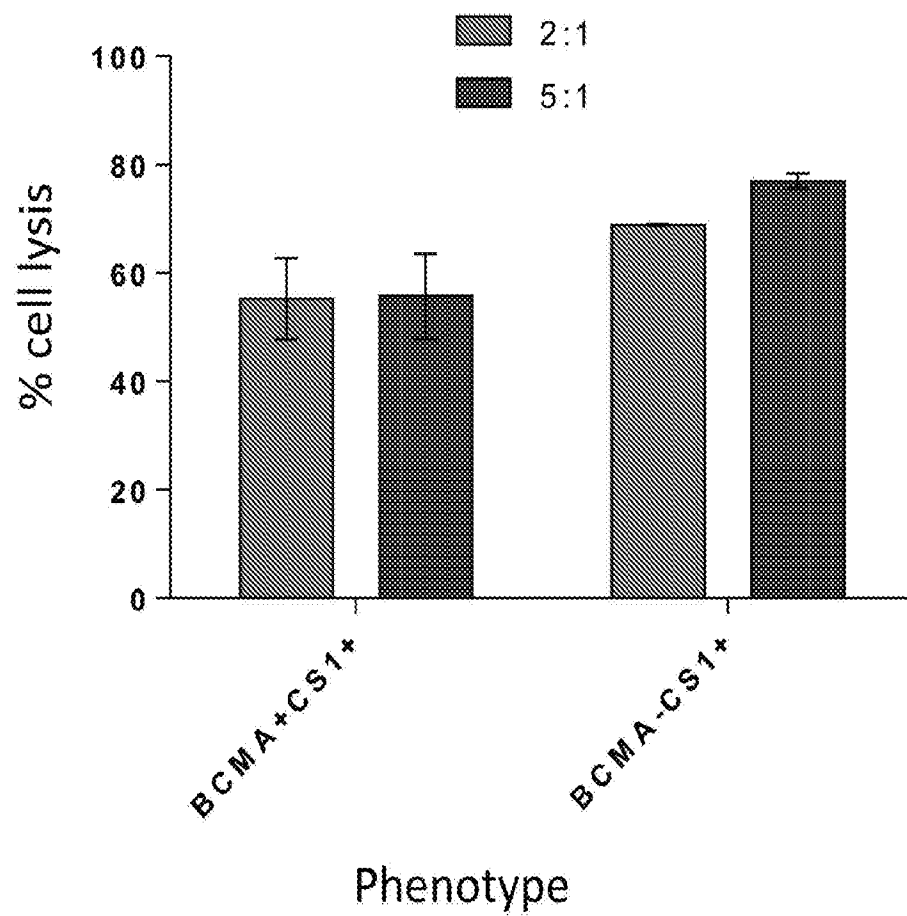


FIG. 19A

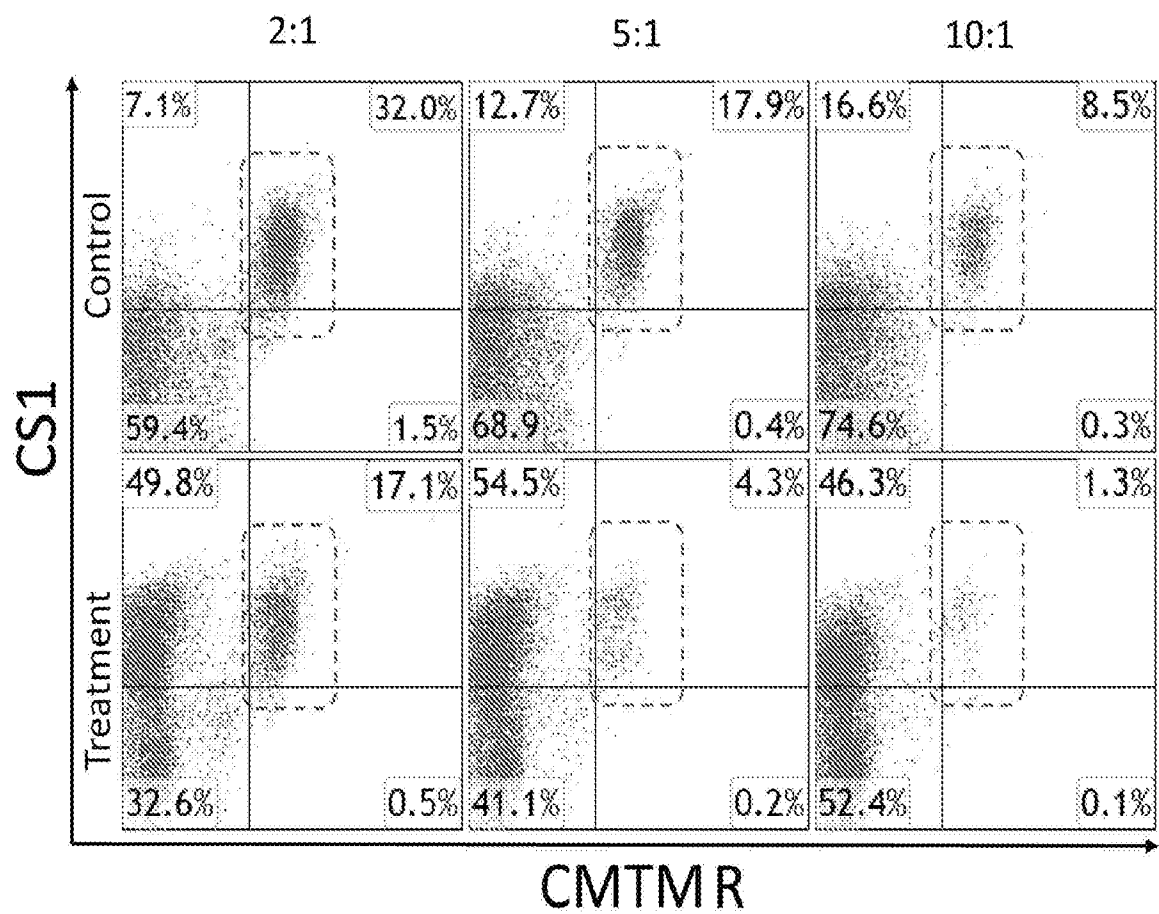


FIG. 19B

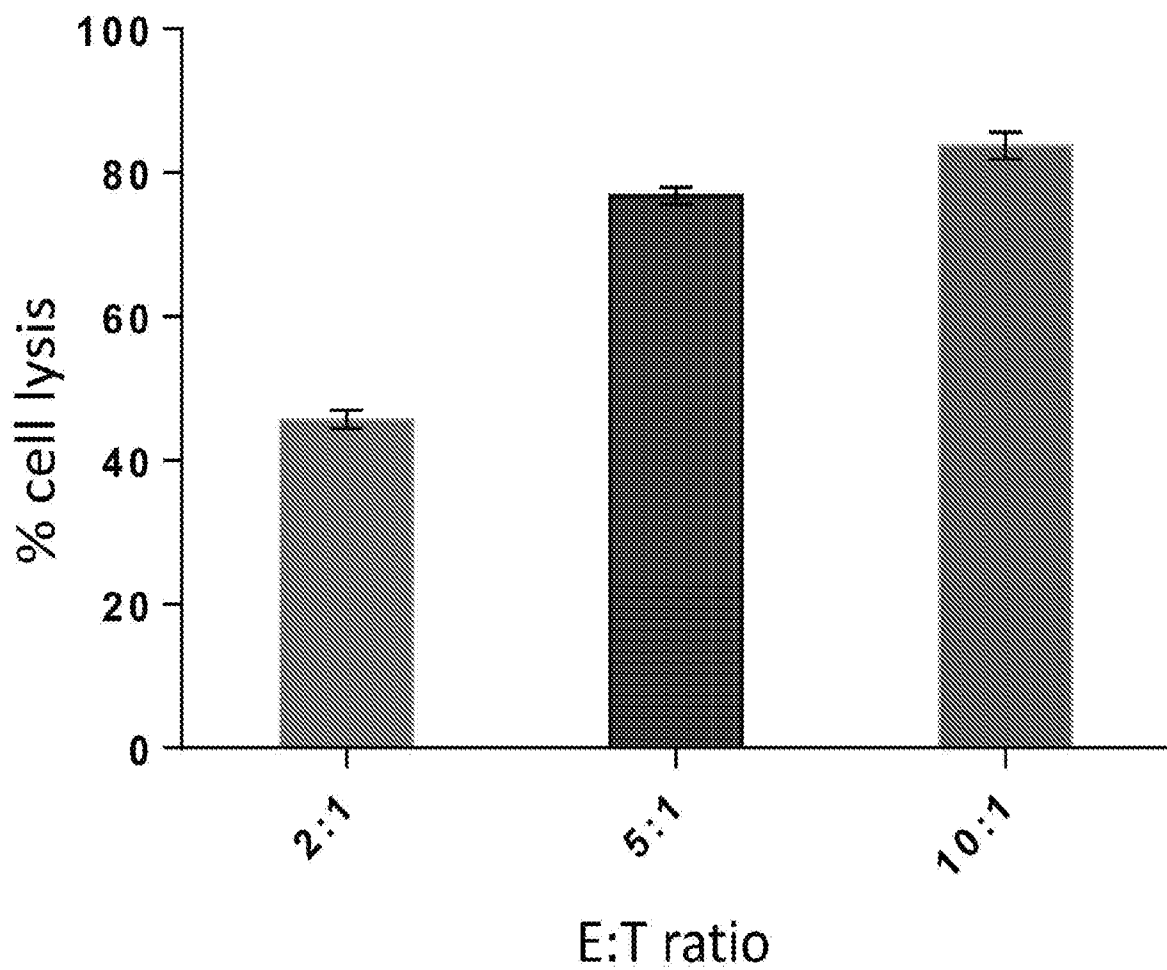


FIG. 20A

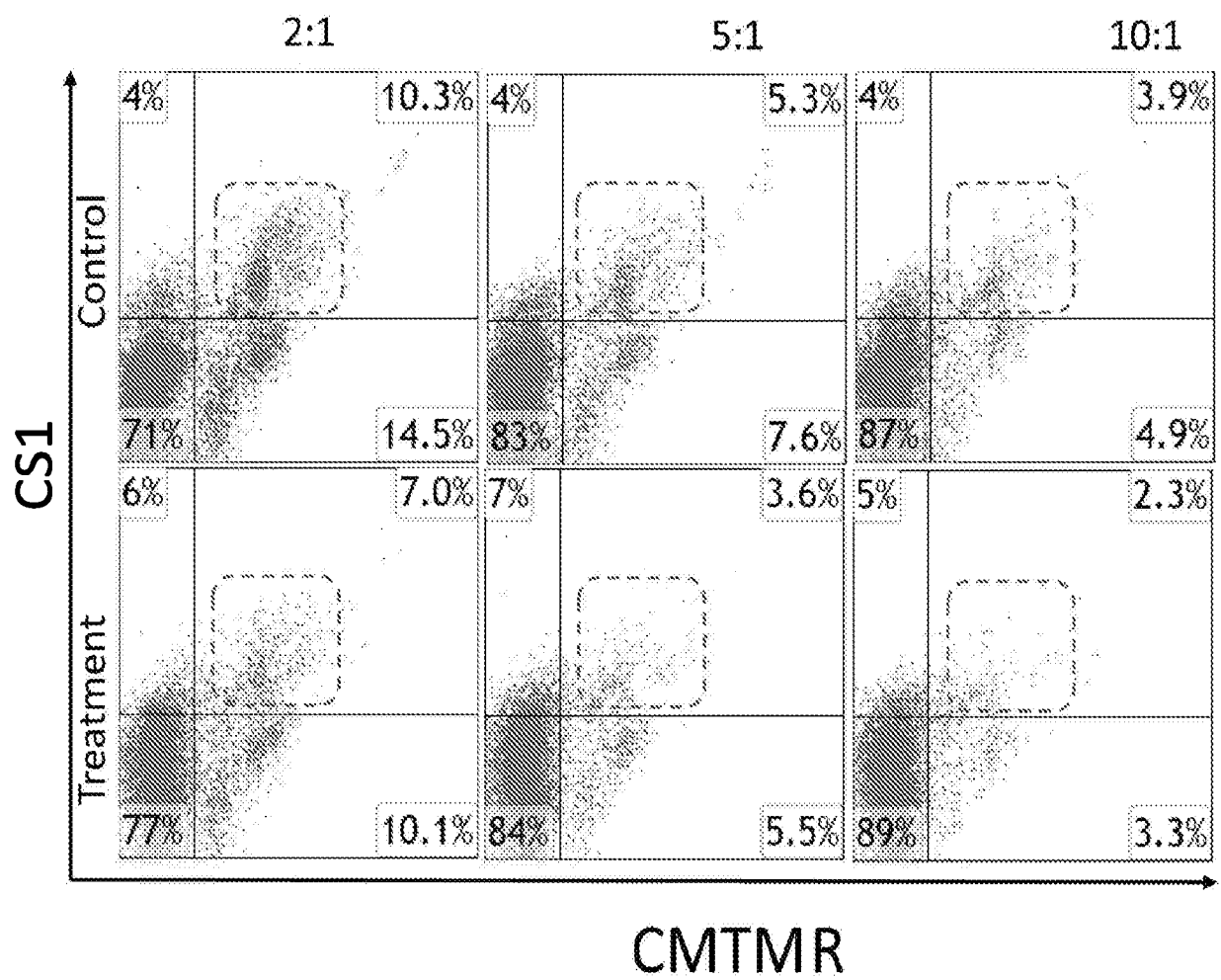




FIG. 20B

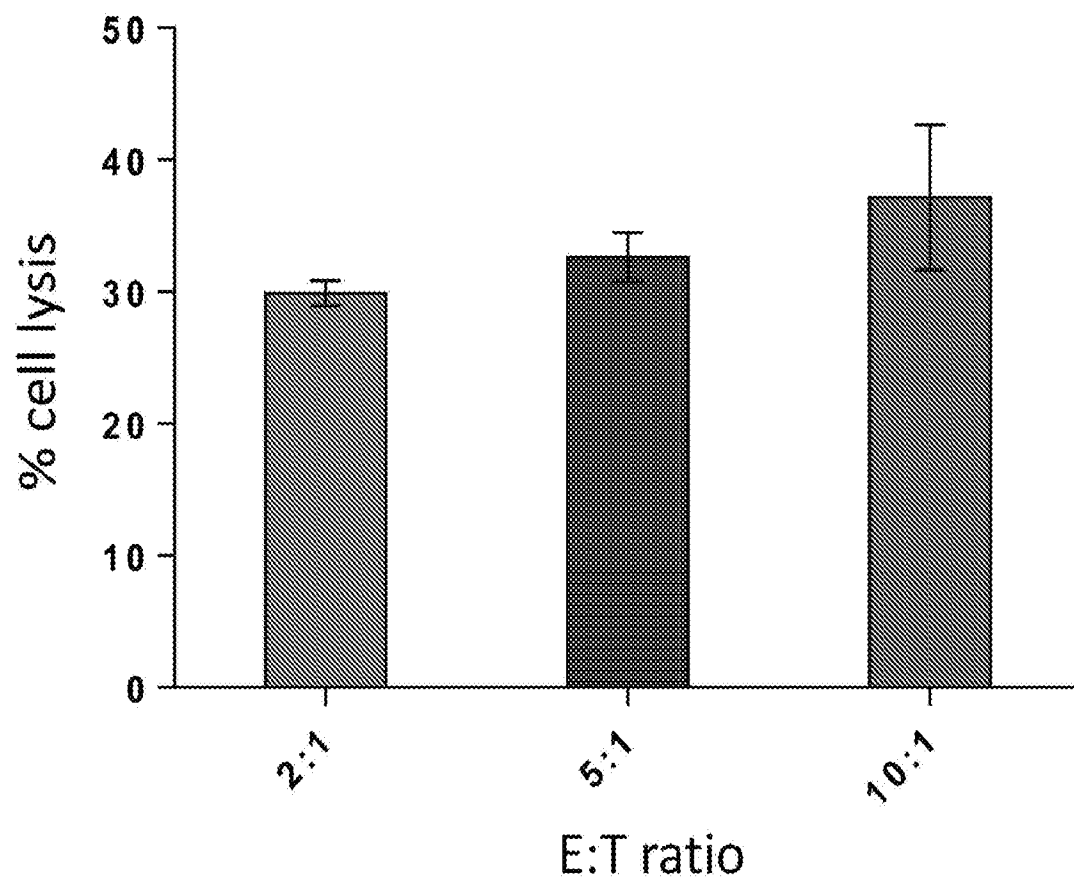


FIG. 21

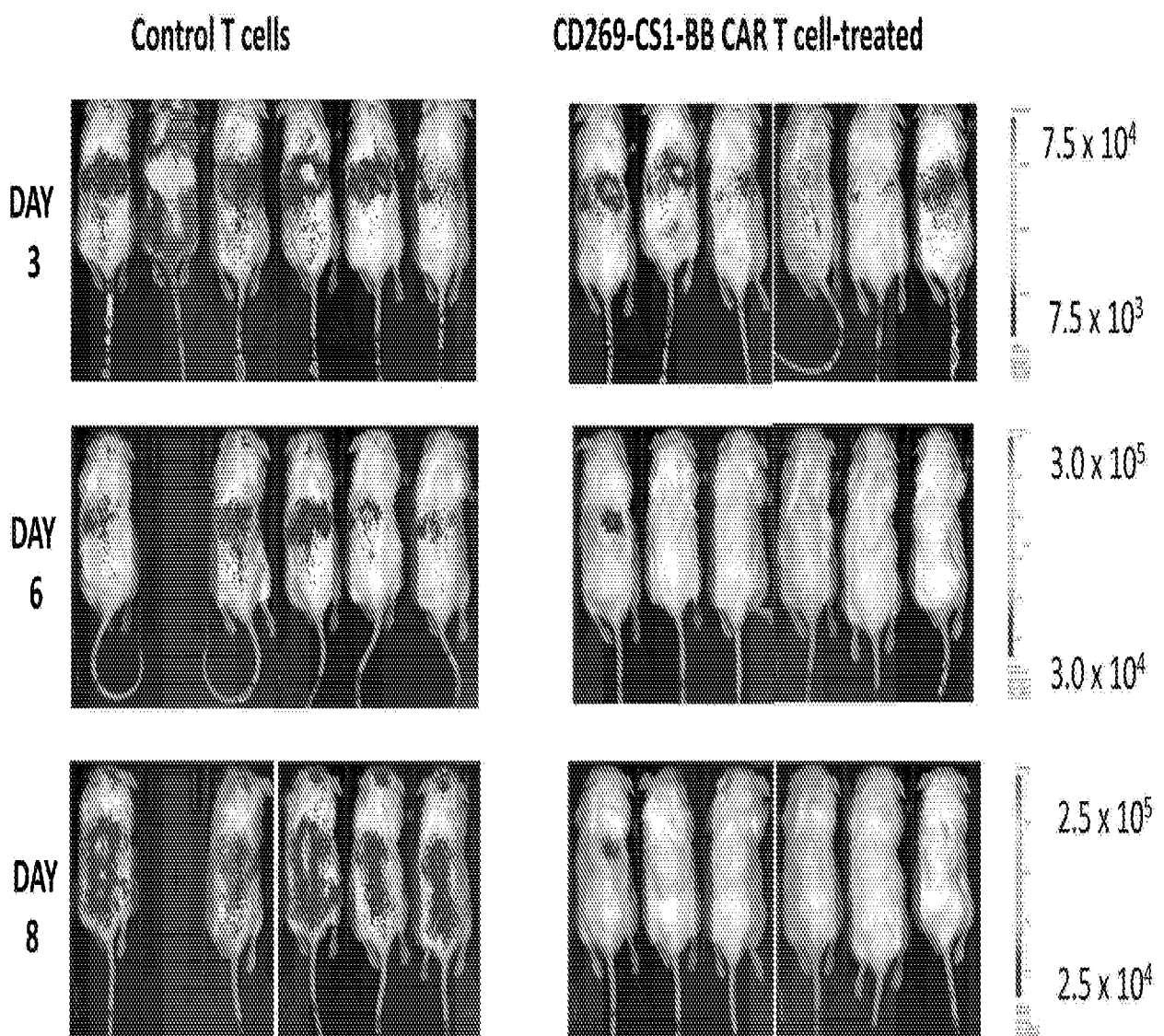


FIG. 22

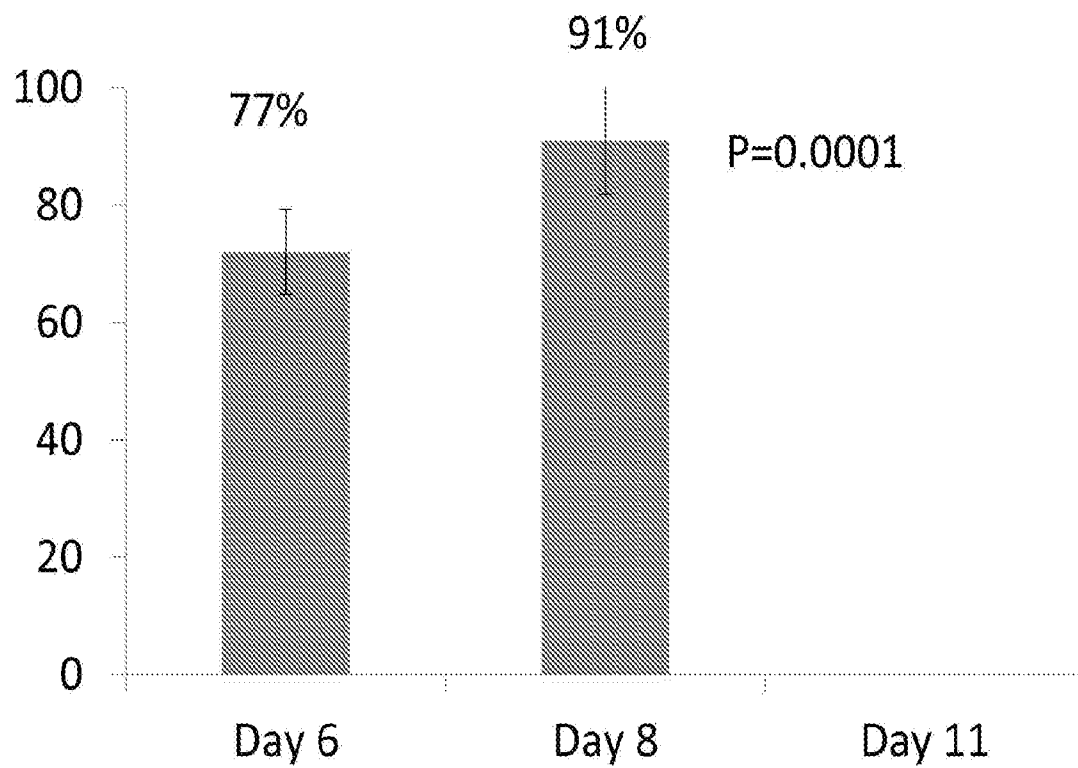


FIG. 23

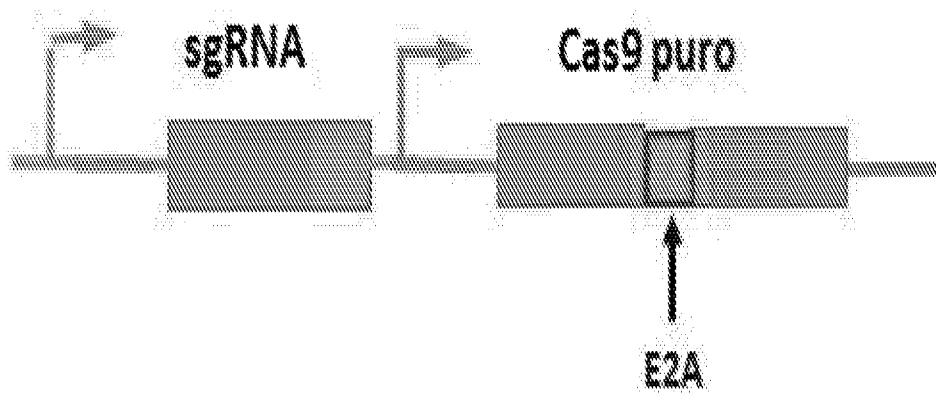
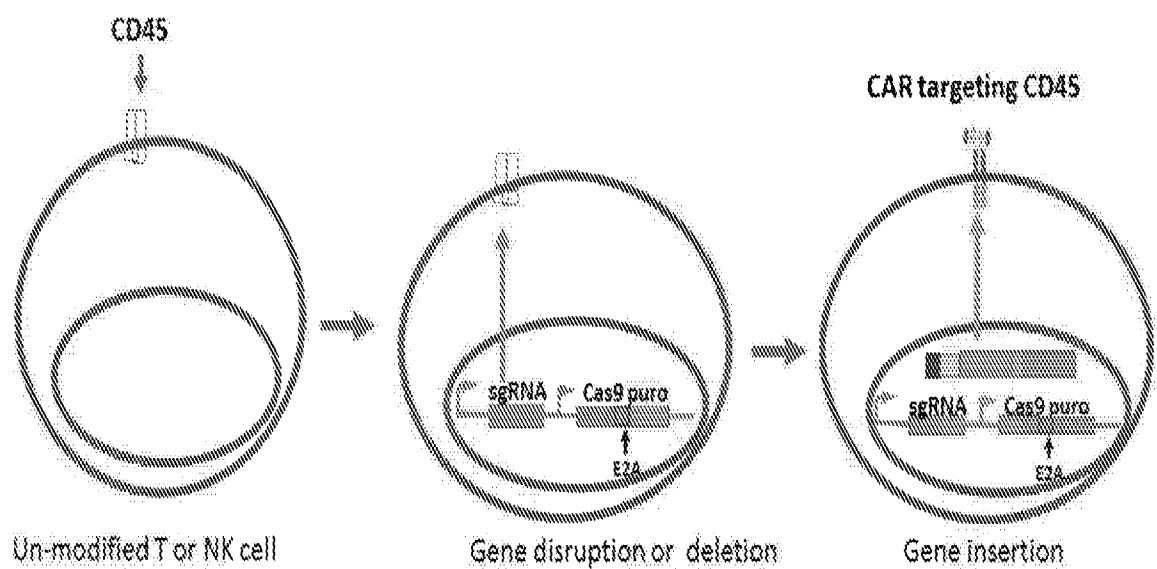


FIG. 24



- Steps:
1. Donor T cells or NK cells
  2. Gene disruption or deletion of CD45
  3. Insert of scFv CAR targeting CD45

FIG. 25

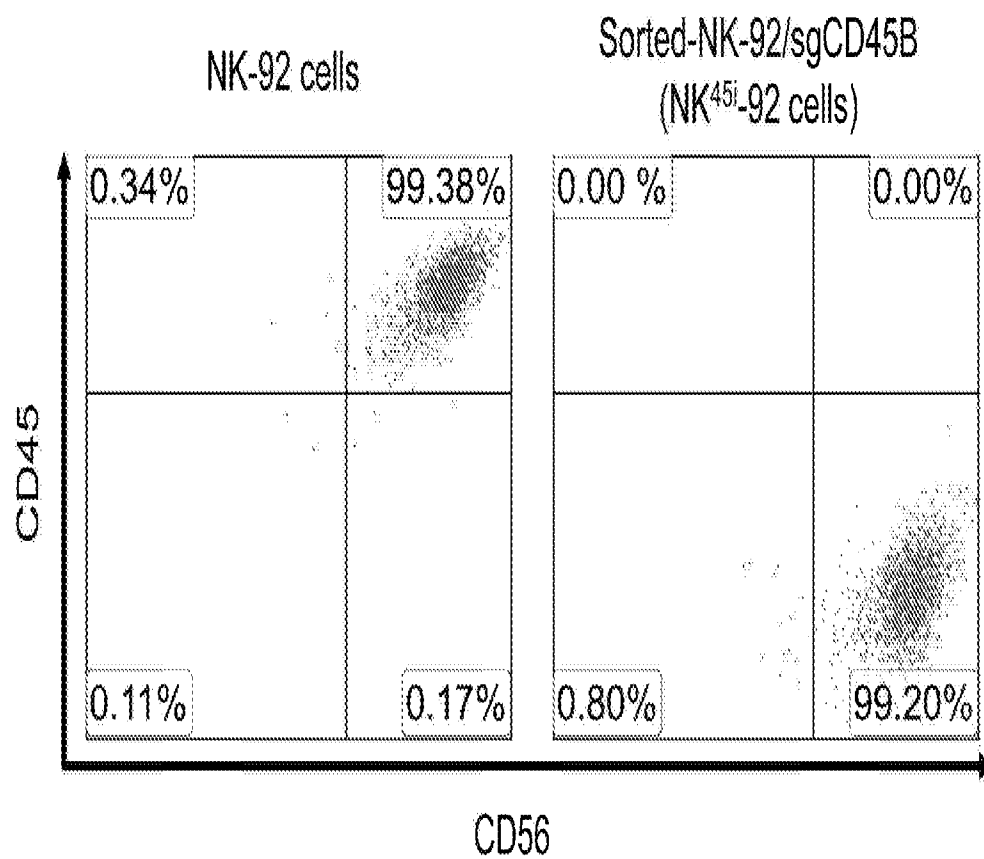
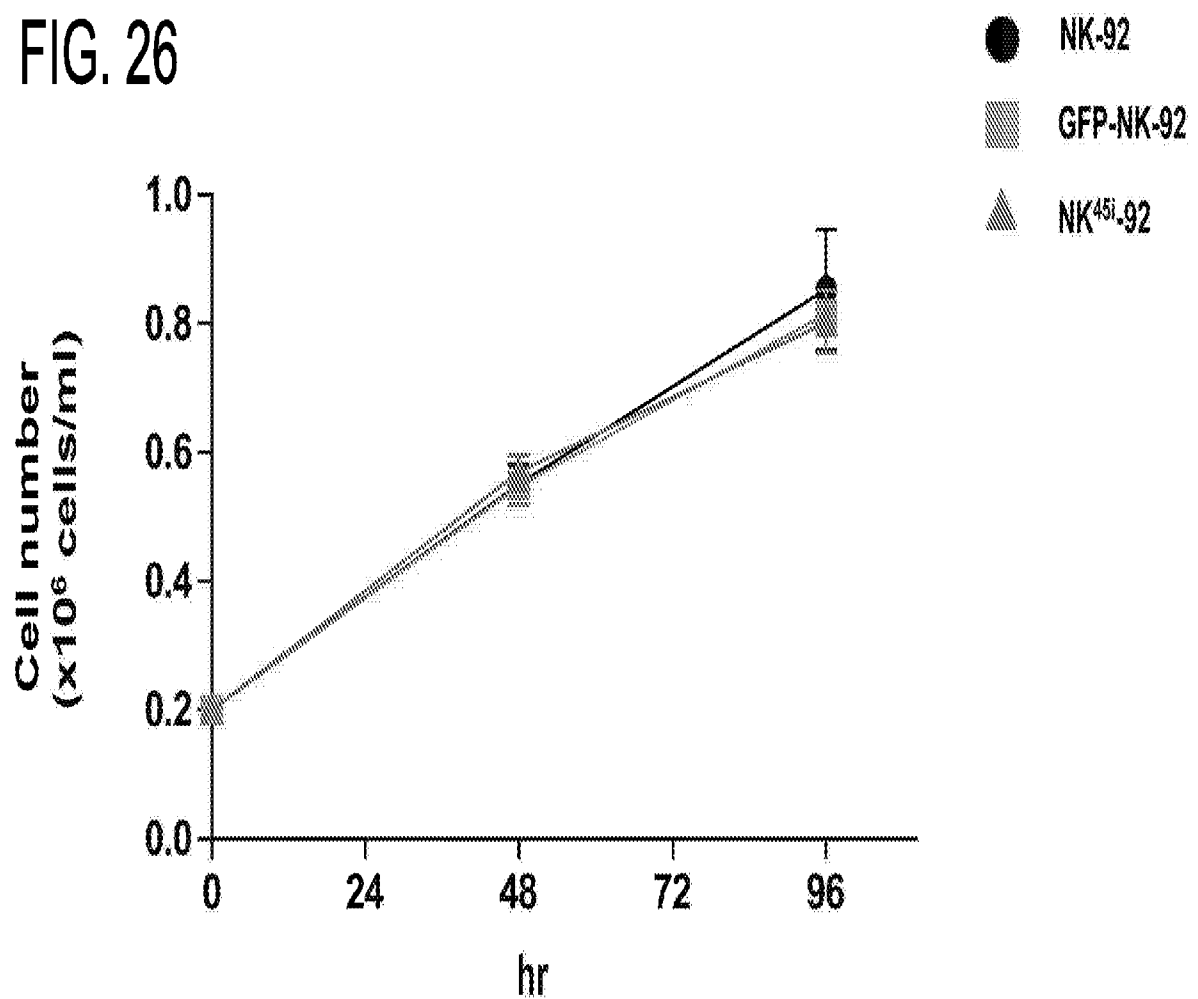


FIG. 26



24 well, duplicate, n=3

IL-2 was added at 48hr time point

FIG. 27A

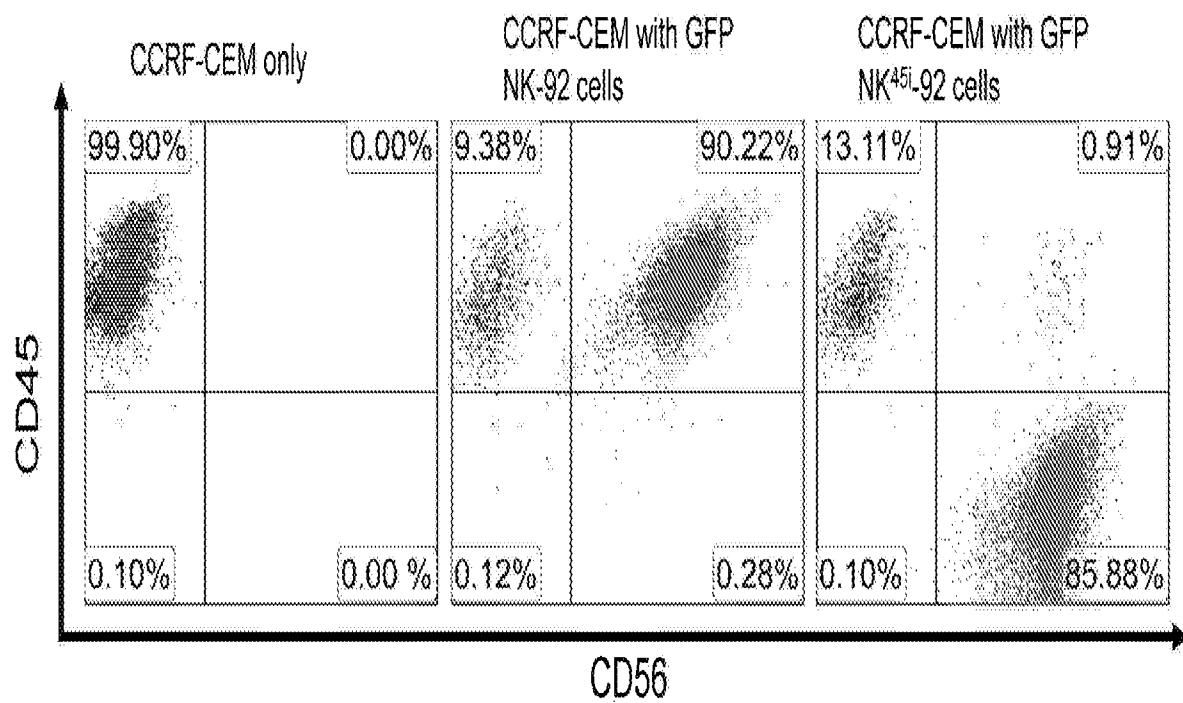




FIG. 27B

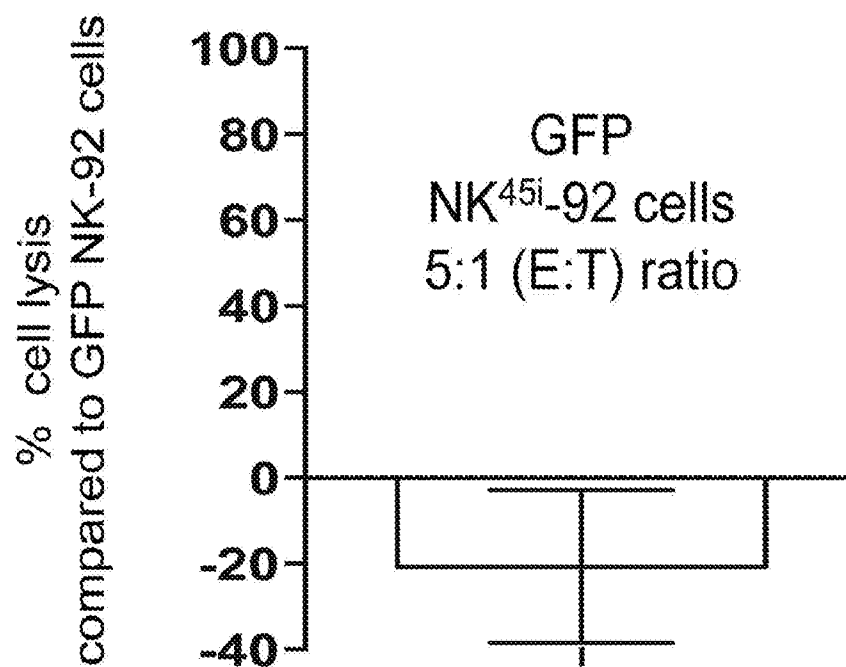


FIG. 28A

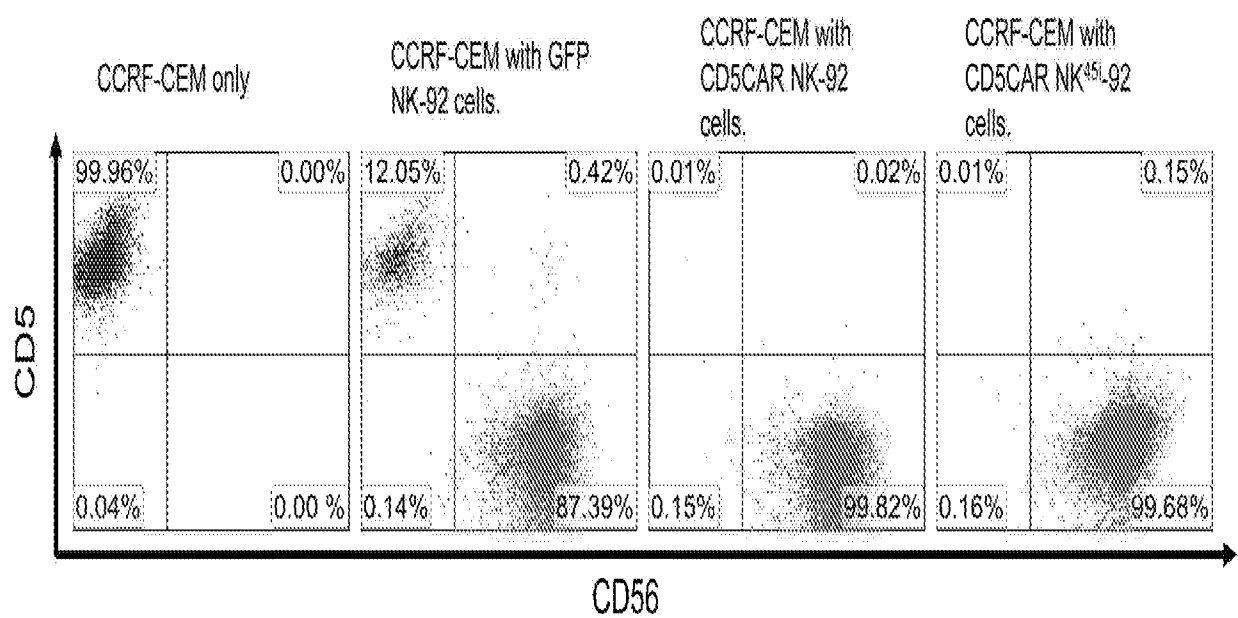


FIG. 28B

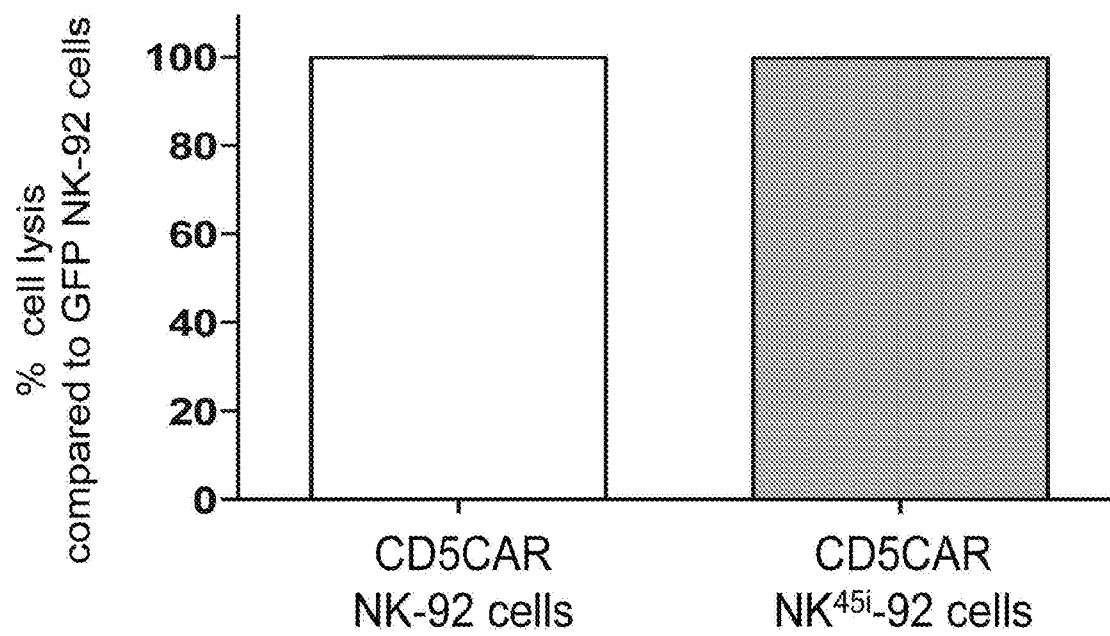


FIG. 29A

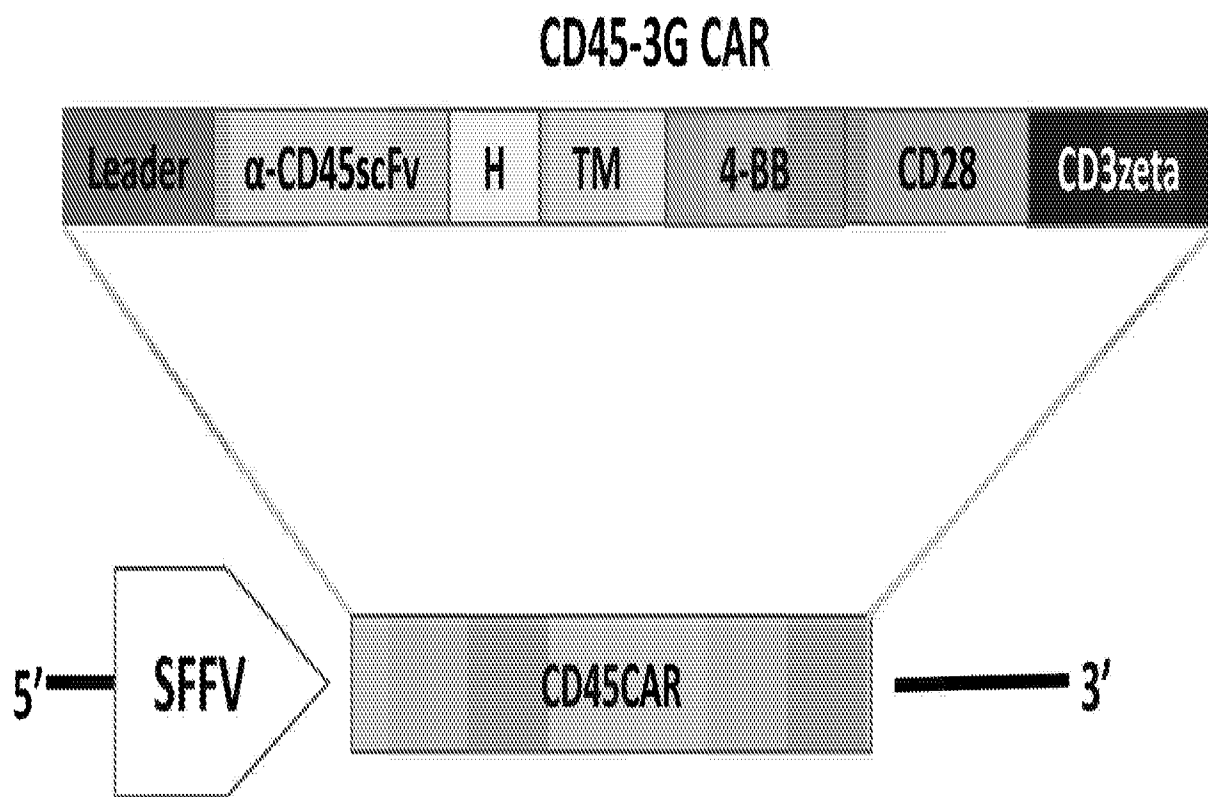


FIG. 29B

**M    GFP    CD45CAR**

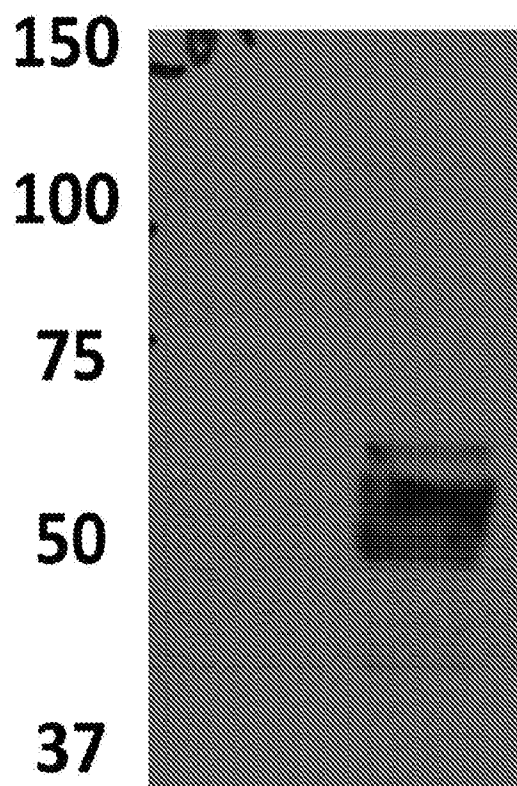


FIG. 30A

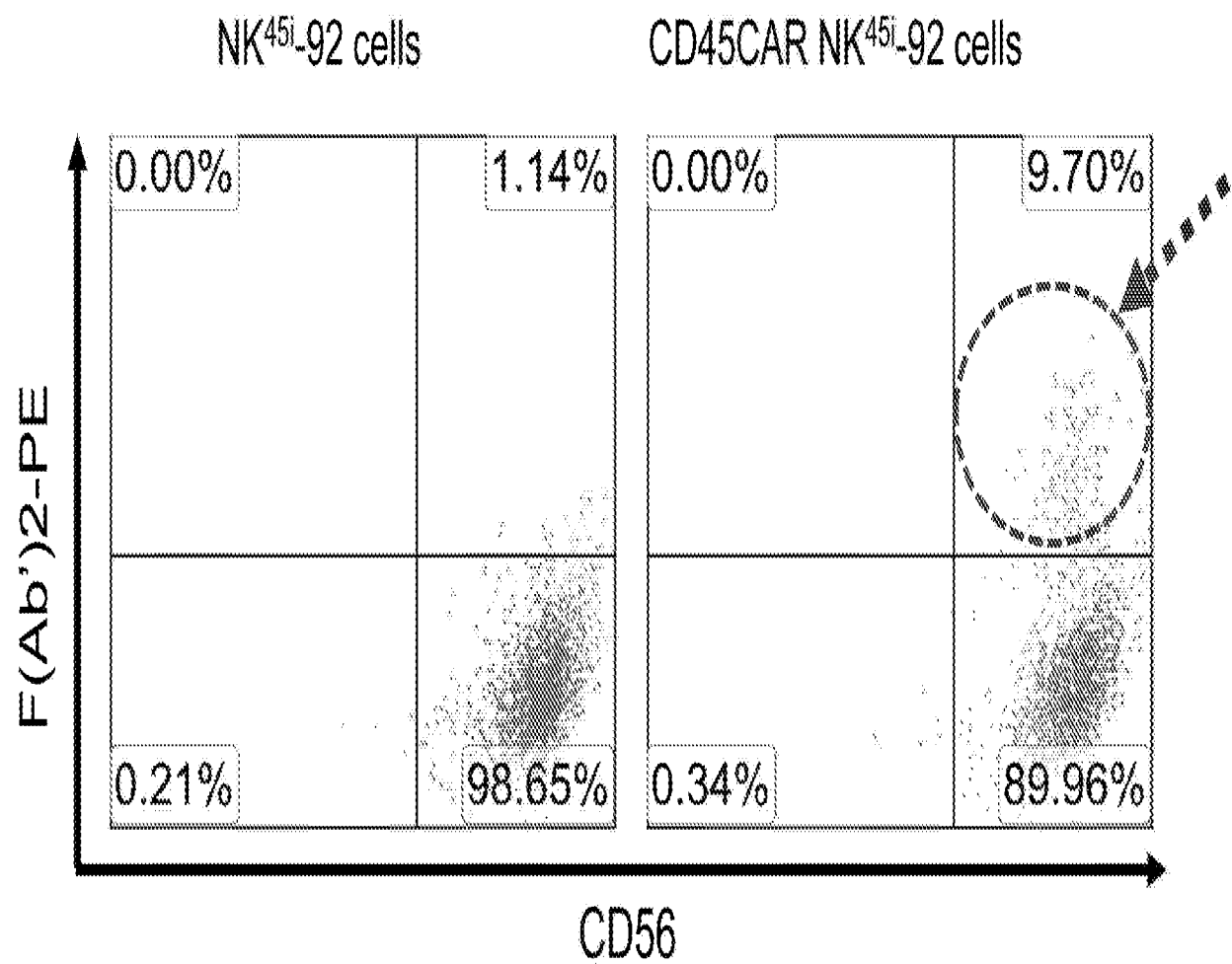


FIG. 30B

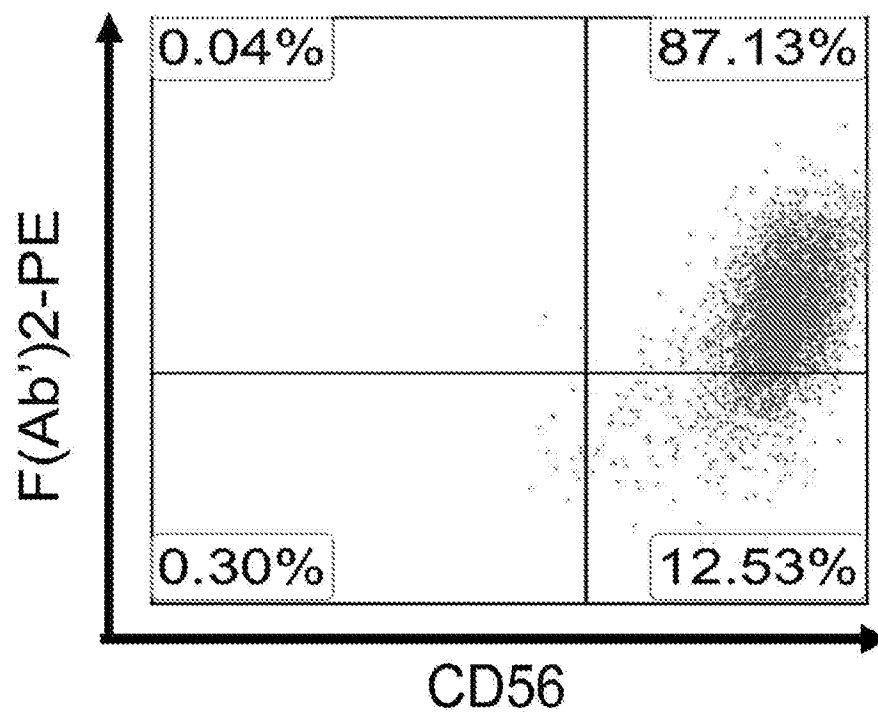


FIG. 31A

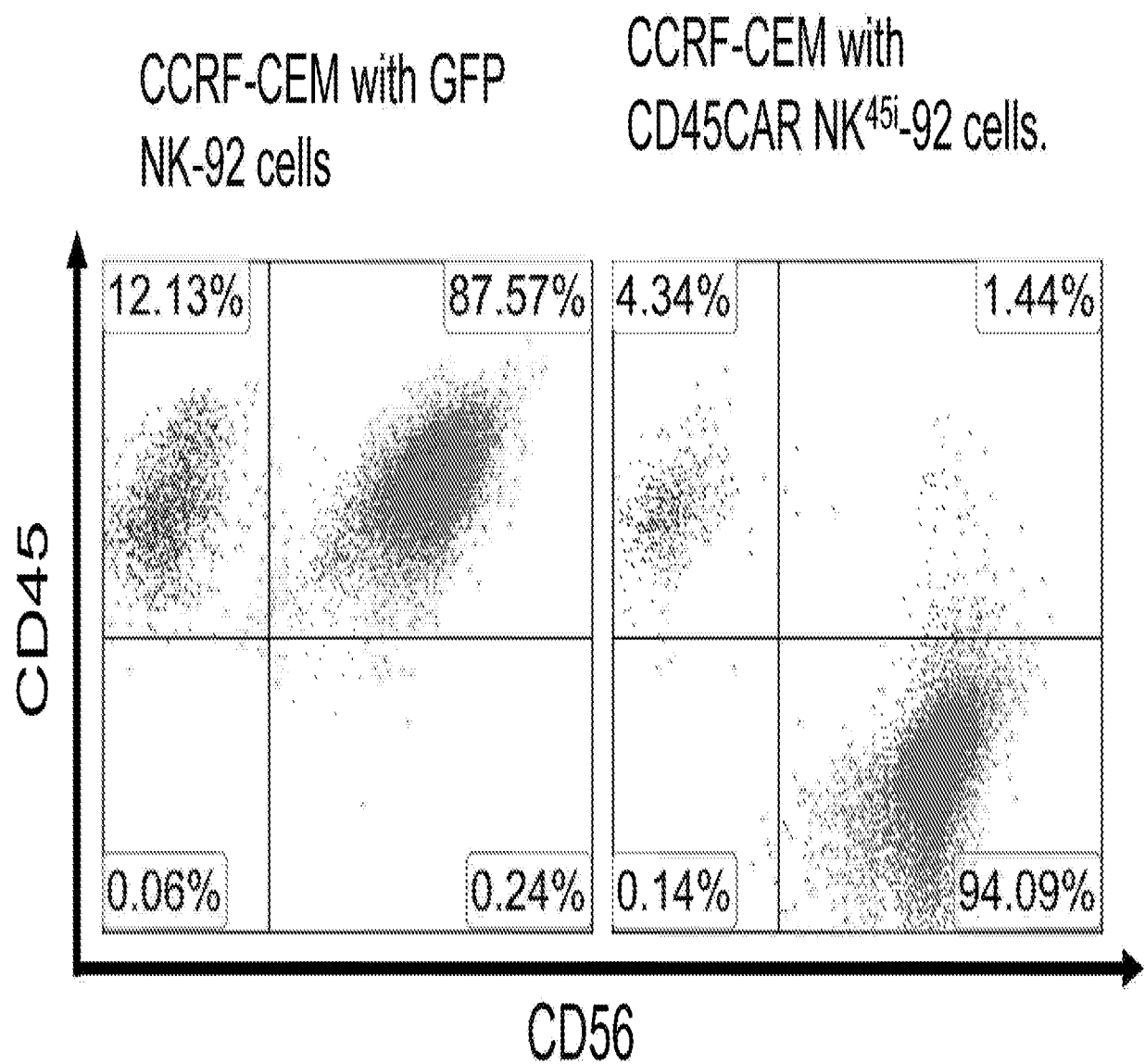




FIG. 31B

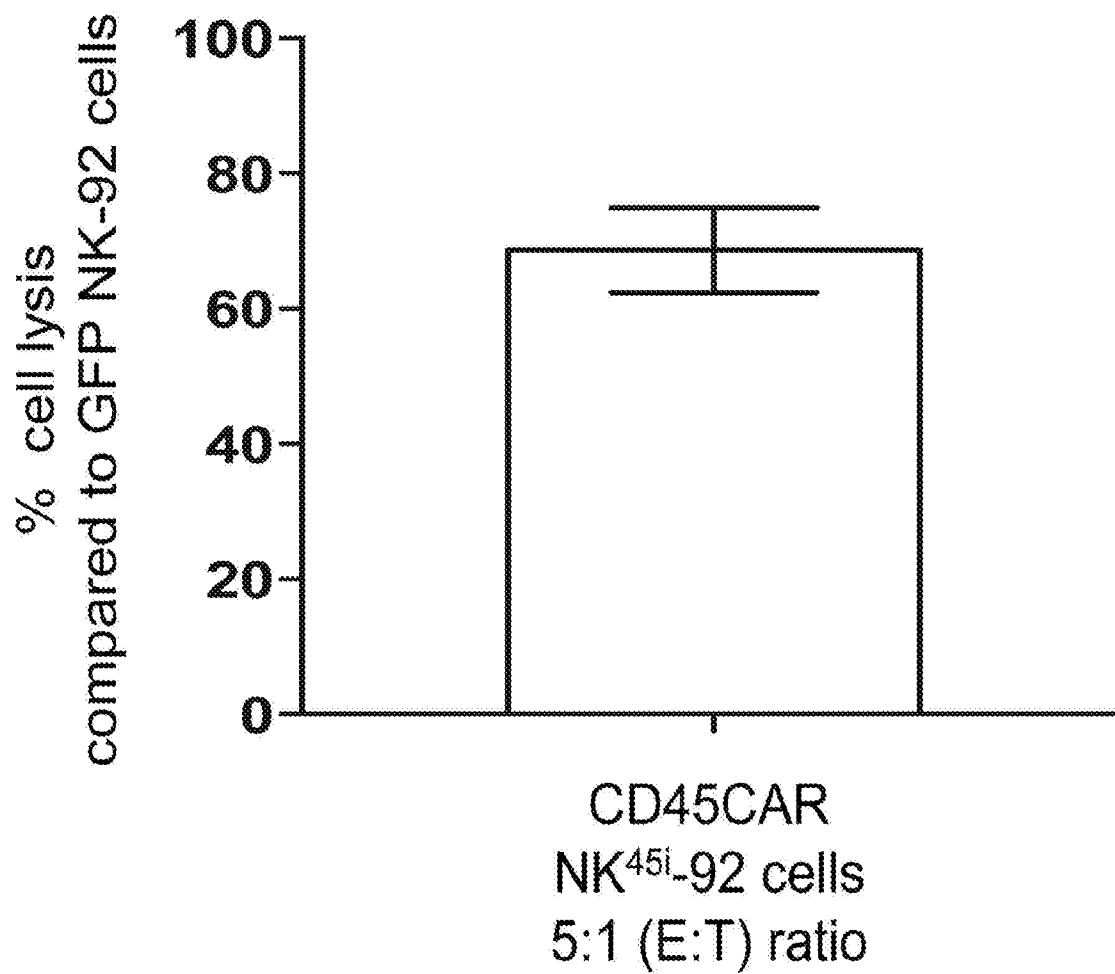


FIG. 32A

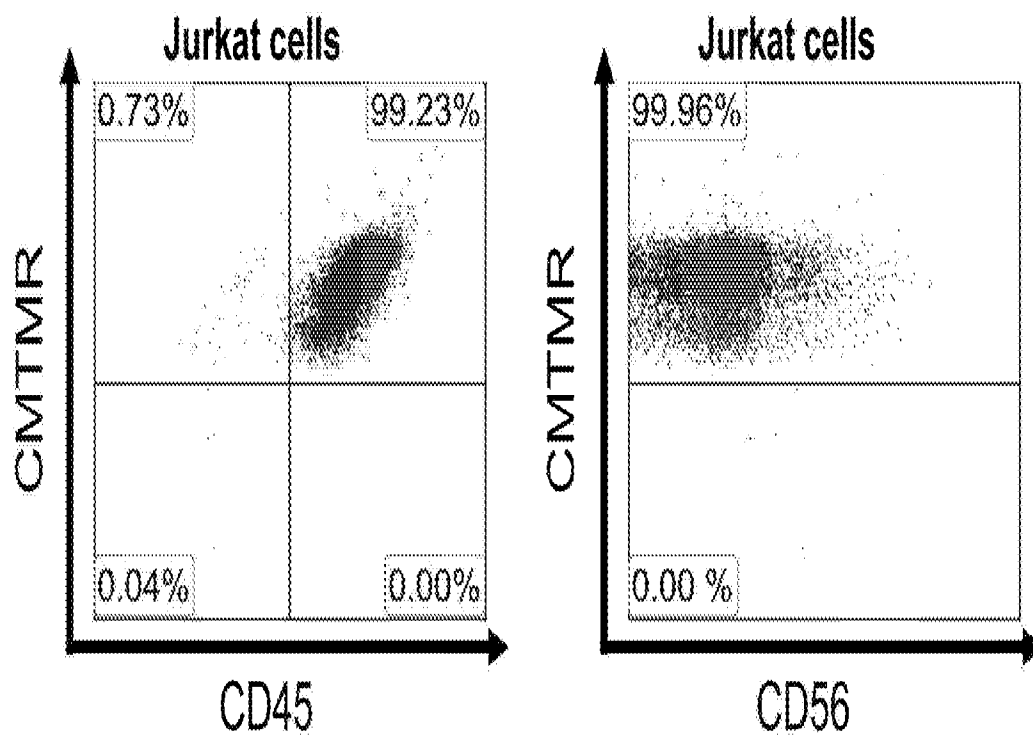


FIG. 32B

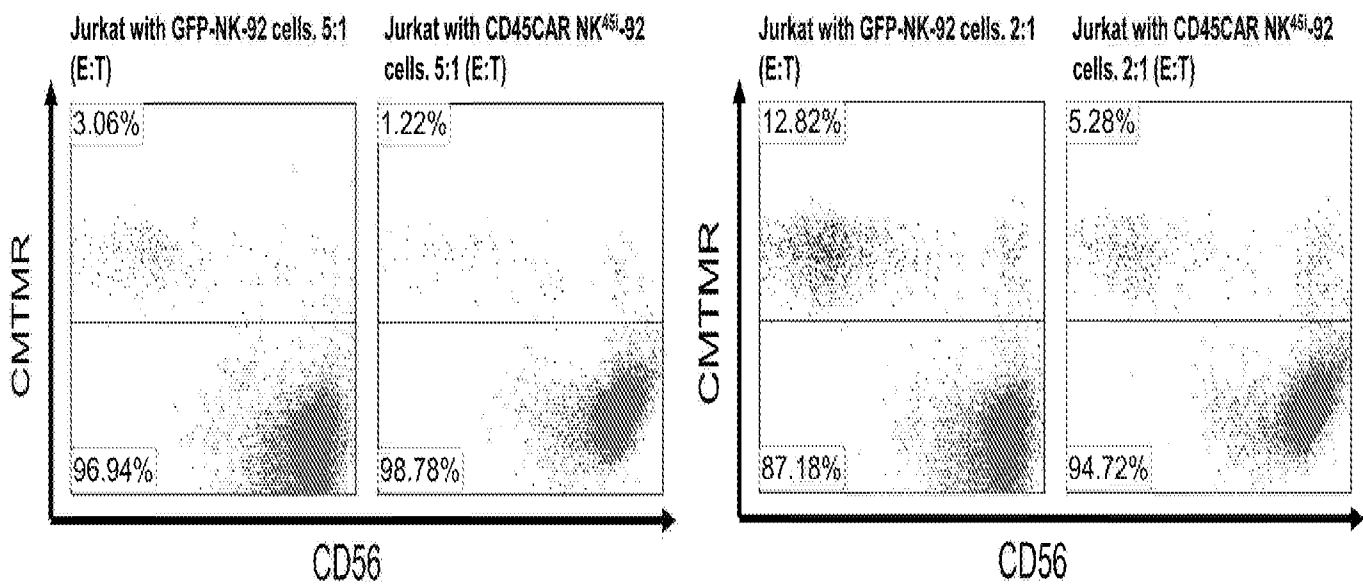


FIG. 32C

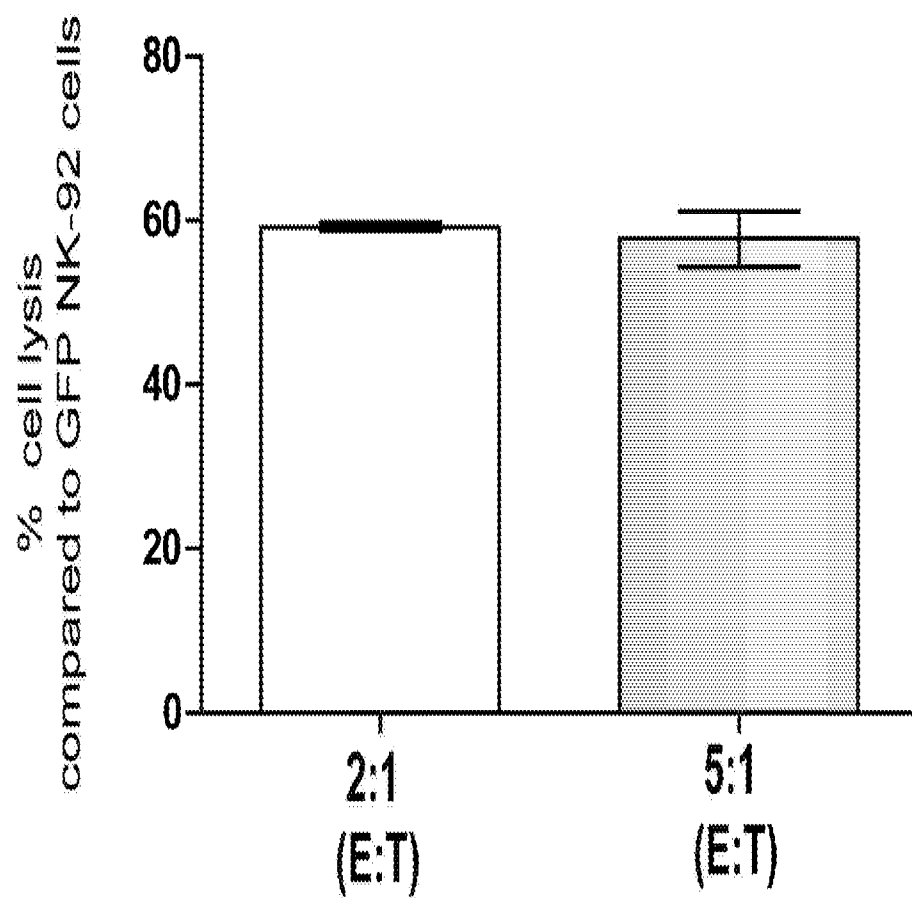


FIG. 33A

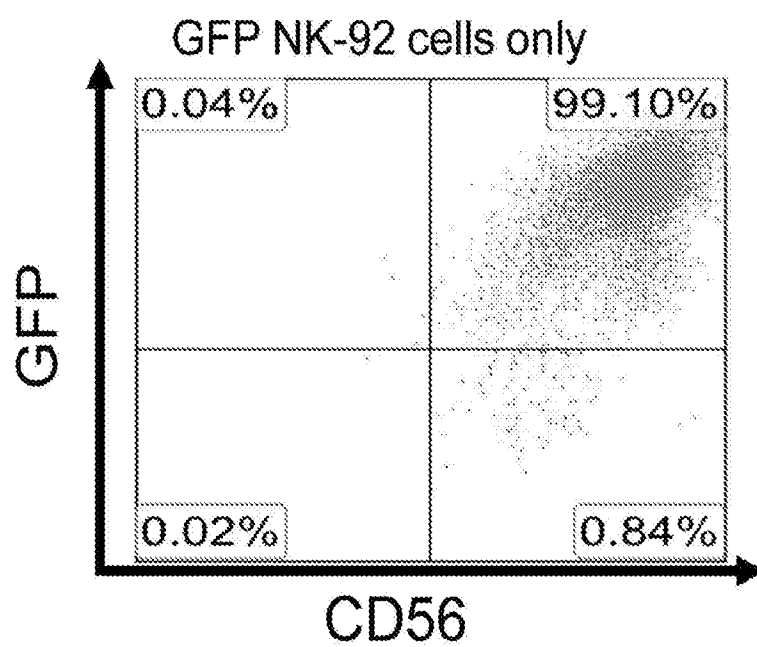


FIG. 33B

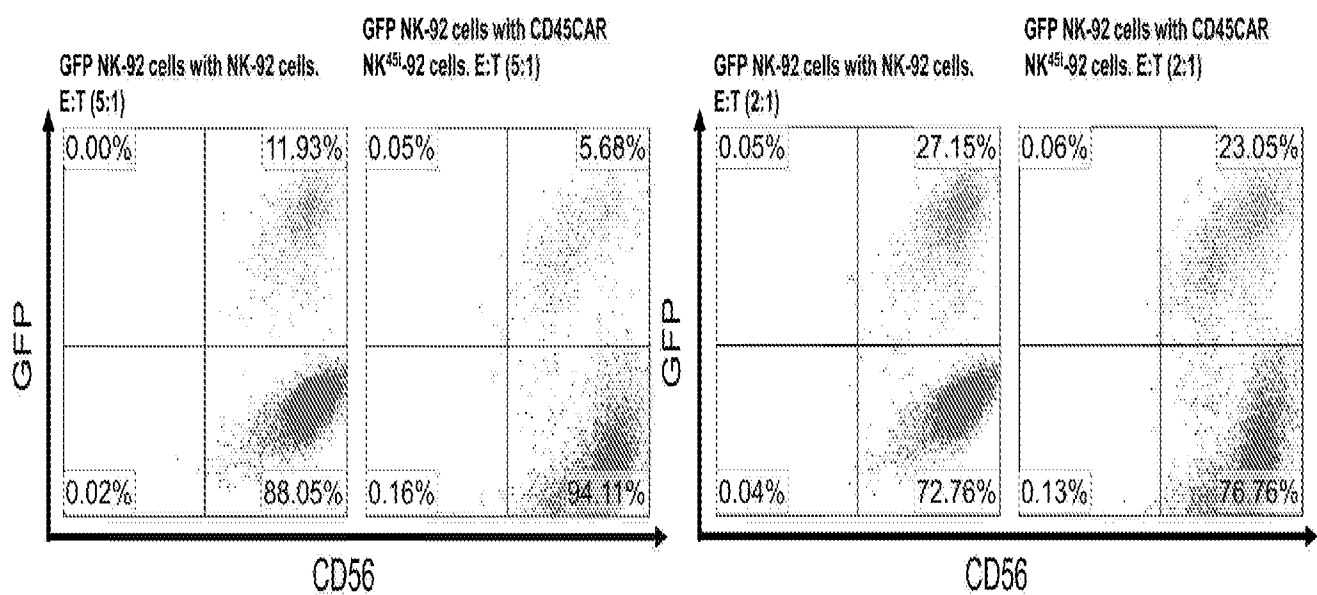


FIG. 33C

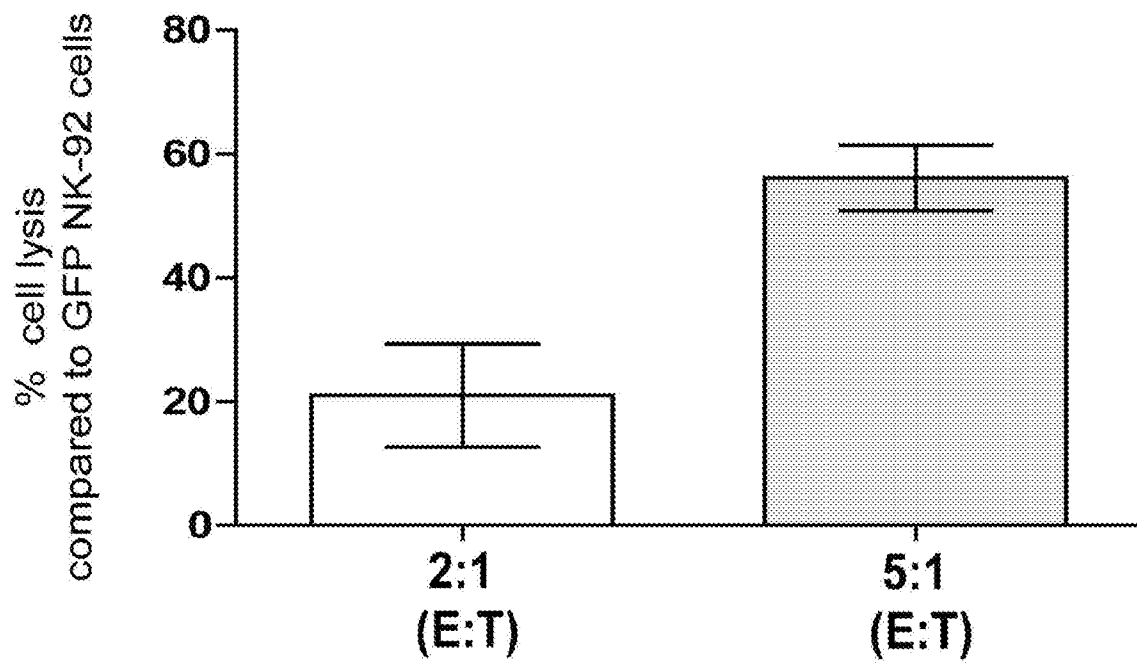


FIG. 33D

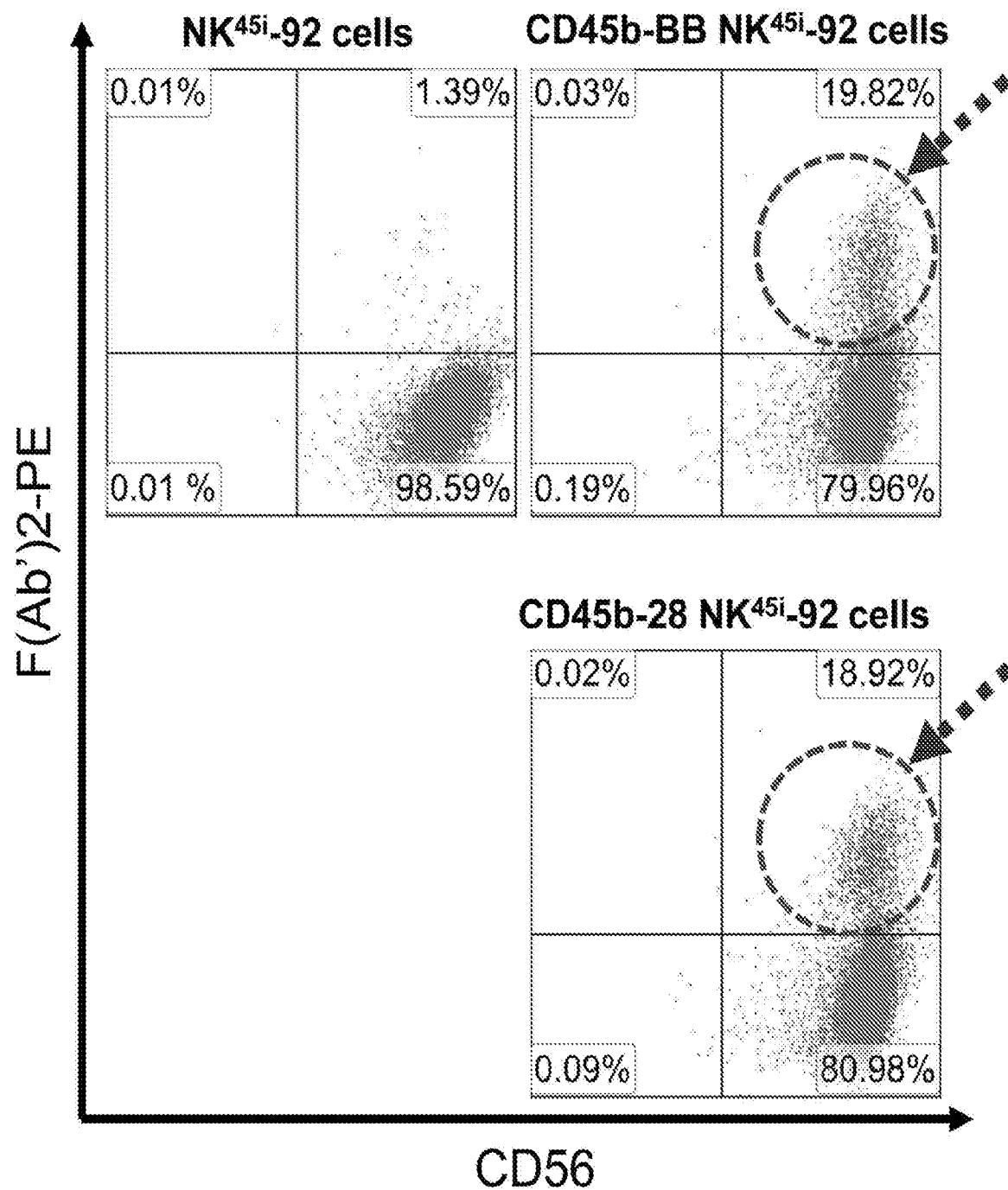




FIG. 33E

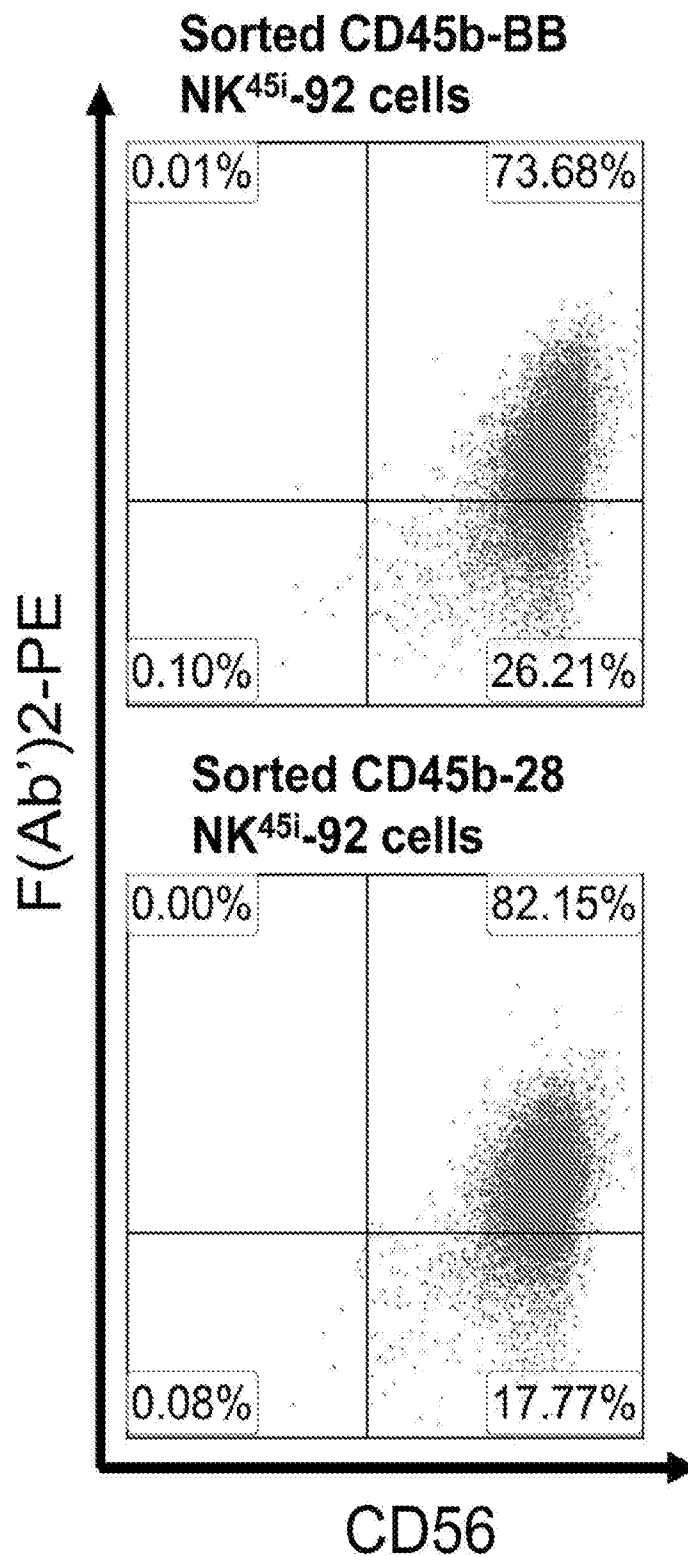


FIG. 33F

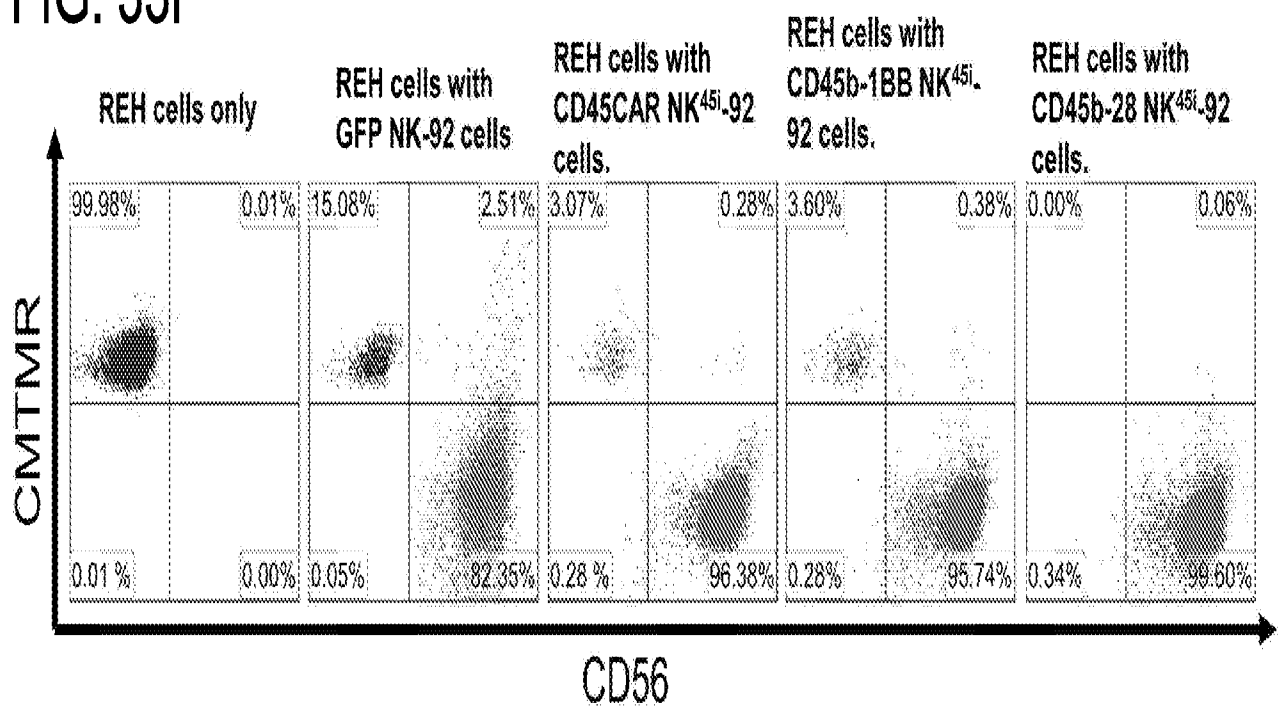


FIG. 33G

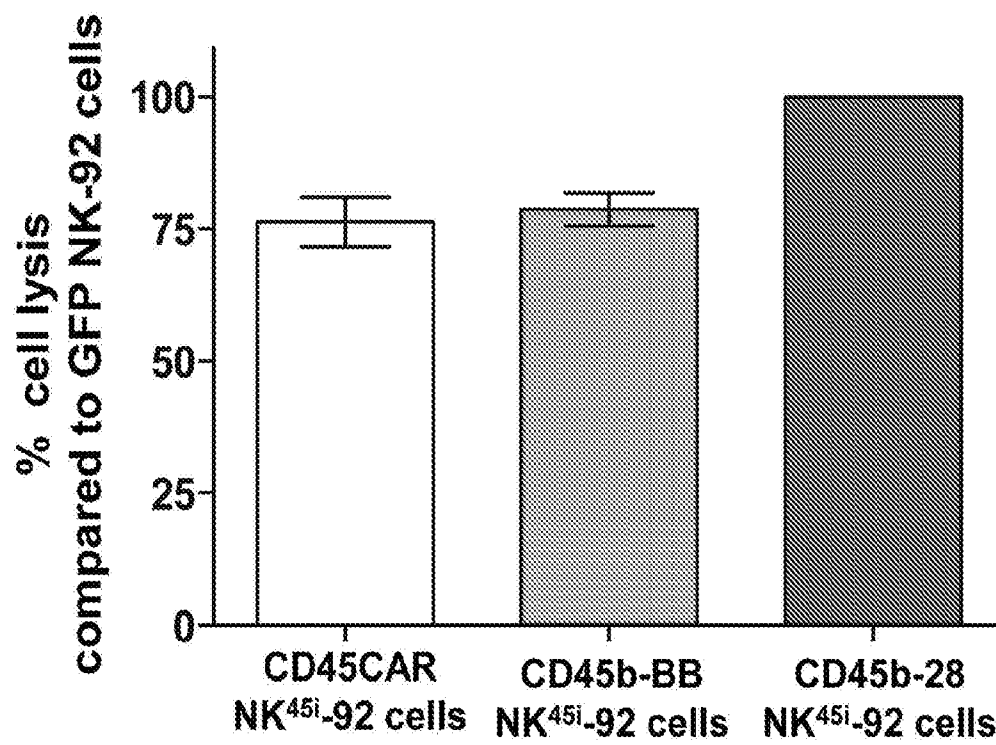


FIG. 34A

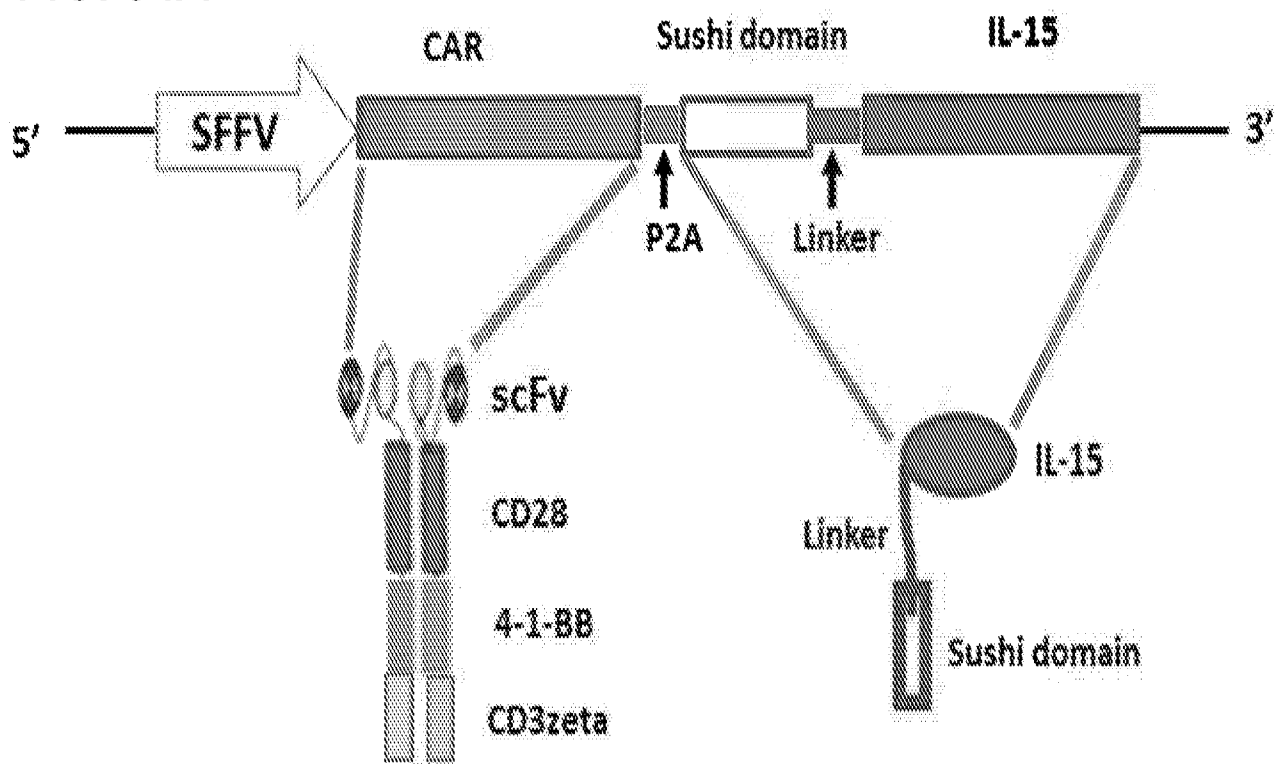


FIG. 34B

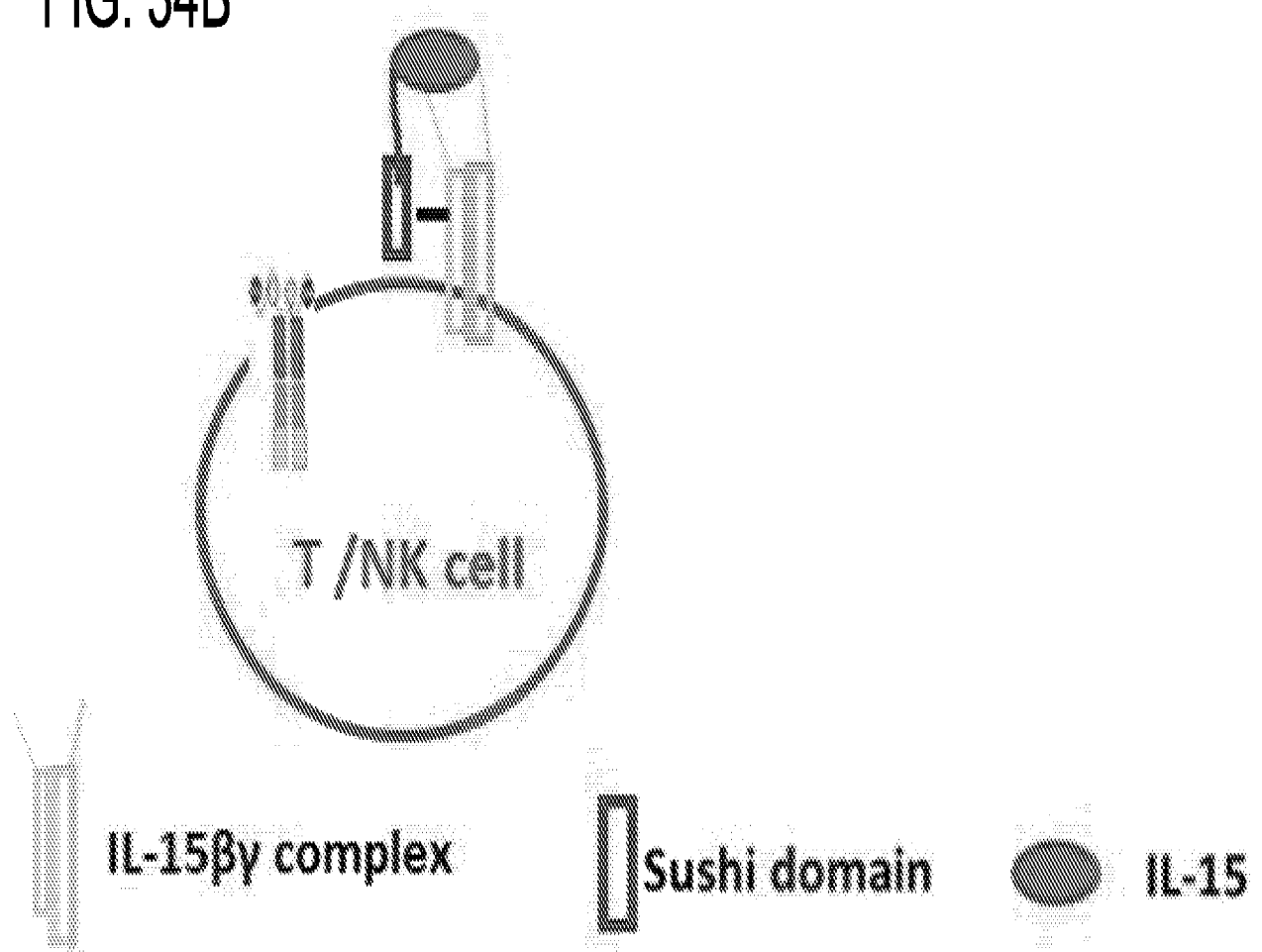


FIG. 35A  
HEK CD4IL15RA

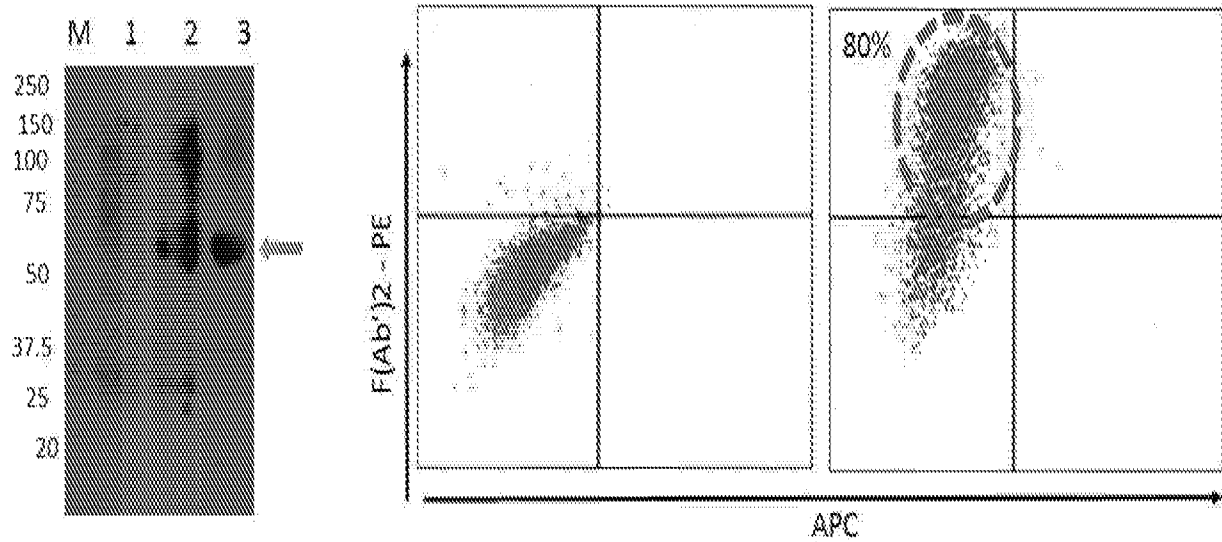


FIG. 35B

FIG. 36

CD4IL15RA NK cells

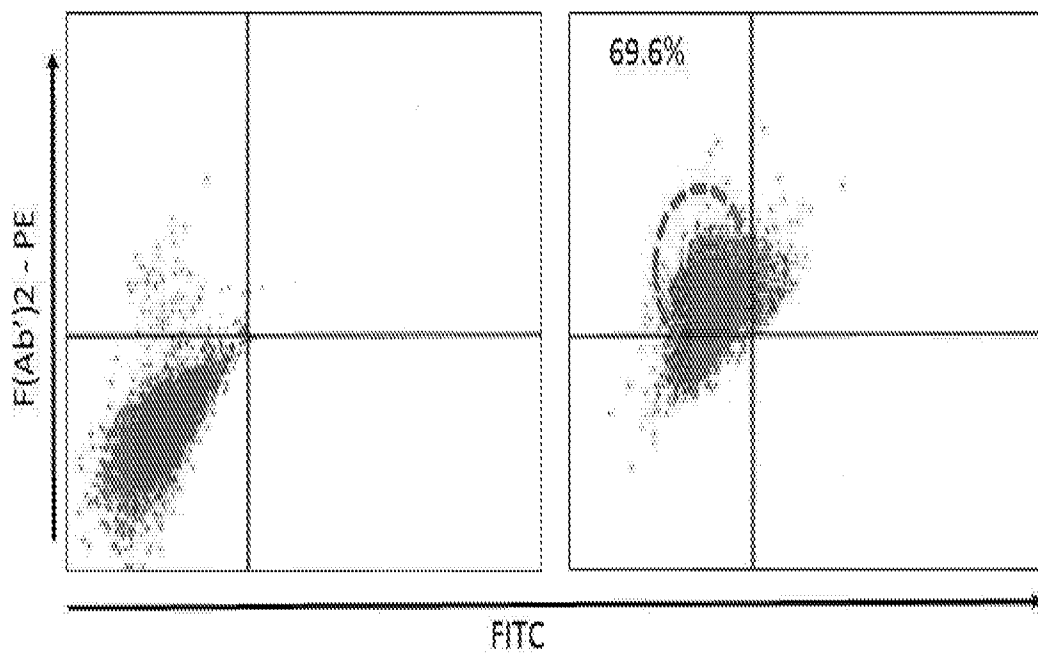


FIG. 37  
CD4IL15RA T cells

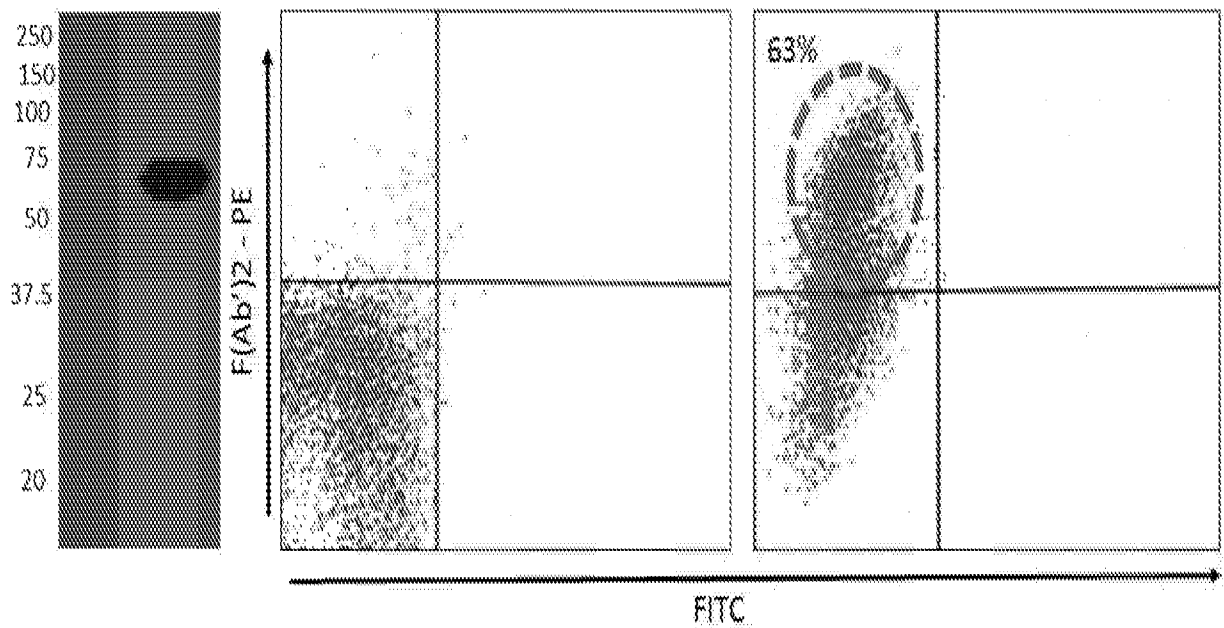
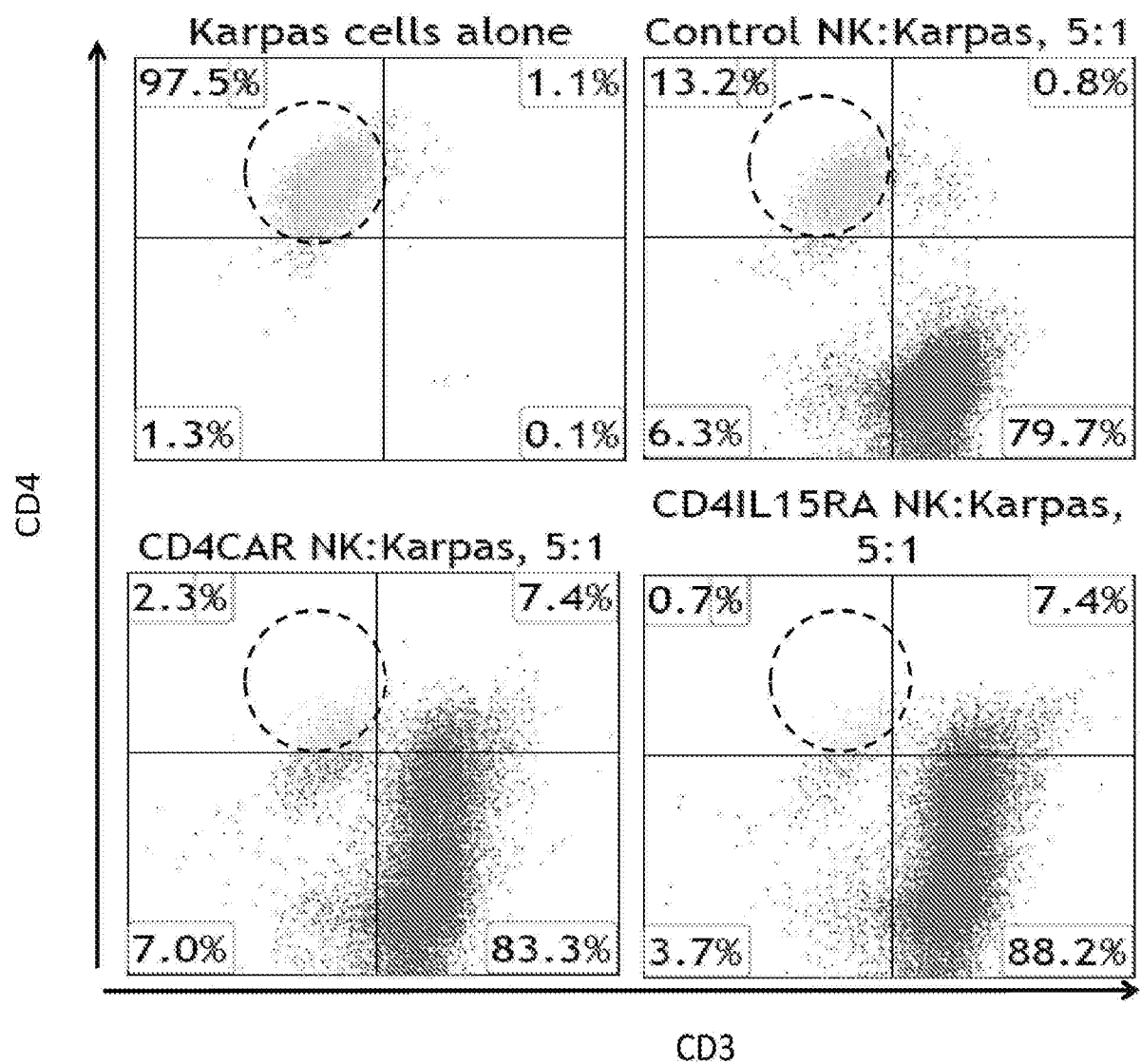




FIG. 38A



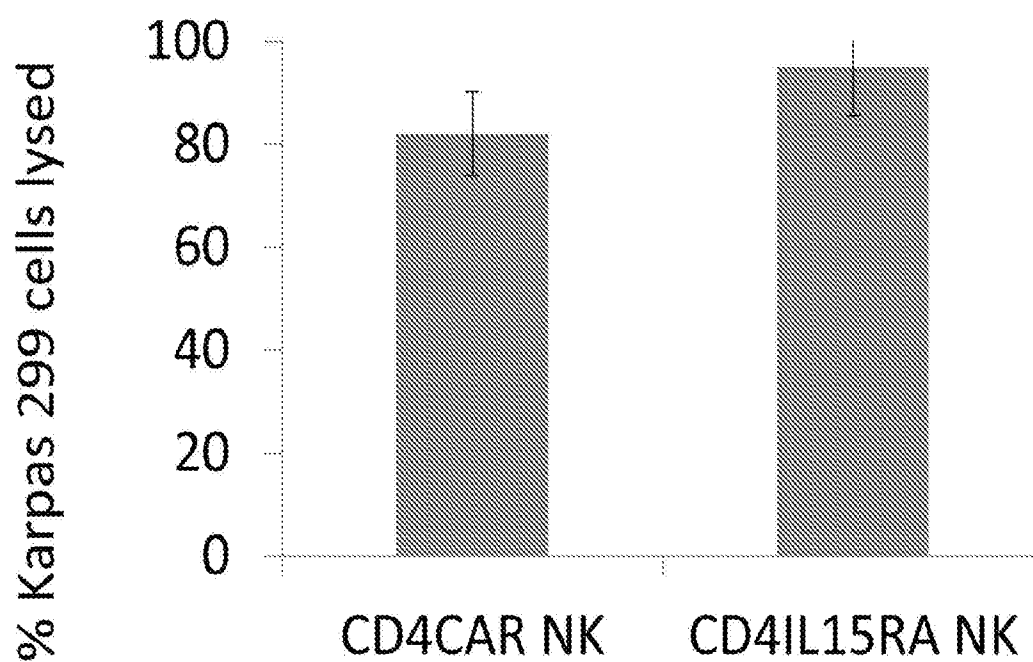
**FIG. 38B**

FIG. 39

CD4 and CD4IL15RA NK CAR cells lyse MOLT4 tumor cells, 1:1

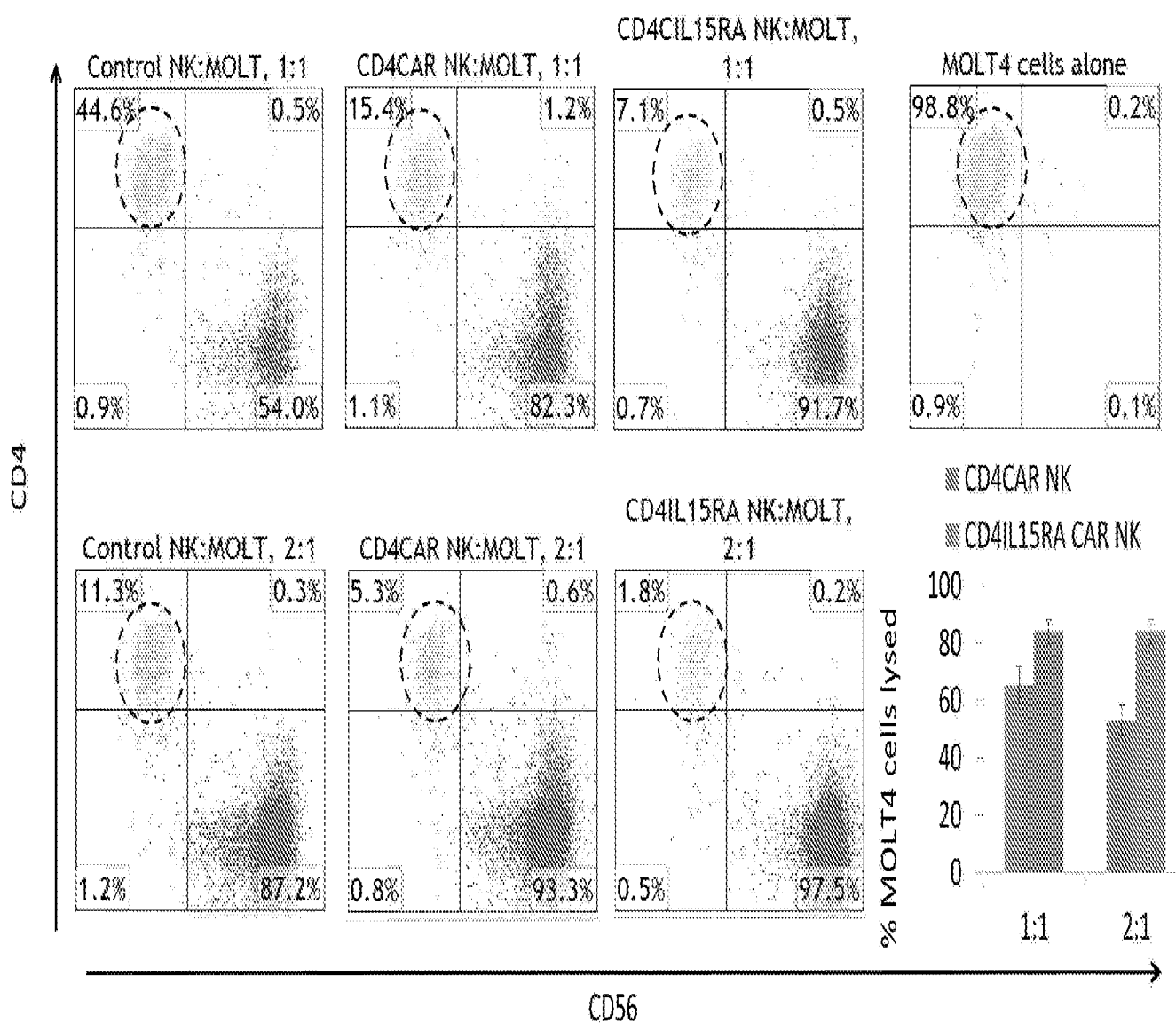


FIG. 40

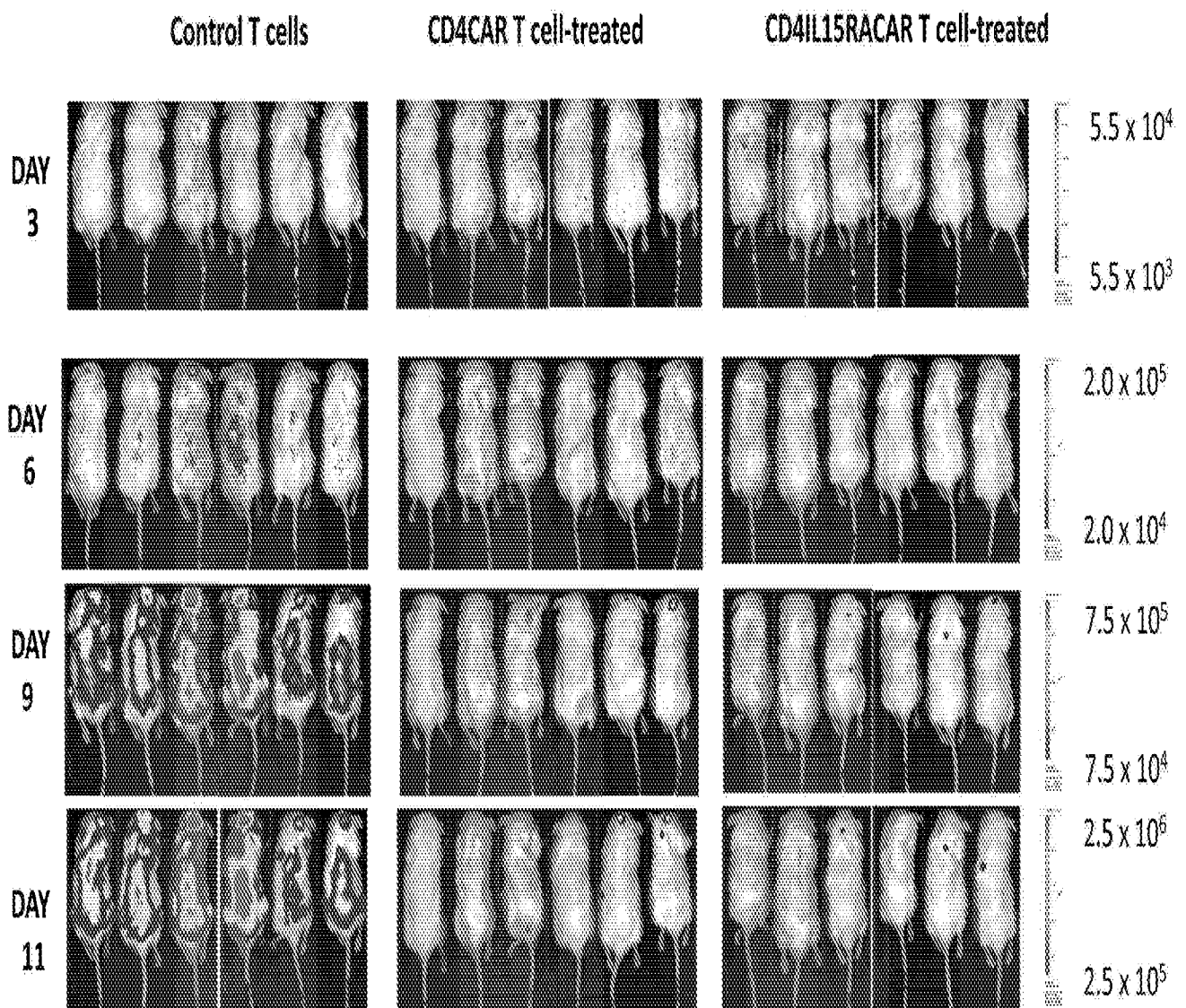


FIG. 41

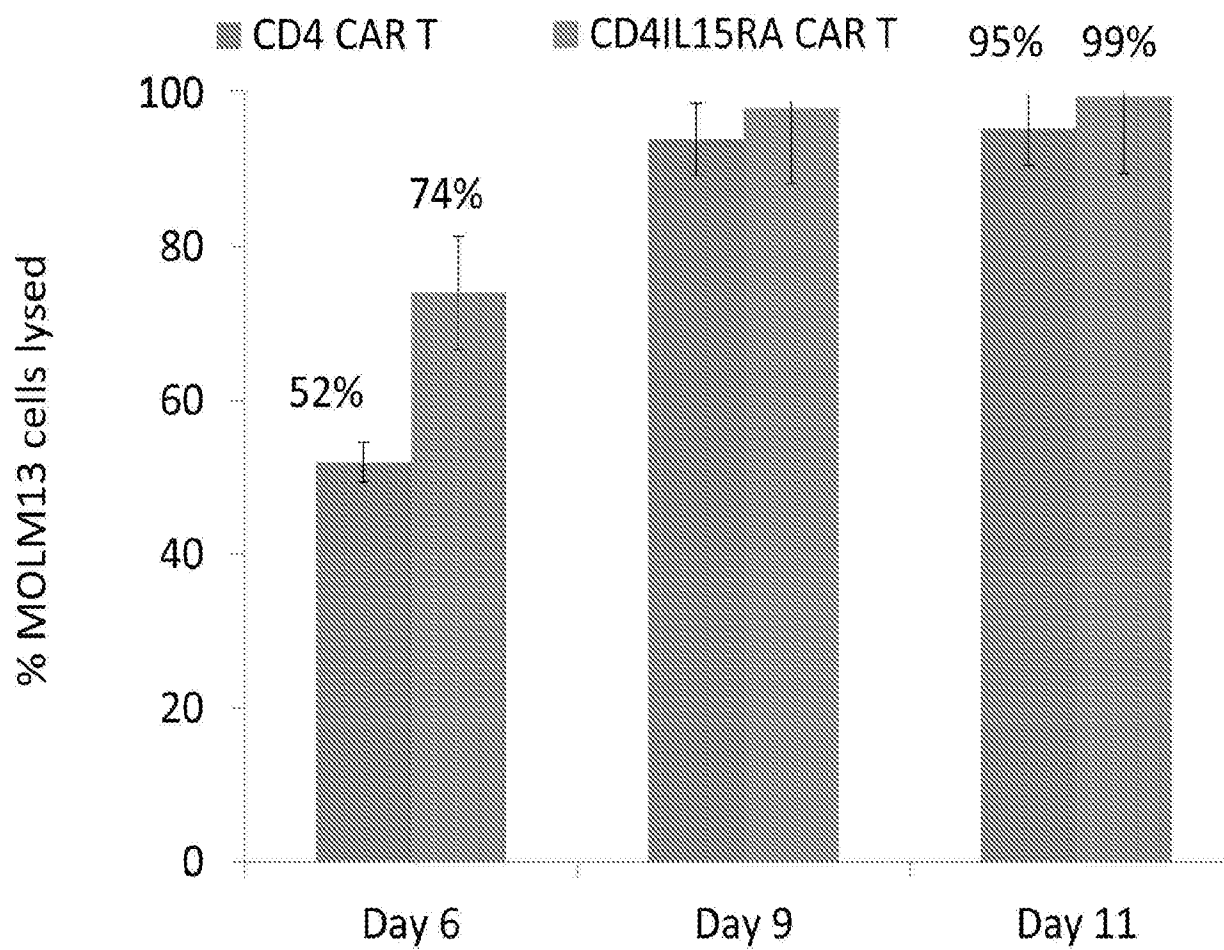


FIG. 42

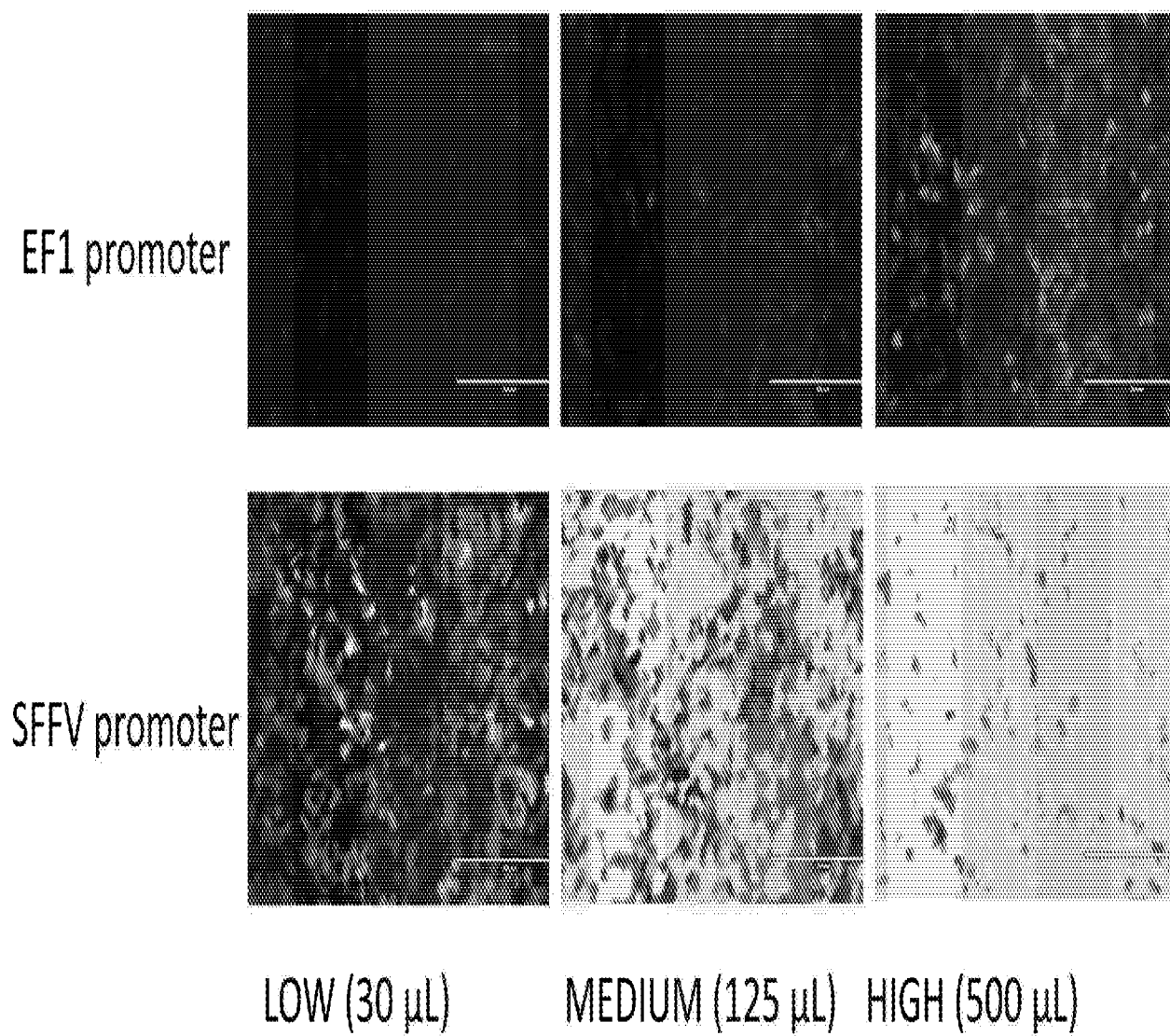


FIG. 43

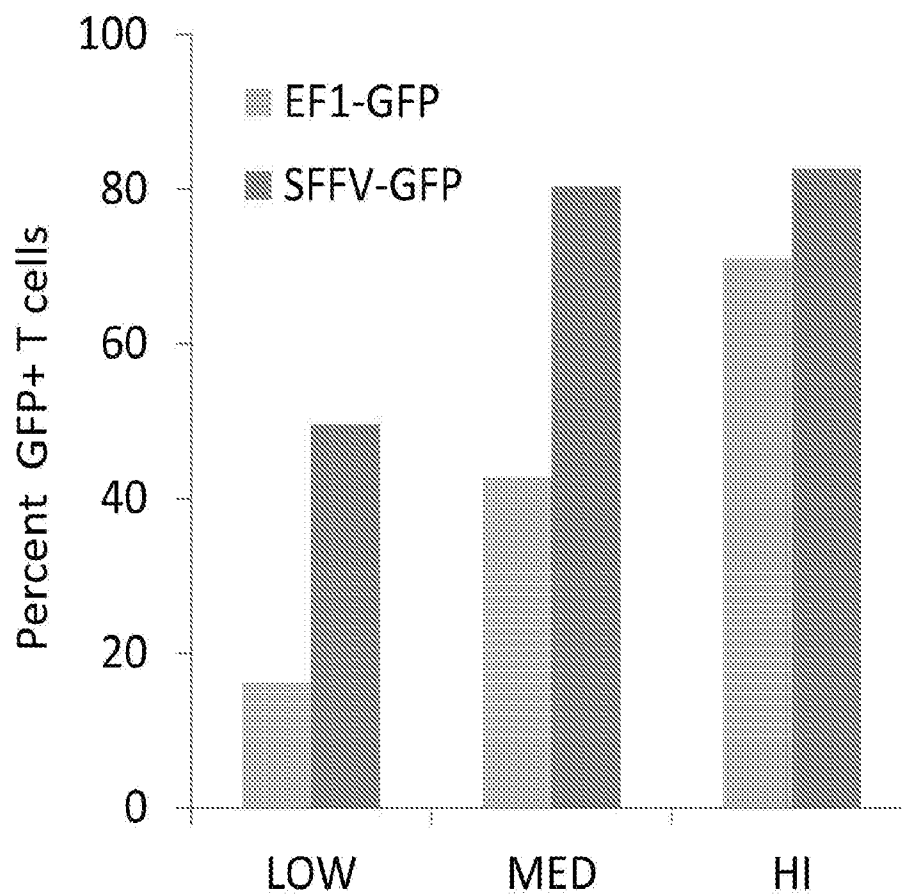


FIG. 44A

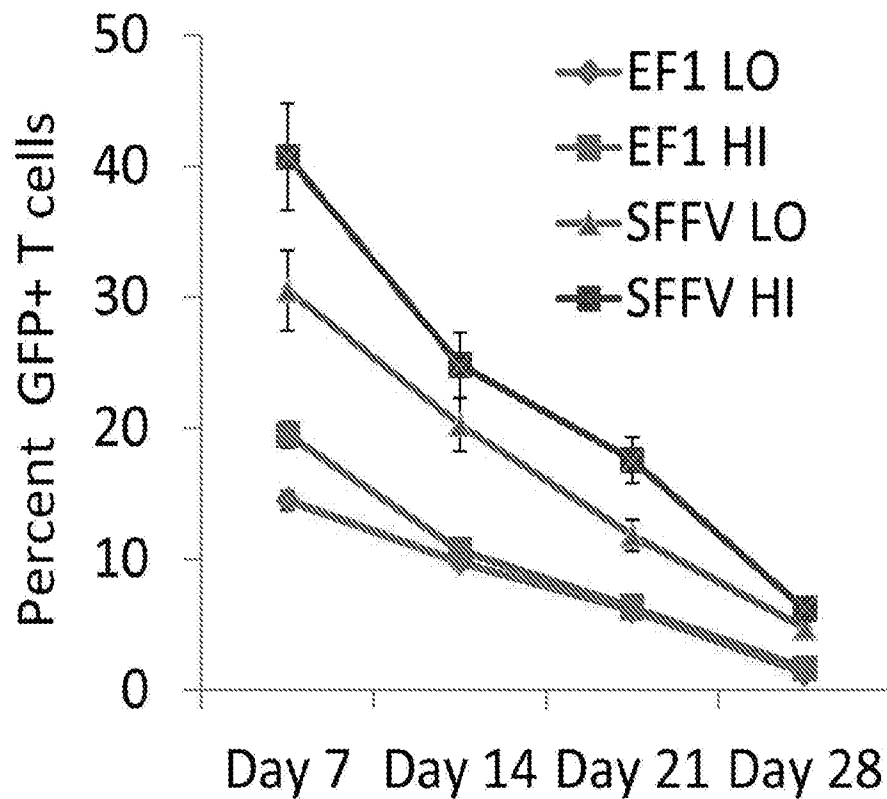


FIG. 44B

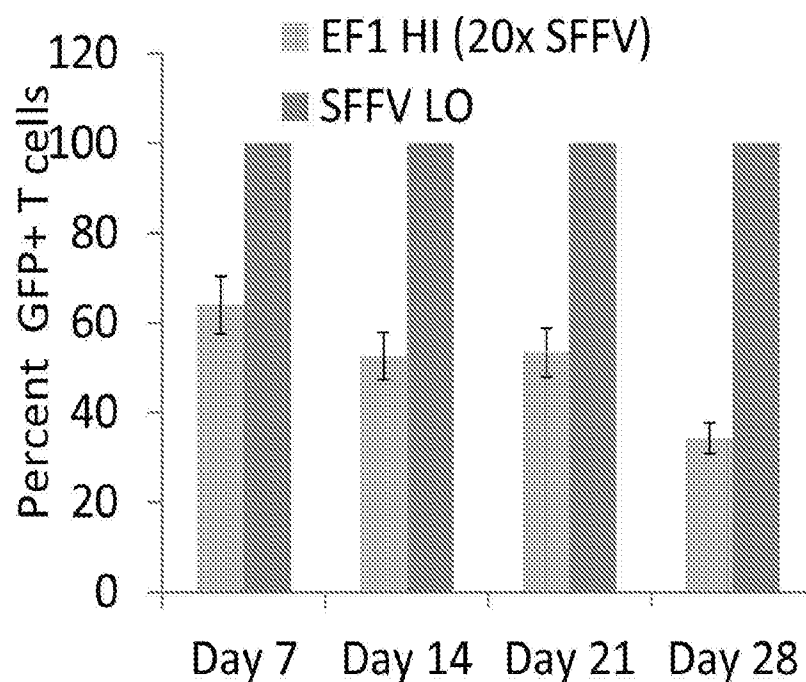
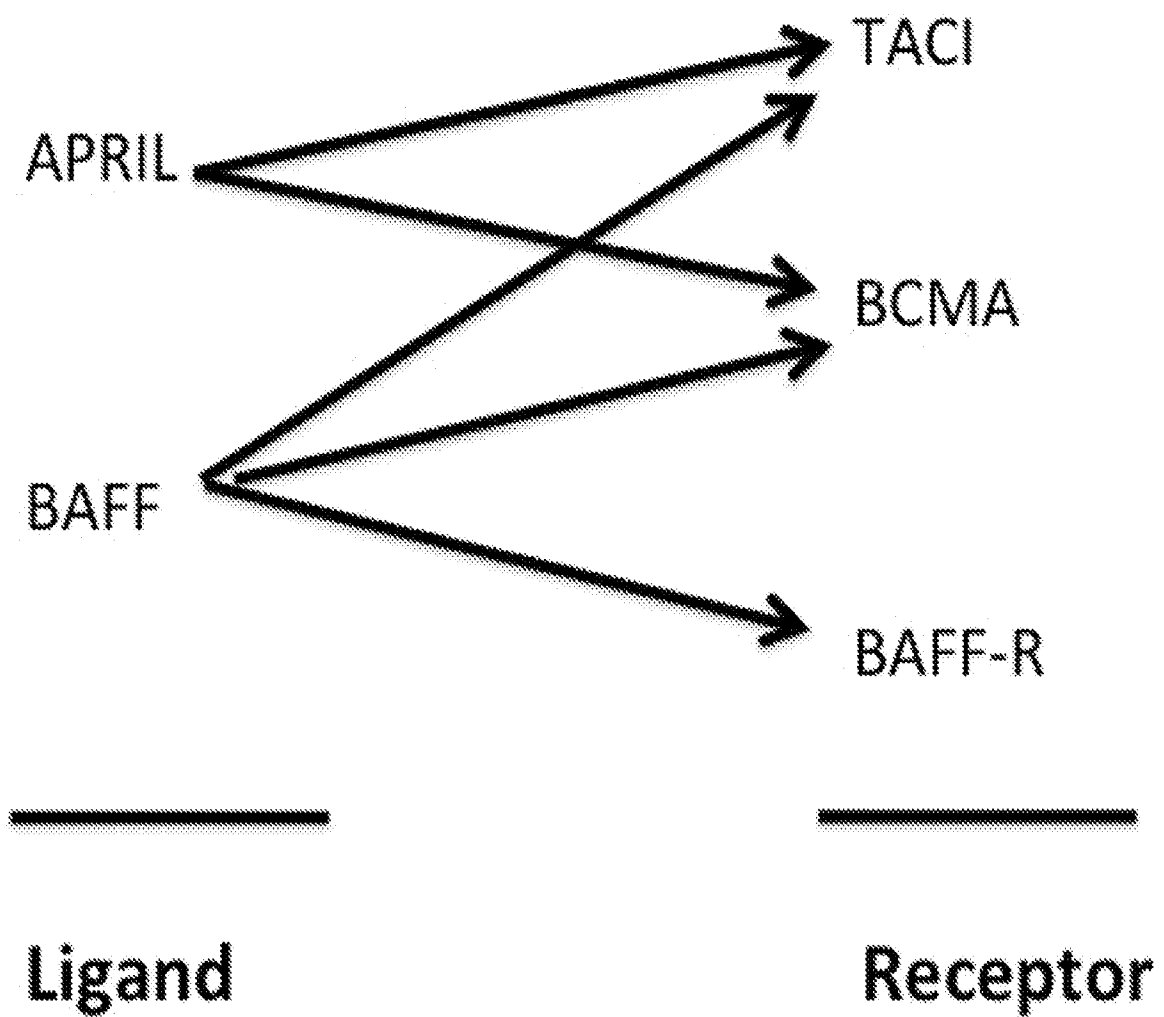




FIG. 45



2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT  
SEQUENCE LISTING

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<120> Chimeric Antigen Receptors (CARs), Compositions and Methods of Use  
<130> 2541-3 PCT  
<140> 0000000  
<141> 2016-06-24  
<150> 62/184,321  
<151> 2015-06-25  
<160> 36  
<170> PatentIn version 3.5  
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<211> 830  
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<213> Artificial Sequence  
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<223> synthetic sequence  
<400> 1

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			20					25					30		
Ala	Val	Ser	Leu	Gly	Glu	Arg	Val	Thr	Met	Asn	Cys	Lys	Ser	Ser	Gln
		35					40					45			
Ser	Leu	Leu	Tyr	Ser	Thr	Asn	Gln	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln
	50					55					60				
Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Thr
65					70					75					80
Arg	Glu	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr
				85					90					95	
Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Val	Ala	Val
			100					105					110		
Tyr	Tyr	Cys	Gln	Gln	Tyr	Tyr	Ser	Tyr	Arg	Thr	Phe	Gly	Gly	Gly	Thr
		115					120					125			
Lys	Leu	Glu	Ile	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly
	130					135					140				
Gly	Gly	Gly	Ser	Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Val	Val
145					150					155					160

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

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 Phe Thr Ser Tyr Val Ile His Trp Val Arg Gln Lys Pro Gly Gln Gly  
 180 185 190  
 Leu Asp Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Asp Tyr  
 195 200 205  
 Asp Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Thr Ser Thr  
 210 215 220  
 Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala  
 225 230 235 240  
 Val Tyr Tyr Cys Ala Arg Glu Lys Asp Asn Tyr Ala Thr Gly Ala Trp  
 245 250 255  
 Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Thr Thr  
 260 265 270  
 Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln  
 275 280 285  
 Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala  
 290 295 300  
 Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala  
 305 310 315 320  
 Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr  
 325 330 335  
 Leu Tyr Cys Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met  
 340 345 350  
 Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro  
 355 360 365  
 Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly Arg  
 370 375 380  
 Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln  
 385 390 395 400  
 Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu  
 405 410 415  
 Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala  
 420 425 430

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu  
435 440 445

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp  
450 455 460

Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly  
465 470 475 480

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu  
485 490 495

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu  
500 505 510

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His  
515 520 525

Met Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr Asn Phe Ser Leu  
530 535 540

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Tyr Arg  
545 550 555 560

Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu Val Thr Asn  
565 570 575

Ser Gly Ile His Val Phe Ile Leu Gly Cys Phe Ser Ala Gly Leu Pro  
580 585 590

Lys Thr Glu Ala Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile  
595 600 605

Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu  
610 615 620

Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu  
625 630 635 640

Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His  
645 650 655

Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser  
660 665 670

Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu  
675 680 685

Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln  
690 695 700

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

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705 710 715 720

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Leu Gl n  
725 730 735

Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala Leu  
740 745 750

Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr Cys  
755 760 765

Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr  
770 775 780

Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg  
785 790 795 800

Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr  
805 810 815

Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg  
820 825 830

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agagggtgac catgaactgc aaaagcagcc agtccctgct gtactccacc aaccagaaga 180  
actacctggc ttggtatcaa cagaagcccg gacagagccc caagctgctg atctattggg 240  
ccagcactag ggaaagcggc gtgcccata ggttcagcgg cagcgggagc ggcacagact 300  
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His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu  
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln  
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr  
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro  
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile  
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly  
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr  
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu  
130 135 140

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

Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly  
165 170 175

Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly  
180 185 190

Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser  
195 200 205

Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys  
210 215 220

Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys  
225 230 235 240

His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly  
245 250 255

Thr Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro  
260 265 270

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Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu  
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp  
290 295 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly  
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg  
325 330 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln  
340 345 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu  
355 360 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala  
370 375 380

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu  
385 390 395 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp  
405 410 415

Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly  
420 425 430

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu  
435 440 445

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu  
450 455 460

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His  
465 470 475 480

Met Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr Asn Phe Ser Leu  
485 490 495

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala Leu  
500 505 510

Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu His Ala Ala  
515 520 525

Arg Pro Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser  
530 535 540



2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser  
545 550 555 560

Tyr Ile His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp  
565 570 575

Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser  
580 585 590

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu  
595 600 605

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro  
610 615 620

Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly  
625 630 635 640

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Gln  
645 650 655

Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Met Ser  
660 665 670

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Trp Val  
675 680 685

Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile Gly Ala Ile Tyr Pro  
690 695 700

Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr  
705 710 715 720

Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser  
725 730 735

Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Ser Thr Tyr  
740 745 750

Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly Ala Gly Thr Thr Val  
755 760 765

Thr Val Ser Ala Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala  
770 775 780

Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg  
785 790 795 800

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

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu  
820 825 830

Leu Ser Leu Val Ile Thr Leu Tyr Cys Arg Ser Lys Arg Ser Arg Leu  
835 840 845

Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr  
850 855 860

Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr  
865 870 875 880

Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln  
885 890 895

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu  
900 905 910

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly  
915 920 925

Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu  
930 935 940

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys  
945 950 955 960

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu  
965 970 975

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980 985 990

Pro Pro Arg  
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 35 40 45

Ser Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp  
 50 55 60

Gly Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly  
 65 70 75 80

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu  
 85 90 95

Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln  
 100 105 110

Gln Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu  
 115 120 125

Ile Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly

130

135

140

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

Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp  
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Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp  
180 185 190

Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu  
195 200 205

Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe  
210 215 220

Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys  
225 230 235 240

Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly  
245 250 255

Gln Gly Thr Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg  
260 265 270

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg  
275 280 285

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly  
290 295 300

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr  
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Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg  
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Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu  
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370 375 380

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu  
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Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly  
Page 12

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gl n Gl u  
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Gl u Ile Gly Met Lys Gly Gl u Arg Arg Arg Gly Lys Gly Hi s Asp Gly  
450 455 460

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Leu Leu Lys Gl n Ala Gly Asp Val Gl u Gl u Asn Pro Gly Pro Met Ala  
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Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu Hi s Ala  
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Ala Arg Pro Asp Ile Gl n Met Thr Gl n Ser Pro Ser Ser Leu Ser Ala  
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Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gl n Ser Ile  
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Val Hi s Ser Val Gly Asn Thr Phe Leu Gl u Trp Tyr Gl n Gl n Lys Pro  
565 570 575

Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser  
580 585 590

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
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Leu Thr Ile Ser Ser Leu Gl n Pro Gl u Asp Phe Ala Thr Tyr Tyr Cys  
610 615 620

Phe Gl n Gly Ser Gl n Phe Pro Tyr Thr Phe Gly Gl n Gly Thr Lys Val  
625 630 635 640

Gl u Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly  
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660 665 670

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Gl u Phe Ser

675

680

685

Arg Ser Trp Met Asn Trp Val Arg Gl n Al a Pro Gly Lys Gly Leu Gl u  
690 695 700

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Lys Phe Lys Gly Arg Phe Thr Ile Ser Al a Asp Thr Ser Lys Asn Thr  
725 730 735

Al a Tyr Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr  
740 745 750

Tyr Cys Al a Arg Asp Gly Ser Ser Trp Asp Trp Tyr Phe Asp Val Trp  
755 760 765

Gly Gl n Gly Thr Leu Val Thr Val Ser Ser Thr Thr Thr Pro Al a Pro  
770 775 780

Arg Pro Pro Thr Pro Al a Pro Thr Ile Al a Ser Gl n Pro Leu Ser Leu  
785 790 795 800

Arg Pro Gl u Al a Cys Arg Pro Al a Al a Gly Gly Al a Val Hi s Thr Arg  
805 810 815

Gly Leu Asp Phe Al a Cys Asp Ile Tyr Ile Trp Al a Pro Leu Al a Gly  
820 825 830

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys  
835 840 845

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gl n Pro Phe Met Arg  
850 855 860

Pro Val Gl n Thr Thr Gl n Gl u Gl u Asp Gly Cys Ser Cys Arg Phe Pro  
865 870 875 880

Gl u Gl u Gl u Gl u Gly Gly Cys Gl u Leu Arg Val Lys Phe Ser Arg Ser  
885 890 895

Al a Asp Al a Pro Al a Tyr Gl n Gl n Gly Gl n Asn Gl n Leu Tyr Asn Gl u  
900 905 910

Leu Asn Leu Gly Arg Arg Gl u Gl u Tyr Asp Val Leu Asp Lys Arg Arg  
915 920 925

Gly Arg Asp Pro Gl u Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gl n  
930 935 940

Gl u Gly Leu Tyr Asn Gl u Leu Gl n Lys Asp Lys Met Al a Gl u Al a Tyr  
Page 14

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<220>  
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tggagatcaa	agggggggga	ggaagcggcg	gaggagggag	cggaggcggg	ggatccgaag	1980
tgcaactggg	cgaatccgga	ggggggctgg	tccagccttg	agggtccttg	agactgagct	2040
gcgccgcaag	cggctacgag	ttctccaggt	cctggatgaa	ctgggtgagg	caggccccag	2100
gaaaagggct	ggaatgggtg	ggcaggatct	accctggcga	cggcgatacc	aactactccg	2160
gaaagttaa	gggcagggtc	actatcagcg	ccgacactag	caagaatacc	gcctacctgc	2220
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gctgggattg	gtacttcgac	gtgtggggcc	agggcactct	ggtgactgtg	agcagcacca	2340
cgacgccagc	gccgcgacca	ccaacaccgg	cgcccaccat	cgcgtcgcag	cccctgtccc	2400
tgcgcccaga	ggcgtgccgg	ccagcggcgg	ggggcgcagt	gcacacgagg	gggctggact	2460
tcgcctgtga	tatctacatc	tgggcgccct	tggccgggac	ttgtggggtc	cttctcctgt	2520
cactggttat	caccctttac	tgcaaacggg	gcagaaagaa	actcctgtat	atattcaaac	2580
aaccatttat	gagaccagta	caaactactc	aagaggaaga	tggtgttagc	tgccgatttc	2640
cagaagaaga	agaaggagga	tgtgaactga	gagtgaagtt	cagcaggagc	gcagacgccc	2700
ccgcgtacca	gcaggggccag	aaccagctct	ataacgagct	caatctagga	cgaagagagg	2760
agtacgatgt	tttgacaag	agacgtggcc	gggaccctga	gatgggggga	aagccgagaa	2820
ggaagaaccc	tcaggaaggc	ctgtacaatg	aactgcagaa	agataagatg	gcggaggcct	2880
acagtgagat	tgggatgaaa	ggcgagcgcc	ggaggggcaa	ggggcacgat	ggcctttacc	2940
aggggtctcag	tacagccacc	aaggacacct	acgacgccct	tcacatgcag	gccctgcccc	3000
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<210> 7  
 <211> 990  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 7

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

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 Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu  
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln  
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr  
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro  
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile  
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly  
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr  
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu  
130 135 140

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

Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly  
165 170 175

Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly  
180 185 190

Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser  
195 200 205

Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys  
210 215 220

Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys  
225 230 235 240

His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly  
245 250 255

Thr Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro  
260 265 270

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu  
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp  
290 295 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly  
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg  
325 330 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln  
340 345 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu  
355 360 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala  
370 375 380

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu  
385 390 395 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp  
405 410 415

Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly  
420 425 430

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu  
435 440 445

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu  
450 455 460

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His  
465 470 475 480

Met Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr Asn Phe Ser Leu  
485 490 495

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala Leu  
500 505 510

Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu His Ala Ala  
515 520 525

Arg Pro Asp Val Gln Ile Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser  
530 535 540

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Pro Gly Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser  
545 550 555 560

Lys Asp Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu  
565 570 575

Leu Ile Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe  
580 585 590

Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu  
595 600 605

Glu Pro Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Lys Tyr  
610 615 620

Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly  
625 630 635 640

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu  
645 650 655

Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Lys Leu  
660 665 670

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Asn Trp  
675 680 685

Val Lys Gln Arg Pro Asp Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp  
690 695 700

Pro Tyr Asp Ser Glu Thr His Tyr Asn Gln Lys Phe Lys Asp Lys Ala  
705 710 715 720

Ile Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser  
725 730 735

Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Gly Asn  
740 745 750

Trp Asp Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Thr  
755 760 765

Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser  
770 775 780

Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly  
785 790 795 800

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

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile  
820 825 830

Thr Leu Tyr Cys Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr  
835 840 845

Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln  
850 855 860

Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys  
865 870 875 880

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln  
885 890 895

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu  
900 905 910

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg  
915 920 925

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys  
930 935 940

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg  
945 950 955 960

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys  
965 970 975

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
980 985 990

<210> 8  
<211> 2989  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

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acagagtcac catcagttgc agggcaagtc aggacattag taaatatitaa aattgggtatc 180  
agcagaaacc agatggaact gttaaactcc tgatctacca tacatcaaga ttacactcag 240  
gagtcccatc aaggttcagt ggcagtgggt ctggaacaga ttattctctc accattagca 300  
acctggagca agaagatatt gccacttact tttgccaaca gggtaatacg cttccgtaca 360  
cgttcggagg ggggaccaag ctggagatca caggtggcgg tggctcgggc ggtggtgggt 420

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT

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gctggattcg	ccagcctcca	cgaaagggtc	tggagtggct	gggagtaata	tggggtagt	600
aaaccacata	ctataattca	gctctcaa	ccagactgac	catcatcaag	gacaactcca	660
agagccaagt	tttcttaaaa	atgaacagtc	tgcaaactga	tgacacagcc	atttactact	720
gtgcaaaca	ttattactac	ggtggtagct	atgctatgga	ctactggggc	caaggaacct	780
cagtcaccgt	ctcctcaacc	acgacgccag	cgccgcgacc	accaacaccg	gcgcccacca	840
tcgcgtcgca	gcccctgtcc	ctgcgcccag	aggcgtgccg	gccagcggcg	gggggcgcag	900
tgacacagag	ggggctggac	ttcgctgtg	atatctacat	ctgggcgccc	ttggccggga	960
cttgtgggg	ccttctcctg	tcactggtta	tcacccttta	ctgcaaacgg	ggcagaaaga	1020
aactcctgta	tatattcaaa	caaccattta	tgagaccagt	acaaactact	caagaggaag	1080
atggctgtag	ctgccgattt	ccagaagaag	aagaaggagg	atgtgaactg	agagtgaagt	1140
tcagcaggag	cgagacgcc	cccgcgtacc	agcagggcc	gaaccagctc	tataacgagc	1200
tcaatctagg	acgaagagag	gagtacgatg	ttttggacaa	gagacgtggc	cgggaccctg	1260
agatggggg	aaagccgcag	agaaggaaga	accctcagga	aggcctgtac	aatgaactgc	1320
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gcaaggggca	cgatggcctt	taccagggtc	tcagtacagc	caccaaggac	acctacgagc	1440
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agcaggccgg	cgacgtggag	gagaaccccg	gccccatggc	cttaccagt	accgccttgc	1560
tcctgccgct	ggccttgctg	ctccacgccg	ccaggccgga	cgtgcagatc	accagagacc	1620
ccagctacct	ggccgccagc	cccggcgaga	ccatcaccat	caactgcaga	gccagcaaga	1680
gcatcagcaa	ggacctggcc	tggtagcagg	agaagcccgg	caagaccaac	aagctgctga	1740
tctacagcgg	cagcaccctg	cagagcggca	tccccagcag	attcagcggc	agcggcagcg	1800
gcaccgactt	caccctgacc	atcagcagcc	tggagcccga	ggacttcgcc	atgtactact	1860
gccagcagca	caacaagtac	ccctacacct	tcggcggcgg	caccaagctg	gagatcaagg	1920
gagggggggg	atccggggga	ggaggctccg	gcggaggcgg	aagccagggtg	cagctgcagc	1980
agcccggcgc	cgagctgggtg	agaccggcg	ccagcgtgaa	gctgagctgc	aaggccagcg	2040
gctacacctt	caccagctac	tggatgaact	gggtgaagca	gagacccgac	cagggcctgg	2100
agtggatcgg	cagaatcgac	ccctacgaca	gcgagaccca	ctacaaccag	aagttcaagg	2160
acaaggccat	cctgaccgtg	gacaagagca	gcagcaccgc	ctacatgcag	ctgagcagcc	2220
tgaccagcga	ggacagcgcc	gtgtactact	gcgccagagg	caactgggac	gactactggg	2280
gccagggcac	caccctgacc	gtgagcagca	ccacgacgcc	agcgcgcgga	ccaccaacac	2340
cggcgcccac	catcgctcg	cagcccctgt	ccctgcgccc	agaggcgtgc	cggccagcgg	2400
cggggggcgc	agtgcacacg	agggggctgg	acttcgcctg	tgatatctac	atctgggcgc	2460

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT

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ccttggccgg gacttgtggg gtccttctcc tgtcactggt taccacccct tactgcagga 2520
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gagtgaagtt cagcaggagc gcagacgccc ccgcgtacca gcagggccag aaccagctct 2700
ataacgagct caatctagga cgaagagagg agtacgatgt tttggacaag agacgtggcc 2760
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gccggagggg caaggggcac gatggccttt accaggtctt cagtacagcc accaaggaca 2940
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 <211> 1066  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 9

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu  
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His Ala Ala Arg Pro Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met  
 20 25 30

Ser Ala Ser Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser  
 35 40 45

Ser Ile Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro  
 50 55 60

Lys Leu Trp Ile Tyr Thr Thr Ser Asn Leu Ala Ser Gly Val Pro Ala  
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser  
 85 90 95

Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Ser  
 100 105 110

Thr Tyr Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu Leu Lys Gly  
 115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val  
 130 135 140

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

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Arg Met  
 165 170 175  
 His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr  
 180 185 190  
 Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn Gln Lys Phe Lys Asp  
 195 200 205  
 Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln  
 210 215 220  
 Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg  
 225 230 235 240  
 Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val  
 245 250 255  
 Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr  
 260 265 270  
 Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala  
 275 280 285  
 Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile  
 290 295 300  
 Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser  
 305 310 315 320  
 Leu Val Ile Thr Leu Tyr Cys Arg Ser Lys Arg Ser Arg Leu Leu His  
 325 330 335  
 Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys  
 340 345 350  
 His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser  
 355 360 365  
 Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met  
 370 375 380  
 Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe  
 385 390 395 400  
 Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg  
 405 410 415  
 Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn  
 420 425 430



2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg  
 435 440 445  
 Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro  
 450 455 460  
 Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala  
 465 470 475 480  
 Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His  
 485 490 495  
 Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp  
 500 505 510  
 Ala Leu His Met Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr Asn  
 515 520 525  
 Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro  
 530 535 540  
 Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu  
 545 550 555 560  
 His Ala Ala Arg Pro Asp Val Gln Ile Thr Gln Ser Pro Ser Tyr Leu  
 565 570 575  
 Ala Ala Ser Pro Gly Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys  
 580 585 590  
 Ser Ile Ser Lys Asp Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr  
 595 600 605  
 Asn Lys Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro  
 610 615 620  
 Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 625 630 635 640  
 Ser Ser Leu Glu Pro Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His  
 645 650 655  
 Asn Lys Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 660 665 670  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln  
 675 680 685  
 Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ala Ser  
 690 695 700

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp  
705 710 715 720

Met Asn Trp Val Lys Gln Arg Pro Asp Gln Gly Leu Glu Trp Ile Gly  
725 730 735

Arg Ile Asp Pro Tyr Asp Ser Glu Thr His Tyr Asn Gln Lys Phe Lys  
740 745 750

Asp Lys Ala Ile Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met  
755 760 765

Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala  
770 775 780

Arg Gly Asn Trp Asp Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val  
785 790 795 800

Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr  
805 810 815

Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala  
820 825 830

Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile  
835 840 845

Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser  
850 855 860

Leu Val Ile Thr Leu Tyr Cys Arg Ser Lys Arg Ser Arg Leu Leu His  
865 870 875 880

Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys  
885 890 895

His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser  
900 905 910

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met  
915 920 925

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe  
930 935 940

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg  
945 950 955 960

Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn  
965 970 975

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg  
980 985 990

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro  
995 1000 1005

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu  
1010 1015 1020

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys  
1025 1030 1035

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp  
1040 1045 1050

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
1055 1060 1065

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<211> 3217  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 10  
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2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT

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ccccaccacg cgacttcgca gcctatcgct ccaaacgggg cagaaagaaa ctctgtata	1140
tattcaaaaca accatttatg agaccagtac aaactactca agaggaagat ggctgtagct	1200
gccgatttcc agaagaagaa gaaggaggat gtgaactgag agtgaagttc agcaggagcg	1260
cagacgcccc cgcggtacaag cagggccaga accagctcta taacgagctc aatctaggac	1320
gaagagagga gtacgatgtt ttggacaaga gacgtggccg ggaccctgag atggggggaa	1380
agccgagaag gaagaaccct caggaaggcc tgtacaatga actgcagaaa gataagatgg	1440
cggaggccta cagtgaattt gggatgaaag gcgagcgccg gaggggcaag gggcacgatg	1500
gcctttacca ggggtctcagt acagccacca aggacaccta cgacgccctt cacatgcagg	1560
ccctgcccc tcgcggaagc ggagctacta acttcagcct gctgaagcag gctggagacg	1620
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tgaccatcag cagcctggag cccgaggact tcgccatgta ctactgccag cagcacaaca	1980
agtacccta caccttcggc ggccggcacca agctggagat caagggaggg gggggatccg	2040
ggggaggagg ctccggcgga ggccggaagcc aggtgcagct gcagcagccc ggcgccgagc	2100
tggtagagacc cggcgccagc gtgaagctga gctgcaaggc cagcggctac accttcacca	2160
gctactggat gaactgggtg aagcagagac ccgaccaggg cctggagtgg atcggcagaa	2220
tcgacccta cgacagcgag acccactaca accagaagtt caaggacaag gccatcctga	2280
ccgtggacaa gagcagcagc accgcctaca tgcagctgag cagcctgacc agcaggaca	2340
gcgccgtgta ctactgcgc agaggcaact gggacgacta ctggggccag ggcaccaccc	2400
tgaccgtgag cagcaccacg acgccagcgc cgcgaccacc aacaccggcg cccaccatcg	2460
cgtcgagcc cctgtccctg cgcccagagg cgtgccggcc agcggcgggg ggcgcagtgc	2520
acacgagggg gctggacttc gcctgtgata tctacatctg ggcgcccttg gccgggactt	2580
gtggggtcct tctcctgtca ctggttatca ccctttactg caggagtaag aggagcaggc	2640
tcctgcacag tgactacatg aacatgactc cccgccgcc cgggcccacc cgcaagcatt	2700
accagcccta tgccccacca cgcgacttcg cagcctatcg ctccaaacgg ggcagaaaga	2760
aactcctgta tatattcaaa caaccattta tgagaccagt acaaactact caagaggaag	2820
atggctgtag ctgccgattt ccagaagaag aagaaggagg atgtgaactg agagtgaagt	2880
tcagcaggag cgcagacgcc cccgcgtaca agcagggccga gaaccagctc tataacgagc	2940
tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc cgggaccctg	3000
agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat gaactgcaga	3060

aagataagat ggcgagggcc tacagtgaga ttgggatgaa aggcgagcgc cggaggggca 3120  
 aggggcacga tggcctttac caggggtctca gtacagccac caaggacacc tacgacgccc 3180  
 ttcacatgca ggccctgccc cctcgctaag tttaaac 3217

<210> 11  
 <211> 985  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 11

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 Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met  
 20 25 30

Ser Ala Ser Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser  
 35 40 45

Ser Ile Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro  
 50 55 60

Lys Leu Trp Ile Tyr Thr Thr Ser Asn Leu Ala Ser Gly Val Pro Ala  
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser  
 85 90 95

Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Ser  
 100 105 110

Thr Tyr Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu Leu Lys Gly  
 115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val  
 130 135 140

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

Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Arg Met  
 165 170 175

His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr  
 180 185 190

Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn Gln Lys Phe Lys Asp  
 195 200 205

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Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln  
 210 215 220  
 Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg  
 225 230 235 240  
 Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val  
 245 250 255  
 Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr  
 260 265 270  
 Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala  
 275 280 285  
 Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile  
 290 295 300  
 Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser  
 305 310 315 320  
 Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr  
 325 330 335  
 Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu  
 340 345 350  
 Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu  
 355 360 365  
 Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln  
 370 375 380  
 Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu  
 385 390 395 400  
 Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly  
 405 410 415  
 Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu  
 420 425 430  
 Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly  
 435 440 445  
 Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser  
 450 455 460  
 Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro  
 465 470 475 480

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Pro Arg Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly  
485 490 495

Asp Val Glu Glu Asn Pro Gly Pro Met Ala Leu Pro Val Thr Ala Leu  
500 505 510

Leu Leu Pro Leu Ala Leu Leu Leu His Ala Ala Arg Pro Asp Val Gln  
515 520 525

Ile Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly Glu Thr Ile  
530 535 540

Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Asp Leu Ala Trp  
545 550 555 560

Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile Tyr Ser Gly  
565 570 575

Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser  
580 585 590

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe  
595 600 605

Ala Met Tyr Tyr Cys Gln Gln His Asn Lys Tyr Pro Tyr Thr Phe Gly  
610 615 620

Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Ser Gly Gly Gly  
625 630 635 640

Gly Ser Gly Gly Gly Ser Gln Val Gln Leu Gln Gln Pro Gly Ala  
645 650 655

Glu Leu Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser  
660 665 670

Gly Tyr Thr Phe Thr Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro  
675 680 685

Asp Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Tyr Asp Ser Glu  
690 695 700

Thr His Tyr Asn Gln Lys Phe Lys Asp Lys Ala Ile Leu Thr Val Asp  
705 710 715 720

Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu  
725 730 735

Asp Ser Ala Val Tyr Tyr Cys Ala Arg Gly Asn Trp Asp Asp Tyr Trp  
740 745 750

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Thr Thr Thr Pro Ala Pro  
755 760 765

Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu  
770 775 780

Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg  
785 790 795 800

Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly  
805 810 815

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Arg  
820 825 830

Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro  
835 840 845

Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro  
850 855 860

Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala  
865 870 875 880

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu  
885 890 895

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly  
900 905 910

Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln  
915 920 925

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr  
930 935 940

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp  
945 950 955 960

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala  
965 970 975

Leu His Met Gln Ala Leu Pro Pro Arg  
980 985

<210> 12  
<211> 2974  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic sequence



2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

<400> 12  
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agaaggtgac catcacctgc agcgccagca gcagcatcag ctacatgcac tggttccagc 180  
agaagcccgg caccagcccc aagctgtgga tctacaccac cagcaacctg gccagcggcg 240  
tgcccgccag attcagcggc agcggcagcg gcaccagcta cagcctgacc atcagcagaa 300  
tggaggccga ggacgccgcc acctactact gccaccagag aagcacctac cccctgacct 360  
tcggcagcgg caccaagctg gagctgaagg gagggggggg atccggggga ggaggctccg 420  
gcggaggcgg aagccaggtg cagctgcagc agagcggcgc cgagctggcc aagccccggcg 480  
ccagcgtgaa gatgagctgc aaggccagcg gctacacctt caccagctac agaatgcaact 540  
gggtgaagca gagacccggc cagggcctgg agtggatcgg ctacatcaac cccagcaccg 600  
gctacaccga gtacaaccag aagttcaagg acaaggccac cctgaccgcc gacaagagca 660  
gcagcaccgc ctacatgcag ctgagcagcc tgaccttcga ggacagcgcc gtgtactact 720  
gcgccagagg cggcggcgtg ttcgactact ggggccaggg caccaccctg accgtgagca 780  
gcaccacgac gccagcgccg cgaccaccaa caccggcgcc caccatcgcg tcgcagcccc 840  
tgtccctgcg cccagaggcg tgccggccag cggcgggggg cgagtgacac acgagggggc 900  
tggacttcgc ctgtgatata tacatctggg cgcccttggc cgggacttgt ggggtccttc 960  
tcctgtcact ggttatcacc ctttactgca aacggggcag aaagaaactc ctgtatatat 1020  
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gatttccaga agaagaagaa ggaggatgtg aactgagagt gaagttcagc aggagcgag 1140  
acgccccgcg gtaccagcag ggccagaacc agctctataa cgagctcaat ctaggacgaa 1200  
gagaggagta cgatgttttg gacaagagac gtggccggga ccctgagatg gggggaaagc 1260  
cgagagaag gaagaacctt caggaaggcc tgtacaatga actgcagaaa gataagatgg 1320  
cggaggccta cagtgaattt gggatgaaag gcgagcgccg gaggggcaag gggcacgatg 1380  
gcctttacca gggctcagat acagccacca aggacaccta cgacgccctt cacatgcagg 1440  
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ccagccccgg cgagaccatc accatcaact gcagagccag caagagcatc agcaaggacc 1680  
tggcctggta ccaggagaag cccggcaaga ccaacaagct gctgatctac agcggcagca 1740  
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agtacccta caccttcggc ggcggcacca agctggagat caagggaggg gggggatccg 1920  
ggggaggagg ctccggcgga ggcggaagcc aggtgcagct gcagcagccc ggcgccgagc 1980

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT

tggtgagacc cggcgccagc gtgaagctga gctgcaaggc cagcggctac accttcacca	2040
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tcgacccta cgacagcgag acccactaca accagaagtt caaggacaag gccatcctga	2160
ccgtggacaa gagcagcagc accgcctaca tgcagctgag cagcctgacc agcgaggaca	2220
gcgccgtgta ctactgcgcc agaggcaact gggacgacta ctggggccag ggcaccaccc	2280
tgaccgtgag cagcaccacg acgccagcgc cgcgaccacc aacaccggcg cccaccatcg	2340
cgtcgcagcc cctgtccctg cgcccagagg cgtgccggcc agcggcgggg ggcgcagtgc	2400
acacgagggg gctggacttc gcctgtgata tctacatctg ggcgcccttg gccgggactt	2460
gtgggggtcct tctcctgtca ctggttatca ccctttactg caggagtaag aggagcaggc	2520
tcctgcacag tgactacatg aacatgactc cccgccgccc cggggcccacc cgcaagcatt	2580
accagcccta tgccccacca cgcgacttcg cagcctatcg ctccagagtg aagttcagca	2640
ggagcgcaga cgcccccgcg taccagcagg gccagaacca gctctataac gagtcaatc	2700
taggacgaag agaggagtac gatgttttg acaagagacg tggccgggac cctgagatgg	2760
ggggaaagcc gcagagaagg aagaaccctc aggaaggcct gtacaatgaa ctgcagaaag	2820
ataagatggc ggaggcctac agtgagattg ggatgaaagg cgagcgccgg aggggcaagg	2880
ggcacgatgg cctttaccag ggtctcagta cagccaccaa ggacacctac gacgcccttc	2940
acatgcaggc cctgccccct cgctaagttt aaac	2974

<210> 13  
 <211> 546  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 13

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	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser
			20					25					30		

Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser
		35					40					45			

Lys	Ser	Val	Ser	Thr	Ser	Gly	Tyr	Ser	Tyr	Leu	His	Trp	Tyr	Gln	Gln
	50					55					60				

Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Leu	Ala	Ser	Asn	Leu
65					70					75					80

Glu	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp
				85					90					95	

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Phe Thr Leu Asn 100 Ile His Pro Val Glu 105 Glu Glu Asp Ala 110 Ala Thr Tyr  
 Tyr Cys Glu 115 His Ser Arg Glu 120 Leu Pro Phe Thr Phe Gly 125 Ser Gly Thr  
 Lys Leu Glu 130 Ile Lys Lys 135 Ile Ser Gly Gly Gly Gly 140 Ser Gly Gly Gly  
 Gly 145 Ser Gly Gly Gly Gly 150 Ser Gly Gly Gly Gly 155 Ser Gly Gly Gly Gly 160  
 Ser Glu Val 165 Glu Val Glu Ser Gly 170 Gly Gly Leu Val Glu 175 Pro Gly  
 Gly Ser Leu Lys 180 Leu Ser Cys Ala 185 Ala Ser Gly Phe Asp Phe 190 Ser Arg  
 Tyr Trp Met 195 Ser Trp Val Arg Glu 200 Ala Pro Gly Lys Gly 205 Leu Glu Trp  
 Ile Gly 210 Glu Ile Asn Pro Thr 215 Ser Ser Thr Ile Asn 220 Phe Thr Pro Ser  
 Leu Lys Asp Lys Val 230 Phe Ile Ser Arg Asp Asn 235 Ala Lys Asn Thr Leu 240  
 Tyr Leu Glu Met 245 Ser Lys Val Arg Ser Glu 250 Asp Thr Ala Leu Tyr 255 Tyr  
 Cys Ala Arg Gly 260 Asn Tyr Tyr Arg Tyr 265 Gly Asp Ala Met Asp 270 Tyr Trp  
 Gly Glu Gly 275 Thr Ser Val Thr Val 280 Ser Thr Thr Thr Pro 285 Ala Pro Arg  
 Pro Pro Thr 290 Pro Ala Pro Thr 295 Ile Ala Ser Glu Pro 300 Leu Ser Leu Arg  
 Pro 305 Glu Ala Cys Arg Pro 310 Ala Ala Gly Gly Ala 315 Val His Thr Arg Gly 320  
 Leu Asp Phe Ala Cys 325 Asp Ile Tyr Ile Trp 330 Ala Pro Leu Ala Gly 335 Thr  
 Cys Gly Val 340 Leu Leu Leu Ser Leu Val 345 Ile Thr Leu Tyr Cys 350 Arg Ser  
 Lys Arg Ser 355 Arg Leu Leu His Ser 360 Asp Tyr Met Asn Met 365 Thr Pro Arg

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg  
370 375 380

Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly Arg Lys Lys Leu Leu Tyr  
385 390 395 400

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu  
405 410 415

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu  
420 425 430

Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln  
435 440 445

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu  
450 455 460

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly  
465 470 475 480

Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu  
485 490 495

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly  
500 505 510

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser  
515 520 525

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro  
530 535 540

Pro Arg  
545

<210> 14  
<211> 1657  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 14	
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ccgccaggcc gagcgacatc gtgctgacct agagccccgc cagcctggcc gtgagcctgg	120
gccagagagc caccatcagc tgcagagcca gcaagagcgt gagcaccagc ggctacagct	180
acctgcactg gtaccagcag aagcccggcc agcccccaa gctgctgata tacctggcca	240
gcaacctgga gagcggcgtg cccgccagat tcagcggcag cggcagcggc accgacttca	300

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT

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ccctgaacat ccaccccggtg gaggaggagg acgccgccac ctactactgc cagcacagca 360
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gcggcggcag cggcggcggc ggcagcggcg gcggcggcag cggcggcggc ggcagcggcg 480
gcggcggcag ccaggtgcag ctggtggaga gcggcggcg cctggtgcag cccggcggca 540
gcctgaagct gagctgcgc gccagcggct tcgacttcag cagatactgg atgagctggg 600
tgagacaggc ccccggaag ggcctggagt ggatcggcga gatcaacccc accagcagca 660
ccatcaactt cacccccagc ctgaaggaca aggtgttcat cagcagagac aacgccaaga 720
acaccctgta cctgcagatg agcaaggtga gaagcgagga caccgccctg tactactgcg 780
ccagaggcaa ctactacaga tacggcgacg ccatggacta ctggggccag ggcaccagcg 840
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cgagcccct gtccctgcgc ccagaggcgt gccggccagc ggcggggggc gcagtgcaca 960
cgagggggct ggacttcgc tgtgatctt acatctgggc gcccttggcc gggacttgtg 1020
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tgcacagtga ctacatgaac atgactcccc gccgccccgg gccaccgcg aagcattacc 1140
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atctaggacg aagagaggag tacgatgttt tggacaagag acgtggccgg gaccctgaga 1440
tggggggaaa gccgcagaga aggaagaacc ctcaggaagg cctgtacaat gaactgcaga 1500
aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc cggaggggca 1560
aggggcacga tggcctttac cagggctctc gtacagccac caaggacacc tacgacgccc 1620
ttcacatgca ggccctgccc cctcgctaag tttaaac 1657

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<210> 15  
 <211> 490  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 15

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

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

Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys  
 35 40 45

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Ser Val Ser Thr Ser Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys  
50 55 60

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu  
65 70 75 80

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
85 90 95

Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr  
100 105 110

Cys Gln His Ser Arg Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys  
115 120 125

Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly  
130 135 140

Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln  
145 150 155 160

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe  
165 170 175

Ser Arg Tyr Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
180 185 190

Glu Trp Ile Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Thr  
195 200 205

Pro Ser Leu Lys Asp Lys Val Phe Ile Ser Arg Asp Asn Ala Lys Asn  
210 215 220

Thr Leu Tyr Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu  
225 230 235 240

Tyr Tyr Cys Ala Arg Gly Asn Tyr Tyr Arg Tyr Gly Asp Ala Met Asp  
245 250 255

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Thr Thr Thr Pro Ala  
260 265 270

Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser  
275 280 285

Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr  
290 295 300

Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala  
305 310 315 320

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys  
325 330 335

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr  
340 345 350

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro  
355 360 365

Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser  
370 375 380

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu  
385 390 395 400

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg  
405 410 415

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro  
420 425 430

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala  
435 440 445

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His  
450 455 460

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp  
465 470 475 480

Ala Leu His Met Gln Ala Leu Pro Pro Arg  
485 490

<210> 16  
<211> 1489  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 16  
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agagggccac catcagctgc agggccagca agagcgtgag caccagcggc tacagctacc 180  
tgactggta ccagcagaag cccggccagc cccccaagct gctgatctac ctggccagca 240  
acctggagag cggcgtgccc gccaggttca gcggcagcgg cagcggcacc gacttcaccc 300  
tgaacatcca ccccgaggag gaggaggacg ccgccaccta ctactgccag cacagcaggg 360  
agctgccctt caccttcggc agcggcacca agctggagat caagggaggg gggggatccg 420

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25.TXT

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ggggaggagg ctccggcgga ggcggaagcc aggtgcagct ggtggagagc ggcggcggcc 480
tggtgcagcc cggcggcagc ctgaagctga gctgcgccgc cagcggcttc gacttcagca 540
ggtactggat gagctgggtg aggcaggccc ccggcaaggg cctggagtgg atcggcgaga 600
tcaaccccac cagcagcacc atcaacttca cccccagcct gaaggacaag gtgttcatca 660
gcagggacaa cgccaagaac accctgtacc tgcagatgag caaggtgagg agcgaggaca 720
ccgccctgta ctactgcgc aggggcaact actacaggtta cggcgacgcc atggactact 780
ggggccaggg caccagcgtg accgtgagca ccacgacgcc agcggcgga ccaccaacac 840
cggcgccac catcgcgtcg cagcccctgt ccctgcgccc agaggcgtgc cggccagcgg 900
cggggggcgc agtgcacac agggggctgg acttcgcctg tgatatctac atctgggcgc 960
ccttggccgg gacttgtggg gtccttctcc tgtcactggt taccacctt tactgcagga 1020
gtaagaggag caggctcctg cacagtgact acatgaacat gactccccgc cgccccgggc 1080
ccaccgcaa gcattaccag ccctatgccc caccacgga cttcgagcc tatcgtcca 1140
gagtgaagtt cagcaggagc gcagacgcc ccgcgtacca gcagggccag aaccagctct 1200
ataacgagct caatctagga cgaagagagg agtacgatgt tttggacaag agacgtggcc 1260
gggaccctga gatgggggga aagccgcaga gaaggaagaa ccctcaggaa ggcctgtaca 1320
atgaactgca gaaagataag atggcggagg cctacagtga gattgggatg aaaggcgagc 1380
gccggagggg caaggggcac gatggccttt accagggtct cagtacagcc accaaggaca 1440
cctacgacgc cttcacatg caggccctgc cccctcgcta agtttaaac 1489

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<210> 17  
 <211> 491  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence  
 <400> 17

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 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu  
20 25 30

Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys  
35 40 45

Ser Val Ser Thr Ser Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys  
50 55 60

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu  
65 70 75 80

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe



Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr  
100 105 110

Cys Gln His Ser Arg Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys  
115 120 125

Leu Glu Ile Lys Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly  
130 135 140

Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln  
145 150 155 160

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe  
165 170 175

Ser Arg Tyr Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
180 185 190

Glu Trp Ile Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Thr  
195 200 205

Pro Ser Leu Lys Asp Lys Val Phe Ile Ser Arg Asp Asn Ala Lys Asn  
210 215 220

Thr Leu Tyr Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu  
225 230 235 240

Tyr Tyr Cys Ala Arg Gly Asn Tyr Tyr Arg Tyr Gly Asp Ala Met Asp  
245 250 255

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Thr Thr Thr Pro Ala  
260 265 270

Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser  
275 280 285

Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr  
290 295 300

Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala  
305 310 315 320

Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys  
325 330 335

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met  
340 345 350

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe

355

360

365

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg  
 370 375 380

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn  
 385 390 395 400

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg  
 405 410 415

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn  
 420 425 430

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu  
 435 440 445

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly  
 450 455 460

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr  
 465 470 475 480

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
 485 490

<210> 18  
 <211> 1492  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 18  
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 ccgccaggcc ggacatcgtg ctgaccaga gccccgccag cctggccgtg agcctgggcc 120  
 agagggccac catcagctgc agggccagca agagcgtgag caccagcggc tacagctacc 180  
 tgcactggta ccagcagaag cccggccagc cccccaagct gctgatctac ctggccagca 240  
 acctggagag cggcgtgccc gccaggttca gcggcagcgg cagcggcacc gacttcaccc 300  
 tgaacatcca ccccgaggag gaggaggacg ccgccaccta ctactgccag cacagcaggg 360  
 agctgccctt caccttcggc agcggcacca agctggagat caagggaggg gggggatccg 420  
 ggggaggagg ctccggcgga ggcggaagcc aggtgcagct ggtggagagc ggcggcggcc 480  
 tgggtcagcc cggcggcagc ctgaagctga gctgcgccgc cagcggcttc gacttcagca 540  
 ggtactggat gagctgggtg aggcaggccc ccggcaaggg cctggagtgg atcggcgaga 600  
 tcaacccac cagcagcacc atcaacttca cccccagcct gaaggacaag gtgttcatca 660  
 gcagggacaa cgccaagaac accctgtacc tgcagatgag caaggtgagg agcaggagaca 720

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ccgccctgta ctactgcgcc aggggcaact actacaggta cggcgacgcc atggactact 780
ggggccaggg caccagcgtg accgtgagca ccacgacgcc agcgccgcga ccaccaacac 840
cggcgcccac catcgctcg cagcccctgt ccctgcgcc agaggcgtgc cggccagcgg 900
cggggggcgc agtgcacacg agggggctgg acttcgcctg tgatatctac atctgggcgc 960
ccttggccgg gacttgtggg gtccttctcc tgtcactggt taccacctt tactgcaaac 1020
ggggcagaaa gaaactcctg tatatatcca aacaaccatt tatgagacca gtacaaacta 1080
ctcaagagga agatggctgt agctgccgat ttccagaaga agaagaagga ggatgtgaac 1140
tgagagtga gttcagcagg agcgacgacg ccccgcgta ccagcagggc cagaaccagc 1200
tctataacga gctcaatcta ggacgaagag aggagtacga tgttttggac aagagacgtg 1260
gccgggaccc tgagatgggg ggaaagccgc agagaaggaa gaaccctcag gaaggcctgt 1320
acaatgaact gcagaaagat aagatggcgg aggcctacag tgagattggg atgaaaggcg 1380
agcgccggag gggcaagggg cacgatggcc ttaccaggg tctcagtaca gccaccaagg 1440
acacctacga cgcccttcac atgcaggccc tgccccctcg ctaagtttaa ac 1492

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<210> 19  
 <211> 997  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 19

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 Asp Val Val Met Thr Gln Ser His Arg Phe Met  
 20 25 30

Ser Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Arg Ala Ser Gln  
 35 40 45

Asp Val Asn Thr Ala Val Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ser  
 50 55 60

Pro Lys Leu Leu Ile Phe Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro  
 65 70 75 80

Asp Arg Phe Thr Gly Ser Gly Ser Gly Ala Asp Phe Thr Leu Thr Ile  
 85 90 95

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His  
 100 105 110

Tyr Ser Thr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 115 120 125

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln  
 130 135 140  
 Ile Gln Leu Val Gln Ser Gly Pro Asp Leu Lys Lys Pro Gly Glu Thr  
 145 150 155 160  
 Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Phe Gly  
 165 170 175  
 Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Phe Lys Trp Met Ala  
 180 185 190  
 Trp Ile Asn Thr Thr Arg Tyr Thr Gly Glu Ser Tyr Phe Ala Asp Asp  
 195 200 205  
 Phe Lys Gly Arg Phe Ala Phe Ser Val Glu Thr Ser Ala Thr Thr Ala  
 210 215 220  
 Tyr Leu Gln Ile Asn Asn Leu Lys Thr Glu Asp Thr Ala Thr Tyr Phe  
 225 230 235 240  
 Cys Ala Arg Gly Glu Ile Tyr Tyr Gly Tyr Asp Gly Gly Phe Ala Tyr  
 245 250 255  
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Thr Thr Thr Pro Ala  
 260 265 270  
 Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser  
 275 280 285  
 Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr  
 290 295 300  
 Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala  
 305 310 315 320  
 Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys  
 325 330 335  
 Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met  
 340 345 350  
 Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe  
 355 360 365  
 Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg  
 370 375 380  
 Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn  
 385 390 395 400

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg  
 405 410 415  
 Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn  
 420 425 430  
 Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu  
 435 440 445  
 Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly  
 450 455 460  
 His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr  
 465 470 475 480  
 Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr  
 485 490 495  
 Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly  
 500 505 510  
 Pro Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu  
 515 520 525  
 His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu  
 530 535 540  
 Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln  
 545 550 555 560  
 Asp Val Gly Ile Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val  
 565 570 575  
 Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg His Thr Gly Val Pro  
 580 585 590  
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 595 600 605  
 Ser Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr  
 610 615 620  
 Ser Ser Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 625 630 635 640  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu  
 645 650 655  
 Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser  
 660 665 670

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr Trp  
675 680 685

Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly  
690 695 700

Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Ala Pro Ser Leu Lys  
705 710 715 720

Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu  
725 730 735

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
740 745 750

Arg Pro Asp Gly Asn Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr  
755 760 765

Leu Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr  
770 775 780

Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala  
785 790 795 800

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

Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val  
820 825 830

Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Arg Ser Lys Arg Ser  
835 840 845

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly  
850 855 860

Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala  
865 870 875 880

Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala  
885 890 895

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg  
900 905 910

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu  
915 920 925

Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr  
930 935 940

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly  
945 950 955 960

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln  
965 970 975

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln  
980 985 990

Ala Leu Pro Pro Arg  
995

<210> 20  
<211> 2994  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 20  
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ccggatgtgg tgatgacca gagccatcgc tttatgagca ccagcgtggg cgatcgcgtg 120  
agcattacct gccgcgcgag ccaggatgtg aacaccgcgg tgagctggta tcagcagaaa 180  
ccggggccaga gcccgaact gctgattttt agcgcgagct atcgctatac cggcgtgccg 240  
gatcgcttta ccggcagcgg cagcggcgcg gattttaccc tgaccattag cagcgtgcag 300  
gcggaagatc tggcgggtgta ttattgccag cagcattata gcaccccggtg gacctttggc 360  
ggcggcacca aactggaaat taaaggaggg gggggatccg ggggaggagg ctccggcgga 420  
ggcgggaagcc agattcagct ggtgcagagc ggcccggatc tgaaaaaacc gggcgaaacc 480  
gtgaaactga gctgcaaagc gagcggctat acctttacca actttggcat gaactgggtg 540  
aaacaggcgc cgggcaaagg ctttaaatgg atggcgtgga ttaacaccac ccgctatacc 600  
ggcgaaagct attttgcgga tgattttaaa ggccgctttg cgtttagcgt ggaaaccagc 660  
gcgaccaccg cgtatctgca gattaacaac ctgaaaaccg aagataccgc gacctatfff 720  
tgcgcgcgcg gcgaaattta ttatggctat gatggcggct ttgcgtattg gggccagggc 780  
accctggtga ccgtgagcgc gaccacgacg ccagcgccgc gaccaccaac accggcgccc 840  
accatcgcgt cgagcccct gtccctgcgc ccagaggcgt gccggccagc ggcggggggc 900  
gcagtgcaca cgaggggggt ggacttcgcc tgtgatattt acatctgggc gcccttggcc 960  
gggacttgtg gggtccttct cctgtcactg gttatcacc tttactgcaa acggggcaga 1020  
aagaaactcc tgtatatatt caaacaacca tttatgagac cagtacaaac tactcaagag 1080  
gaagatggct gtagctgccg atttcagaa gaagaagaag gaggatgtga actgagagt 1140  
aagttcagca ggagcgcaga cggcccgcg taccagcagg gccagaacca gctctataac 1200  
gagctcaatc taggacgaag agaggagtac gatgttttgg acaagagacg tggccgggac 1260

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cctgagatgg	ggggaaagcc	gcagagaagg	aagaaccctc	aggaaggcct	gtacaatgaa	1320
ctgcagaaag	ataagatggc	ggaggcctac	agtgagattg	ggatgaaagg	cgagcgccgg	1380
aggggcaagg	ggcacgatgg	cctttaccag	ggtctcagta	cagccaccaa	ggacacctac	1440
gacgcccttc	acatgcaggc	cctgccccct	cgcggaagcg	gagccaccaa	cttcagcctg	1500
ctgaagcagg	ccggcgacgt	ggaggagaac	cccggccccg	ccttaccagt	gaccgccttg	1560
ctcctgccgc	tggccttgct	gctccacgcc	gccaggccgg	atattcagat	gacccagagc	1620
ccgagcagcc	tgagcgcgag	cgtgggcgat	cgcgtgacca	ttacctgcaa	agcgagccag	1680
gatgtgggca	ttgcggtggc	gtggtatcag	cagaaaccgg	gcaaagtgcc	gaaactgctg	1740
atttattggg	cgagcacccg	ccataccggc	gtgccggatc	gctttagcgg	cagcggcagc	1800
ggcaccgatt	ttaccctgac	cattagcagc	ctgcagccgg	aagatgtggc	gacctattat	1860
tgccagcagt	atagcagcta	tccgtatacc	tttggccagg	gcaccaaagt	ggaaattaaa	1920
ggaggggggg	gatccggggg	aggaggctcc	ggcggaggcg	gaagcgaagt	gcagctggtg	1980
gaaagcggcg	gcggcctggt	gcagccgggc	ggcagcctgc	gcctgagctg	cgcggcgagc	2040
ggctttgatt	ttagccgcta	ttggatgagc	tgggtgcgcc	aggcgccggg	caaaggcctg	2100
gaatggattg	gcgaaattaa	cccggatagc	agcaccatta	actatgcgcc	gagcctgaaa	2160
gataaattta	ttattagccg	cgataacgcg	aaaaacagcc	tgtatctgca	gatgaacagc	2220
ctgcgcgcgg	aagataccgc	ggtgtattat	tgcgcgcgcc	cggatggcaa	ctattggtat	2280
tttgatgtgt	ggggccaggg	caccctggtg	accgtgagca	gcaccacgac	gccagcgccg	2340
cgaccaccaa	caccggcgcc	caccatcgcg	tcgcagcccc	tgtccctgcg	cccagaggcg	2400
tgccggccag	cgcggggggg	cgcagtgcac	acgagggggc	tggacttcgc	ctgtgatatc	2460
tacatctggg	cgcccttggc	cgggacttgt	ggggtccttc	tcctgtcact	ggttatcacc	2520
ctttactgca	ggagtaagag	gagcaggctc	ctgcacagtg	actacatgaa	catgactccc	2580
cgccgccccg	ggcccacccg	caagcattac	cagccctatg	ccccaccacg	cgacttcgca	2640
gcctatcgct	ccagagtga	gttcagcagg	agcgagagcg	ccccgcgta	ccagcagggc	2700
cagaaccagc	tctataacga	gctcaatcta	ggacgaagag	aggagtacga	tgttttggac	2760
aagagacgtg	gccgggaccc	tgagatgggg	ggaaagccgc	agagaaggaa	gaaccctcag	2820
gaaggcctgt	acaatgaact	gcagaaagat	aagatggcgg	aggcctacag	tgagattggg	2880
atgaaaggcg	agcgccggag	gggcaagggg	cacgatggcc	tttaccaggg	tctcagtaca	2940
gccaccaagg	acacctacga	cgcccttcac	atgcaggccc	tgccccctcg	ctaa	2994

<210> 21  
 <211> 997  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic sequence

<400> 21



2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

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 Asp Val Val Met Thr Gln Ser His Arg Phe Met  
20 25 30

Ser Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Arg Ala Ser Gln  
35 40 45

Asp Val Asn Thr Ala Val Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ser  
50 55 60

Pro Lys Leu Leu Ile Phe Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro  
65 70 75 80

Asp Arg Phe Thr Gly Ser Gly Ser Gly Ala Asp Phe Thr Leu Thr Ile  
85 90 95

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His  
100 105 110

Tyr Ser Thr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Asp Ile Lys  
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln  
130 135 140

Ile Gln Leu Val Gln Ser Gly Pro Asp Leu Lys Lys Pro Gly Glu Thr  
145 150 155 160

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

Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Phe Lys Trp Met Ala  
180 185 190

Trp Ile Asn Thr Tyr Thr Gly Glu Ser Tyr Phe Ala Asp Asp Phe Lys  
195 200 205

Gly Arg Phe Ala Phe Ser Val Glu Thr Ser Ala Thr Thr Ala Tyr Leu  
210 215 220

Gln Ile Asn Asn Leu Lys Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala  
225 230 235 240

Arg Gly Glu Ile Tyr Tyr Gly Tyr Asp Gly Gly Phe Ala Tyr Trp Gly  
245 250 255

Gln Gly Thr Leu Val Thr Val Ser Ala Thr Thr Thr Pro Ala Pro Arg  
260 265 270

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg  
275 280 285

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly  
290 295 300

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr  
305 310 315 320

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg  
325 330 335

Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro  
340 345 350

Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu  
355 360 365

Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala  
370 375 380

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu  
385 390 395 400

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly  
405 410 415

Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln  
420 425 430

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr  
435 440 445

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp  
450 455 460

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala  
465 470 475 480

Leu His Met Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr Asn Phe  
485 490 495

Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met  
500 505 510

Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu His  
515 520 525

Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser  
530 535 540

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp  
545 550 555 560

Val Gly Ile Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro  
565 570 575

Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg His Thr Gly Val Pro Asp  
580 585 590

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
595 600 605

Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser  
610 615 620

Ser Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly  
625 630 635 640

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val  
645 650 655

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu  
660 665 670

Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr Trp Met  
675 680 685

Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Glu  
690 695 700

Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Ala Pro Ser Leu Lys Asp  
705 710 715 720

Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln  
725 730 735

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg  
740 745 750

Pro Asp Gly Asn Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu  
755 760 765

Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro  
770 775 780

Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys  
785 790 795 800

Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala  
805 810 815

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Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu  
820 825 830

Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys  
835 840 845

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr  
850 855 860

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly  
865 870 875 880

Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala  
885 890 895

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg  
900 905 910

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu  
915 920 925

Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr  
930 935 940

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly  
945 950 955 960

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln  
965 970 975

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln  
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Ala Leu Pro Pro Arg  
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gcagaaaccg ggccagagcc cgaaactgct gatttttagc gcgagctatc gctataccgg 240  
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gaaagataaa	tttattatta	gccgcgataa	cgcgaaaaac	agcctgtatc	tgcagatgaa	2220
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gagaccagta caaactactc aagaggaaga tggctgtagc tgccgatttc cagaagaaga    2640
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ggtgagcagt ttcggccccg gcccgggggc aagaacagat ggtcaccgca gtttcggccc    180
cggcccagg ccaagaacag atggtcccca gatatggccc aaccctcagc agtttcttaa    240
gacccatcag atgtttccag gtcccccaa ggacctgaaa tgacctgcg cttatttga    300
attaaccaat cagcctgctt ctgccttctg ttcgcgcgt tctgcttccc gagctctata    360
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<210> 24  
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 <213> Artificial Sequence

<220>  
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Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met  
35 40 45

Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg Thr Cys Ala Ala  
50 55 60

Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp  
65 70 75 80

His Leu Leu Arg Asp Cys Ile Ser Cys Ala Ser Ile Cys Gly Gln His  
85 90 95

Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn Lys Leu Arg Ser Pro Val  
100 105 110

Asn Leu Pro Pro Glu Leu Arg Arg Gln Arg Ser Gly Glu Val Glu Asn  
115 120 125

Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly Leu Glu His Arg Gly Ser  
130 135 140

Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys Leu Ser Ala Asp Gln Val  
145 150 155 160

Ala Leu Val Tyr Ser  
165

<210> 25  
<211> 54  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 25

Met Leu Gln Met Ala Gly Gln Cys Ser Gln Asn Glu Tyr Phe Asp Ser  
1 5 10 15

Leu Leu His Ala Cys Ile Pro Cys Gln Leu Arg Cys Ser Ser Asn Thr  
20 25 30

Pro Pro Leu Thr Cys Gln Arg Tyr Cys Asn Ala Ser Val Thr Asn Ser  
35 40 45

Val Lys Gly Thr Asn Ala  
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<210> 26  
<211> 204  
<212> PRT  
<213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; synthetic sequence

&lt;400&gt; 26

Ser Gly Pro Val Lys Glu Leu Val Gly Ser Val Gly Gly Ala Val Thr  
 1 5 10 15

Phe Pro Leu Lys Ser Lys Val Lys Gln Val Asp Ser Ile Val Trp Thr  
 20 25 30

Phe Asn Thr Thr Pro Leu Val Thr Ile Gln Pro Glu Gly Gly Thr Ile  
 35 40 45

Ile Val Thr Gln Asn Arg Asn Arg Glu Arg Val Asp Phe Pro Asp Gly  
 50 55 60

Gly Tyr Ser Leu Lys Leu Ser Lys Leu Lys Lys Asn Asp Ser Gly Ile  
 65 70 75 80

Tyr Tyr Val Gly Ile Tyr Ser Ser Ser Leu Gln Gln Pro Ser Thr Gln  
 85 90 95

Glu Tyr Val Leu His Val Tyr Glu His Leu Ser Lys Pro Lys Val Thr  
 100 105 110

Met Gly Leu Gln Ser Asn Lys Asn Gly Thr Cys Val Thr Asn Leu Thr  
 115 120 125

Cys Cys Met Glu His Gly Glu Glu Asp Val Ile Tyr Thr Trp Lys Ala  
 130 135 140

Leu Gly Gln Ala Ala Asn Glu Ser His Asn Gly Ser Ile Leu Pro Ile  
 145 150 155 160

Ser Trp Arg Trp Gly Glu Ser Asp Met Thr Phe Ile Cys Val Ala Arg  
 165 170 175

Asn Pro Val Ser Arg Asn Phe Ser Ser Pro Ile Leu Ala Arg Lys Leu  
 180 185 190

Cys Glu Gly Ala Ala Asp Asp Pro Asp Ser Ser Met  
 195 200

&lt;210&gt; 27

&lt;211&gt; 46

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; synthetic sequence

&lt;400&gt; 27

Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu



1

5

10

15

Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro  
20 25 30

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys  
35 40 45

&lt;210&gt; 28

&lt;211&gt; 242

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; synthetic sequence

&lt;400&gt; 28

Asp Pro Asn Phe Trp Leu Gln Val Gln Glu Ser Val Thr Val Gln Glu  
1 5 10 15

Gly Leu Cys Val Leu Val Pro Cys Thr Phe Phe His Pro Ile Pro Tyr  
20 25 30

Tyr Asp Lys Asn Ser Pro Val His Gly Tyr Trp Phe Arg Glu Gly Ala  
35 40 45

Ile Ile Ser Arg Asp Ser Pro Val Ala Thr Asn Lys Leu Asp Gln Glu  
50 55 60

Val Gln Glu Glu Thr Gln Gly Arg Phe Arg Leu Leu Gly Asp Pro Ser  
65 70 75 80

Arg Asn Asn Cys Ser Leu Ser Ile Val Asp Ala Arg Arg Arg Asp Asn  
85 90 95

Gly Ser Tyr Phe Phe Arg Met Glu Arg Gly Ser Thr Lys Tyr Ser Tyr  
100 105 110

Lys Ser Pro Gln Leu Ser Val His Val Thr Asp Leu Thr His Arg Pro  
115 120 125

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

Thr Cys Ser Val Ser Trp Ala Cys Glu Gln Gly Thr Pro Pro Ile Phe  
145 150 155 160

Ser Trp Leu Ser Ala Ala Pro Thr Ser Leu Gly Pro Arg Thr Thr His  
165 170 175

Ser Ser Val Leu Ile Ile Thr Pro Arg Pro Gln Asp His Gly Thr Asn  
180 185 190

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Leu Thr Cys Gln Val Lys Phe Ala Gly Ala Gly Val Thr Thr Glu Arg  
195 200 205

Thr Ile Gln Leu Asn Val Thr Tyr Val Pro Gln Asn Pro Thr Thr Gly  
210 215 220

Ile Phe Pro Gly Asp Gly Ser Gly Lys Gln Glu Thr Arg Ala Gly Val  
225 230 235 240

Val His

<210> 29  
<211> 287  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 29

Thr Lys Glu Asp Pro Asn Pro Pro Ile Thr Asn Leu Arg Met Lys Ala  
1 5 10 15

Lys Ala Gln Gln Leu Thr Trp Asp Leu Asn Arg Asn Val Thr Asp Ile  
20 25 30

Glu Cys Val Lys Asp Ala Asp Tyr Ser Met Pro Ala Val Asn Asn Ser  
35 40 45

Tyr Cys Gln Phe Gly Ala Ile Ser Leu Cys Glu Val Thr Asn Tyr Thr  
50 55 60

Val Arg Val Ala Asn Pro Pro Phe Ser Thr Trp Ile Leu Phe Pro Glu  
65 70 75 80

Asn Ser Gly Lys Pro Trp Ala Gly Ala Glu Asn Leu Thr Cys Trp Ile  
85 90 95

His Asp Val Asp Phe Leu Ser Cys Ser Trp Ala Val Gly Pro Gly Ala  
100 105 110

Pro Ala Asp Val Gln Tyr Asp Leu Tyr Leu Asn Val Ala Asn Arg Arg  
115 120 125

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

Ile Gly Cys Arg Phe Asp Asp Ile Ser Arg Leu Ser Ser Gly Ser Gln  
145 150 155 160

Ser Ser His Ile Leu Val Arg Gly Arg Ser Ala Ala Phe Gly Ile Pro  
Page 57

Cys Thr Asp Lys Phe Val Val Phe Ser Gln Ile Glu Ile Leu Thr Pro  
180 185 190

Pro Asn Met Thr Ala Lys Cys Asn Lys Thr His Ser Phe Met His Trp  
195 200 205

Lys Met Arg Ser His Phe Asn Arg Lys Phe Arg Tyr Glu Leu Gln Ile  
210 215 220

Gln Lys Arg Met Gln Pro Val Ile Thr Glu Gln Val Arg Asp Arg Thr  
225 230 235 240

Ser Phe Gln Leu Leu Asn Pro Gly Thr Tyr Thr Val Gln Ile Arg Ala  
245 250 255

Arg Glu Arg Val Tyr Glu Phe Leu Ser Ala Trp Ser Thr Pro Gln Arg  
260 265 270

Phe Glu Cys Asp Gln Glu Glu Gly Ala Asn Thr Arg Ala Trp Arg  
275 280 285

<210> 30  
<211> 272  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 30

Pro Glu Glu Pro Leu Val Val Lys Val Glu Glu Gly Asp Asn Ala Val  
1 5 10 15

Leu Gln Cys Leu Lys Gly Thr Ser Asp Gly Pro Thr Gln Gln Leu Thr  
20 25 30

Trp Ser Arg Glu Ser Pro Leu Lys Pro Phe Leu Lys Leu Ser Leu Gly  
35 40 45

Leu Pro Gly Leu Gly Ile His Met Arg Pro Leu Ala Ile Trp Leu Phe  
50 55 60

Ile Phe Asn Val Ser Gln Gln Met Gly Gly Phe Tyr Leu Cys Gln Pro  
65 70 75 80

Gly Pro Pro Ser Glu Lys Ala Trp Gln Pro Gly Trp Thr Val Asn Val  
85 90 95

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

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

Leu Gly Cys Gly Leu Lys Asn Arg Ser Ser Glu Gly Pro Ser Ser Pro  
115 120 125

Ser Gly Lys Leu Met Ser Pro Lys Leu Tyr Val Trp Ala Lys Asp Arg  
130 135 140

Pro Glu Ile Trp Glu Gly Glu Pro Pro Cys Leu Pro Pro Arg Asp Ser  
145 150 155 160

Leu Asn Gln Ser Leu Ser Gln Asp Leu Thr Met Ala Pro Gly Ser Thr  
165 170 175

Leu Trp Leu Ser Cys Gly Val Pro Pro Asp Ser Val Ser Arg Gly Pro  
180 185 190

Leu Ser Trp Thr His Val His Pro Lys Gly Pro Lys Ser Leu Leu Ser  
195 200 205

Leu Glu Leu Lys Asp Asp Arg Pro Ala Arg Asp Met Trp Val Met Glu  
210 215 220

Thr Gly Leu Leu Leu Pro Arg Ala Thr Ala Gln Asp Ala Gly Lys Tyr  
225 230 235 240

Tyr Cys His Arg Gly Asn Leu Thr Met Ser Phe His Leu Glu Ile Thr  
245 250 255

Ala Arg Pro Val Leu Trp His Trp Leu Leu Arg Thr Gly Gly Trp Lys  
260 265 270

<210> 31  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 31

Pro Ile Cys Val Thr Val  
1 5

<210> 32  
<211> 47  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> synthetic sequence

<400> 32

Lys Ile Ser His Phe Leu Lys Met Glu Ser Leu Asn Phe Ile Arg Ala  
1 5 10 15

2541\_3\_PCT\_Text\_Sequence\_Listing\_6\_24\_16\_ST25. TXT

His Thr Pro Tyr Ile Asn Ile Tyr Asn Cys Glu Pro Ala Asn Pro Ser  
20 25 30

Glu Lys Asn Ser Pro Ser Thr Gln Tyr Cys Tyr Ser Ile Gln Ser  
35 40 45

<210> 33

<211> 480

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 33

Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu Tyr Ala Trp  
1 5 10 15

Glu Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala Leu Asp Gly  
20 25 30

Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr Asn Lys Asn  
35 40 45

Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr Lys Asp Gly  
50 55 60

Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly Asp Lys Asn  
65 70 75 80

Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu Asn Asp Ser Gly  
85 90 95

Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg  
100 105 110

Ile His Leu Asn Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu  
115 120 125

Pro Pro Glu Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu  
130 135 140

Asn Phe Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu  
145 150 155 160

Gly Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile  
165 170 175

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser  
180 185 190

His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp Gly Lys

195

200

205

Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His Thr Pro Lys  
 210 215 220

Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val Arg Glu Gly Asp  
 225 230 235 240

Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser Asn Pro Glu Tyr Thr  
 245 250 255

Thr Val Ser Trp Leu Lys Asp Gly Thr Ser Leu Lys Lys Gln Asn Thr  
 260 265 270

Phe Thr Leu Asn Leu Arg Glu Val Thr Lys Asp Gln Ser Gly Lys Tyr  
 275 280 285

Cys Cys Gln Val Ser Asn Asp Val Gly Pro Gly Arg Ser Glu Glu Val  
 290 295 300

Phe Leu Gln Val Gln Tyr Ala Pro Glu Pro Ser Thr Val Gln Ile Leu  
 305 310 315 320

His Ser Pro Ala Val Glu Gly Ser Gln Val Glu Phe Leu Cys Met Ser  
 325 330 335

Leu Ala Asn Pro Leu Pro Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys  
 340 345 350

Glu Met Gln Gly Arg Thr Glu Glu Lys Val His Ile Pro Lys Ile Leu  
 355 360 365

Pro Trp His Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly  
 370 375 380

Thr Gly Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln Tyr Pro Pro  
 385 390 395 400

Lys Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly  
 405 410 415

Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser Val  
 420 425 430

Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro Ser Leu  
 435 440 445

Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr Thr Ile Ala  
 450 455 460

Cys Ala Ala Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro Val Ala Leu  
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465

470

475

480

&lt;210&gt; 34

&lt;211&gt; 480

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; synthetic sequence

&lt;400&gt; 34

Gln Ser Pro Thr Pro Ser Pro Thr Gly Leu Thr Thr Ala Lys Met Pro  
1 5 10 15

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

Ser Pro Ala Ser Thr Phe Glu Arg Glu Asn Asp Phe Ser Glu Thr Thr  
35 40 45

Thr Ser Leu Ser Pro Asp Asn Thr Ser Thr Gln Val Ser Pro Asp Ser  
50 55 60

Leu Asp Asn Ala Ser Ala Phe Asn Thr Thr Gly Val Ser Ser Val Gln  
65 70 75 80

Thr Pro His Leu Pro Thr His Ala Asp Ser Gln Thr Pro Ser Ala Gly  
85 90 95

Thr Asp Thr Gln Thr Phe Ser Gly Ser Ala Ala Asn Ala Lys Leu Asn  
100 105 110

Pro Thr Pro Gly Ser Asn Ala Ile Ser Asp Val Pro Gly Glu Arg Ser  
115 120 125

Thr Ala Ser Thr Phe Pro Thr Asp Pro Val Ser Pro Leu Thr Thr Thr  
130 135 140

Leu Ser Leu Ala His His Ser Ser Ala Ala Leu Pro Ala Arg Thr Ser  
145 150 155 160

Asn Thr Thr Ile Thr Ala Asn Thr Ser Asp Ala Tyr Leu Asn Ala Ser  
165 170 175

Glu Thr Thr Thr Leu Ser Pro Ser Gly Ser Ala Val Ile Ser Thr Thr  
180 185 190

Thr Ile Ala Thr Thr Pro Ser Lys Pro Thr Cys Asp Glu Lys Tyr Ala  
195 200 205

Asn Ile Thr Val Asp Tyr Leu Tyr Asn Lys Glu Thr Lys Leu Phe Thr  
210 215 220

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Ala Lys Leu Asn Val Asn Glu Asn Val Glu Cys Gly Asn Asn Thr Cys  
 225 230 235 240

Thr Asn Asn Glu Val His Asn Leu Thr Glu Cys Lys Asn Ala Ser Val  
 245 250 255

Ser Ile Ser His Asn Ser Cys Thr Ala Pro Asp Lys Thr Leu Ile Leu  
 260 265 270

Asp Val Pro Pro Gly Val Glu Lys Phe Gln Leu His Asp Cys Thr Gln  
 275 280 285

Val Glu Lys Ala Asp Thr Thr Ile Cys Leu Lys Trp Lys Asn Ile Glu  
 290 295 300

Thr Phe Thr Cys Asp Thr Gln Asn Ile Thr Tyr Arg Phe Gln Cys Gly  
 305 310 315 320

Asn Met Ile Phe Asp Asn Lys Glu Ile Lys Leu Glu Asn Leu Glu Pro  
 325 330 335

Glu His Glu Tyr Lys Cys Asp Ser Glu Ile Leu Tyr Asn Asn His Lys  
 340 345 350

Phe Thr Asn Ala Ser Lys Ile Ile Lys Thr Asp Phe Gly Ser Pro Gly  
 355 360 365

Glu Pro Gln Ile Ile Phe Cys Arg Ser Glu Ala Ala His Gln Gly Val  
 370 375 380

Ile Thr Trp Asn Pro Pro Gln Arg Ser Phe His Asn Phe Thr Leu Cys  
 385 390 395 400

Tyr Ile Lys Glu Thr Glu Lys Asp Cys Leu Asn Leu Asp Lys Asn Leu  
 405 410 415

Ile Lys Tyr Asp Leu Gln Asn Leu Lys Pro Tyr Thr Lys Tyr Val Leu  
 420 425 430

Ser Leu His Ala Tyr Ile Ile Ala Lys Val Gln Arg Asn Gly Ser Ala  
 435 440 445

Ala Met Cys His Phe Thr Thr Lys Ser Ala Pro Pro Ser Gln Val Trp  
 450 455 460

Asn Met Thr Val Ser Met Thr Ser Asp Asn Ser Met His Val Lys Cys  
 465 470 475 480

<210> 35  
 <211> 65  
 <212> PRT



<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 35

Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val  
1 5 10 15

Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly  
20 25 30

Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn  
35 40 45

Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile  
50 55 60

Arg  
65

<210> 36

<211> 153

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 36

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu  
1 5 10 15

Val Thr Asn Ser Gly Ile His Val Phe Ile Leu Gly Cys Phe Ser Ala  
20 25 30

Gly Leu Pro Lys Thr Glu Ala Asn Trp Val Asn Val Ile Ser Asp Leu  
35 40 45

Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu  
50 55 60

Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys  
65 70 75 80

Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala  
85 90 95

Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser  
100 105 110

Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu  
115 120 125

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Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His  
130 135 140

Ile Val Gln Met Phe Ile Asn Thr Ser  
145 150