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(12) **United States Patent  
Skinner**(10) **Patent No.: US 12,384,830 B2**(45) **Date of Patent: Aug. 12, 2025**(54) **COMPOSITIONS AND METHODS FOR  
IMPROVING IMMUNOTHERAPY**(71) Applicant: **Regents of the University of  
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(US)(73) Assignee: **Regents of the University of  
Minnesota**, Minneapolis, MN (US)(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 734 days.(21) Appl. No.: **15/996,337**(22) Filed: **Jun. 1, 2018**(65) **Prior Publication Data**

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**Related U.S. Application Data**(60) Provisional application No. 62/514,438, filed on Jun.  
2, 2017.(51) **Int. Cl.****C07K 14/715** (2006.01)  
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**A61K 40/30** (2025.01)  
**A61K 40/31** (2025.01)  
**A61K 40/46** (2025.01)  
**A61P 31/18** (2006.01)  
**C07K 14/705** (2006.01)  
**C07K 14/725** (2006.01)  
**C12N 7/00** (2006.01)  
**G01N 33/569** (2006.01)(52) **U.S. Cl.**CPC ..... **C07K 14/7158** (2013.01); **A61K 40/11**  
(2025.01); **A61K 40/30** (2025.01); **A61K 40/31**  
(2025.01); **A61K 40/46** (2025.01); **A61P 31/18**  
(2018.01); **C07K 14/7051** (2013.01); **C07K**  
**14/70521** (2013.01); **C12N 7/00** (2013.01);  
**G01N 33/56983** (2013.01); **C07K 2319/03**  
(2013.01); **C07K 2319/20** (2013.01); **C12N**  
**2740/13043** (2013.01); **C12N 2740/16043**  
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**2800/52** (2013.01)(58) **Field of Classification Search**CPC ..... **C07K 14/158**; **C07K 14/7158**; **C07K**  
**14/7051**; **C07K 14/70521**; **C07K 2319/03**;  
**C07K 2319/20**; **A61K 35/17**; **A61K**  
**2039/5156**; **A61K 2039/5158**; **A61K**  
**39/0011**; **A61P 31/18**; **C12N 7/00**; **C12N**  
**2740/13043**; **C12N 2740/16043**; **G01N**  
**33/56983**; **G01N 2800/26**; **G01N 2800/52**

See application file for complete search history.

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(57) **ABSTRACT**This disclosure provides compositions and methods for  
improving immunotherapy, specifically against diseases like  
HIV or lymphoma that manifest within B cell follicles.**11 Claims, 15 Drawing Sheets****Specification includes a Sequence Listing.**

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FIG. 1A



FIG. 1B

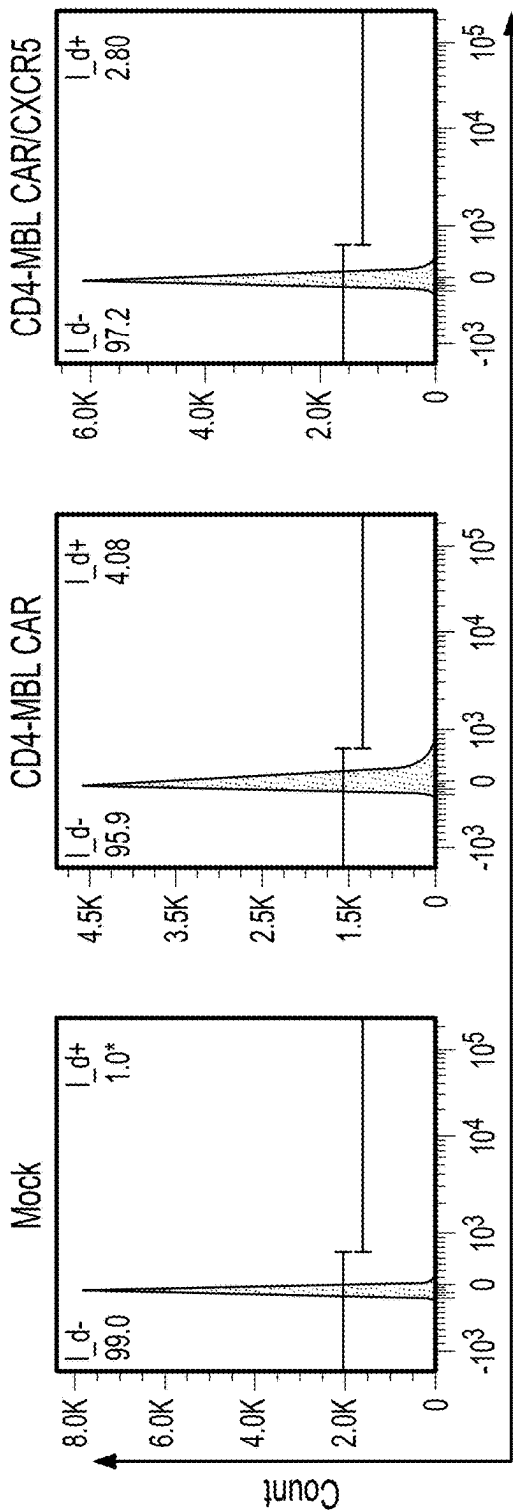


FIG. 2A

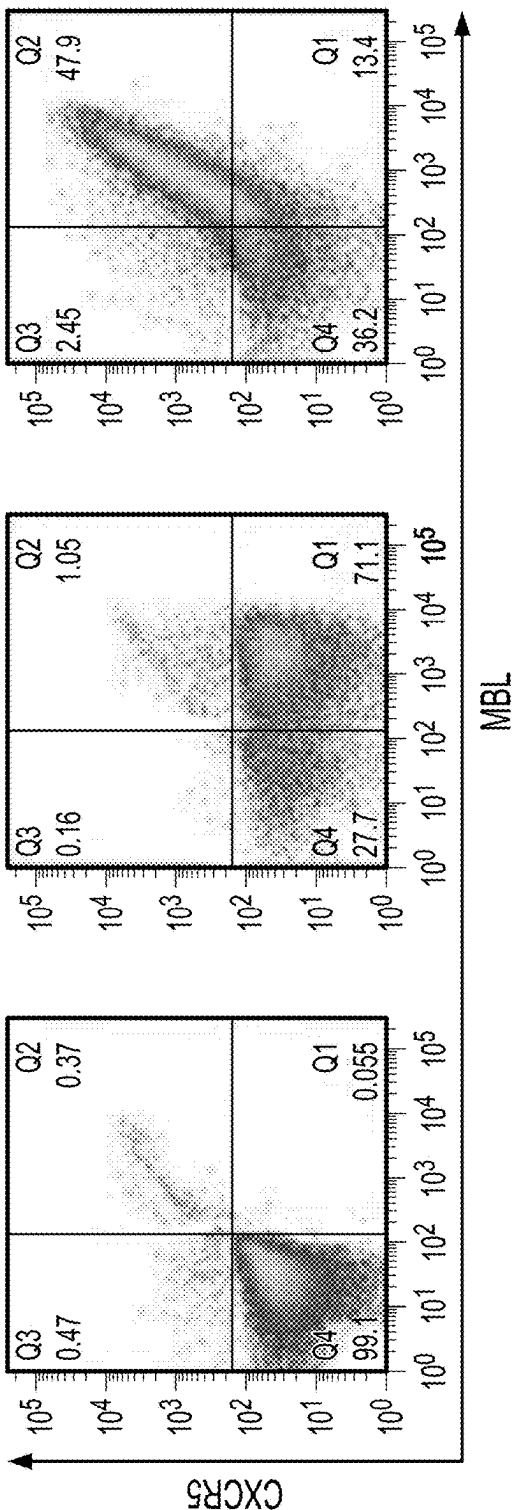


FIG. 2B

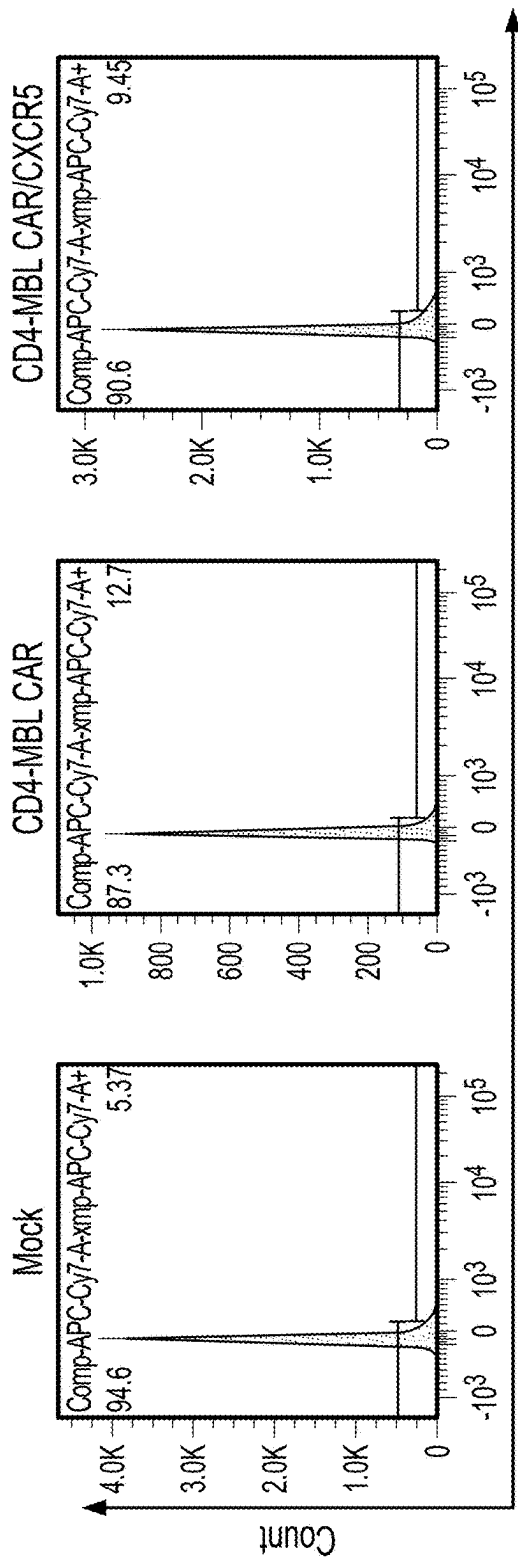


FIG. 3A

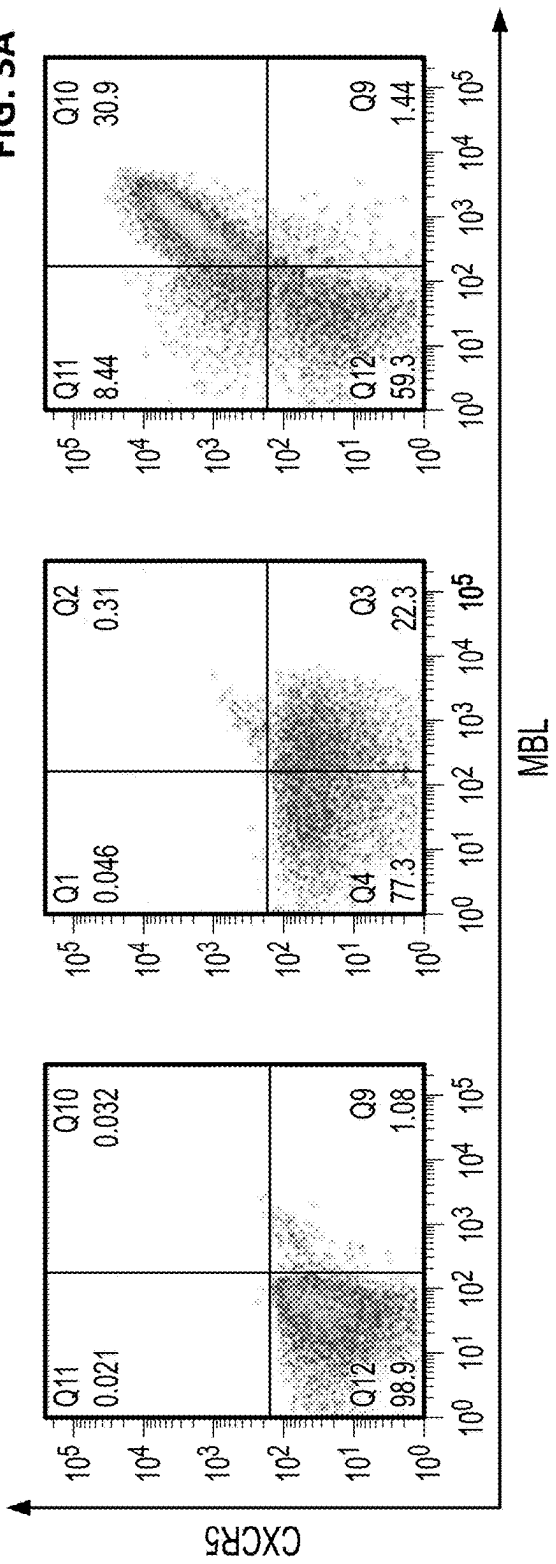


FIG. 3B

CAR+CXCR5\_CD20\_CD3

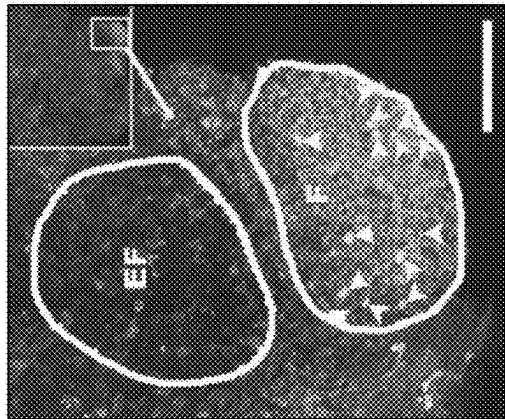


FIG. 4B

CAR\_CD20\_CD3

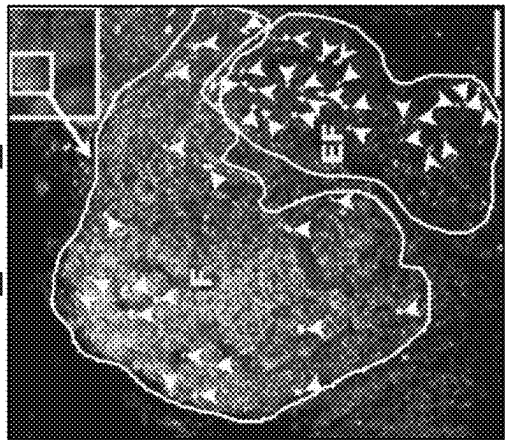


FIG. 4A

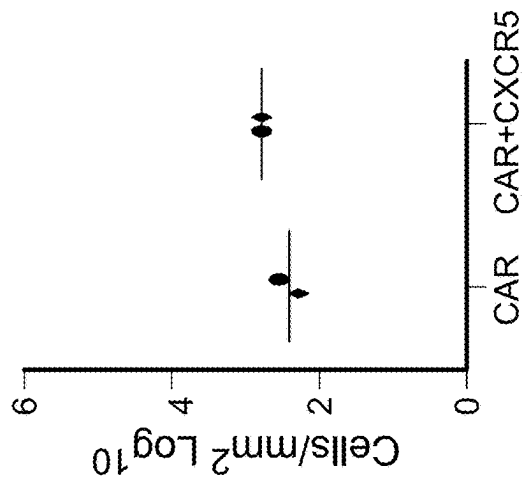


FIG. 4C

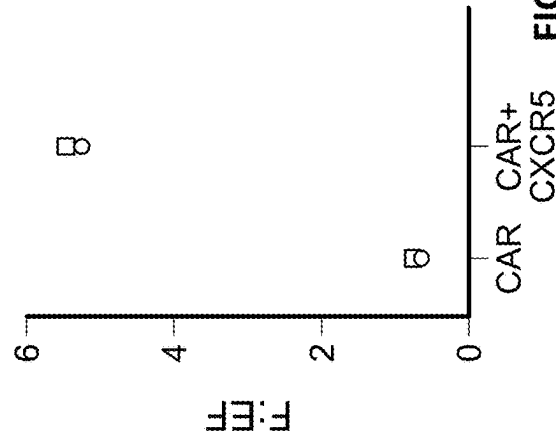


FIG. 4E

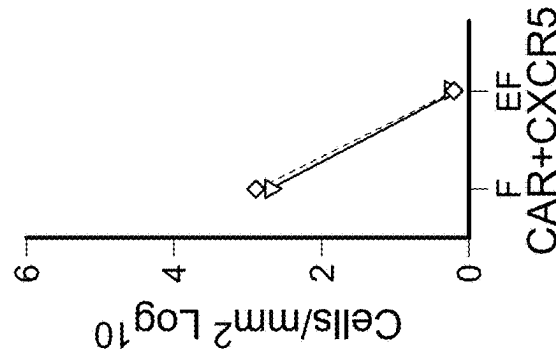
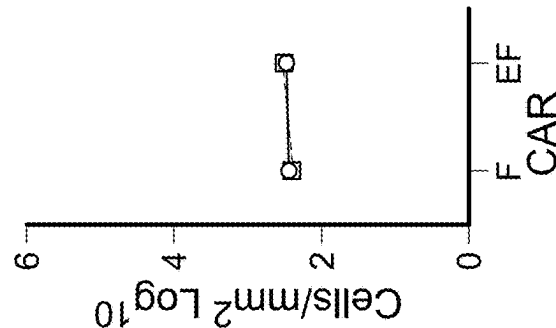


FIG. 4D





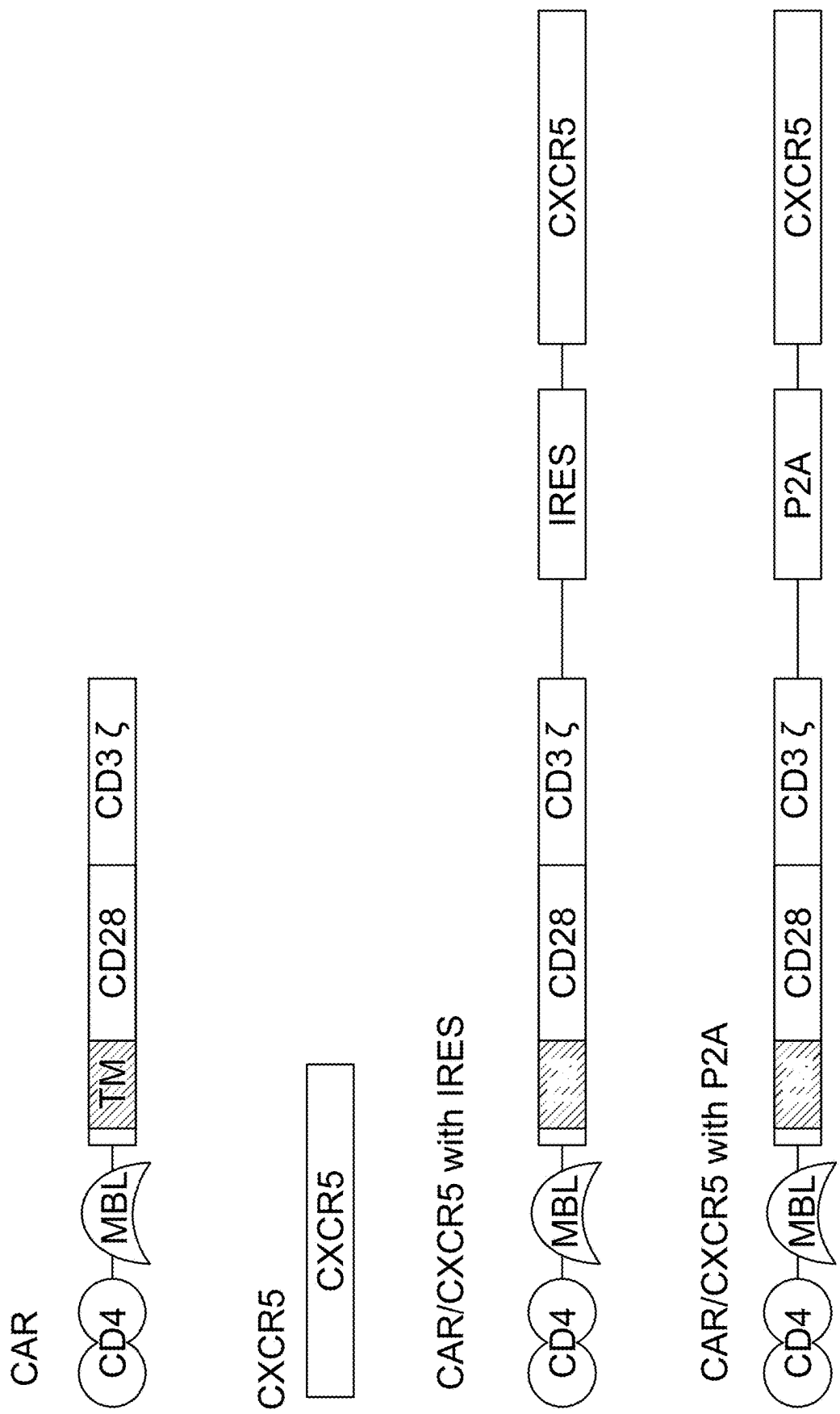
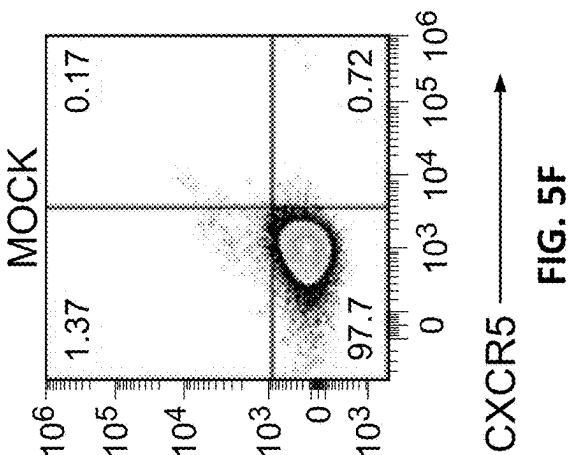
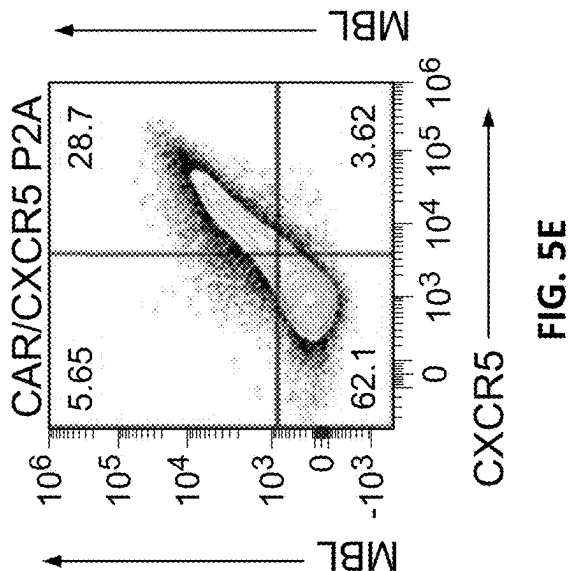
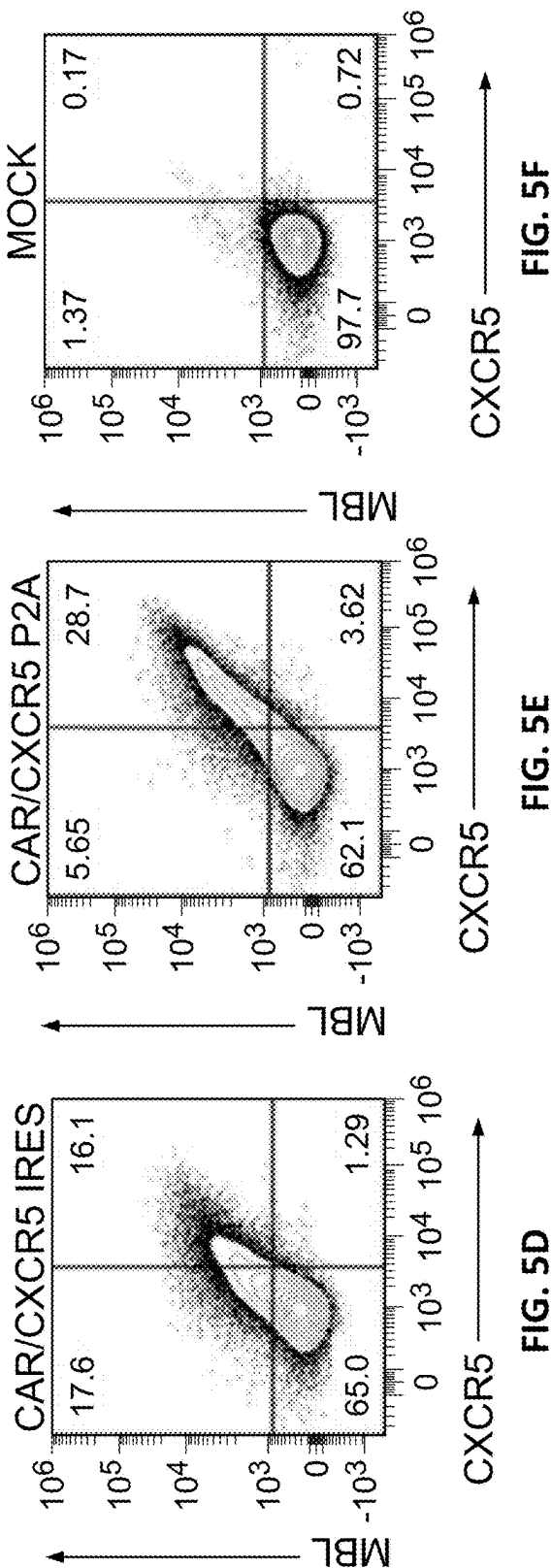
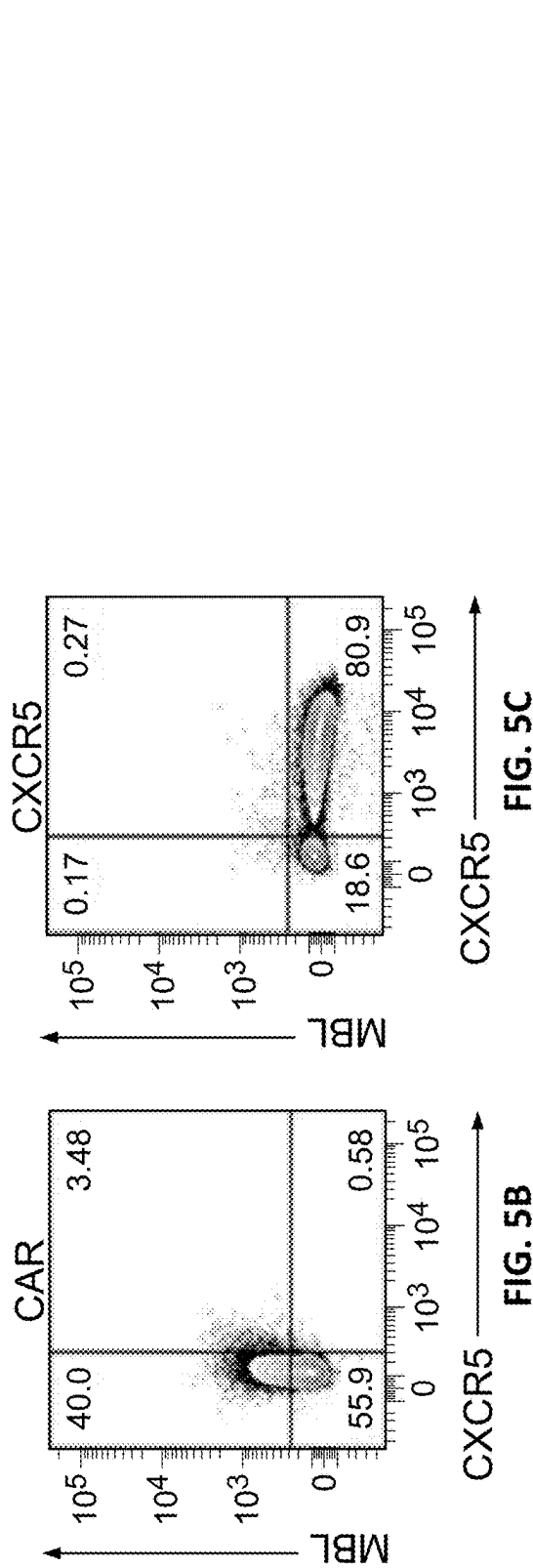


FIG. 5A



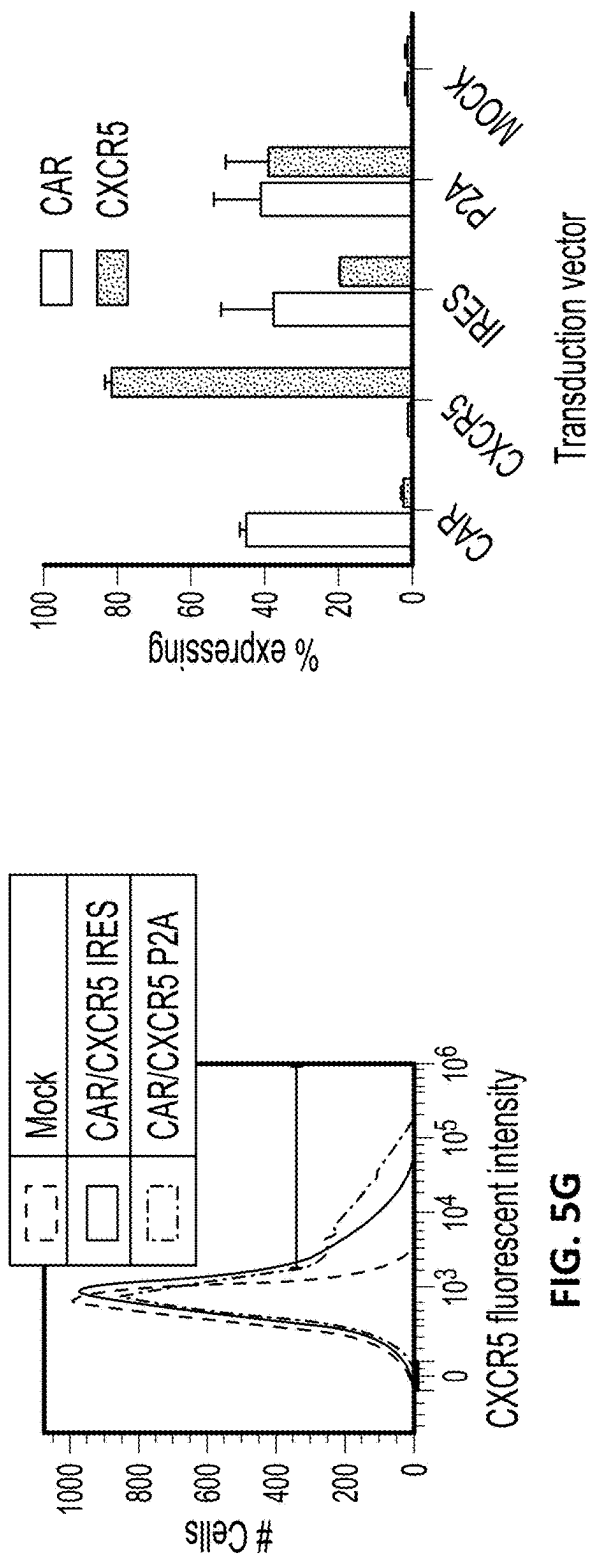


FIG. 5H

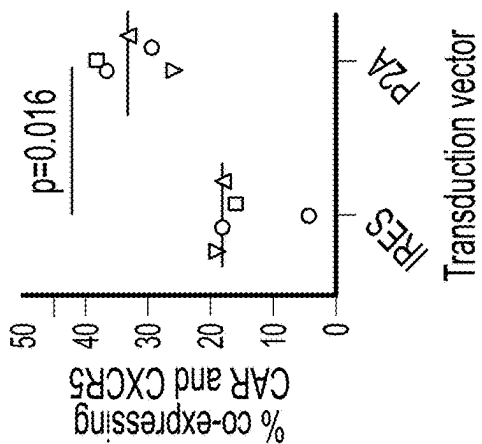


FIG. 5I

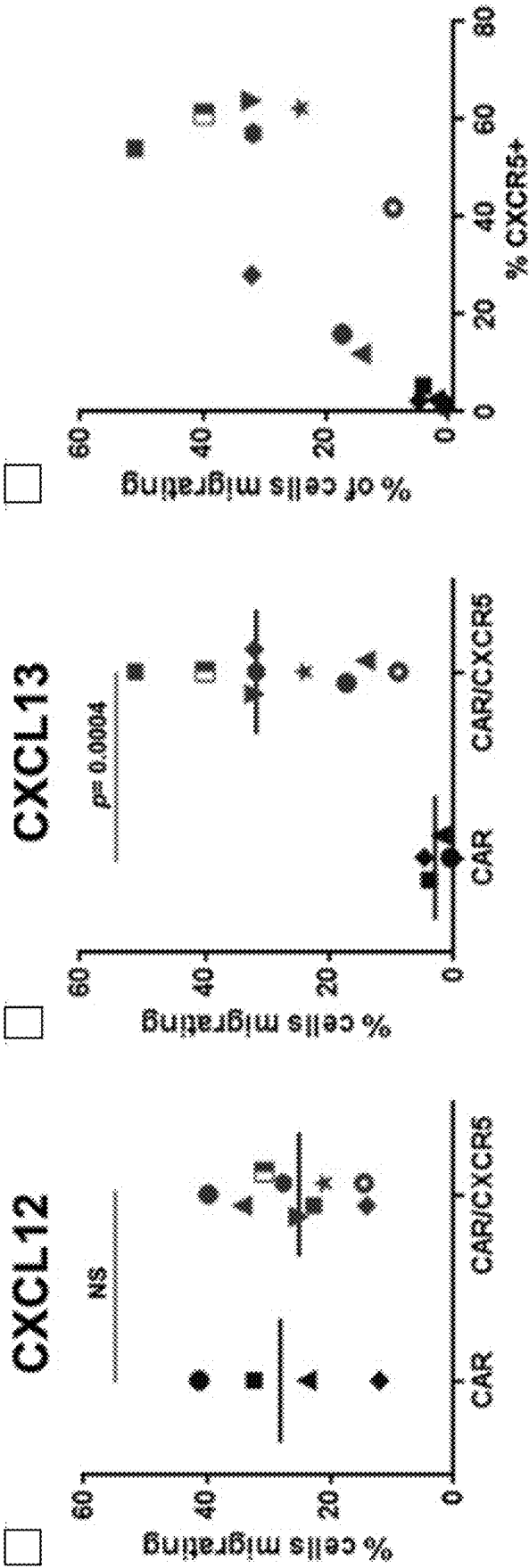


FIG. 6A

FIG. 6B

FIG. 6C

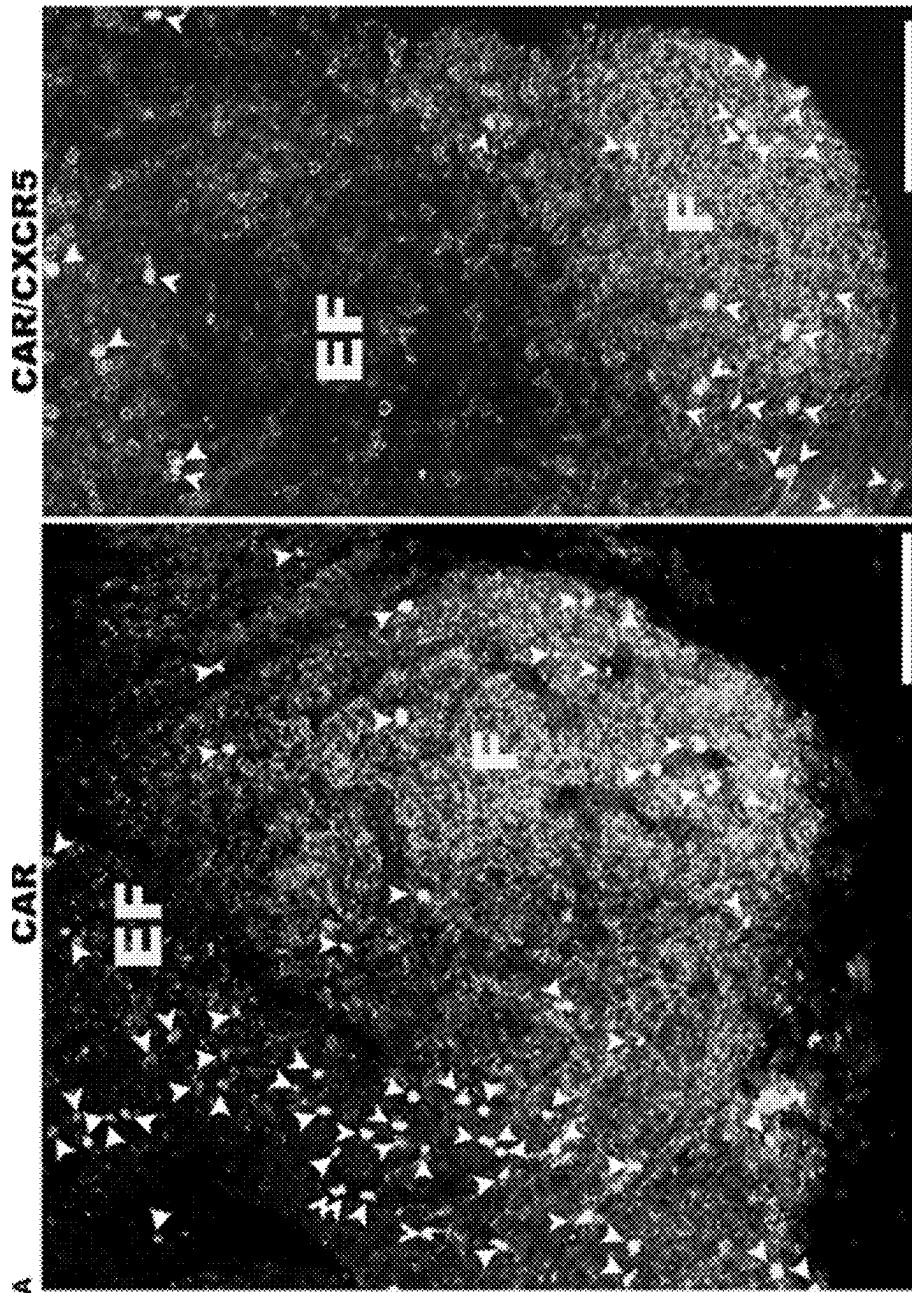


FIG. 7A

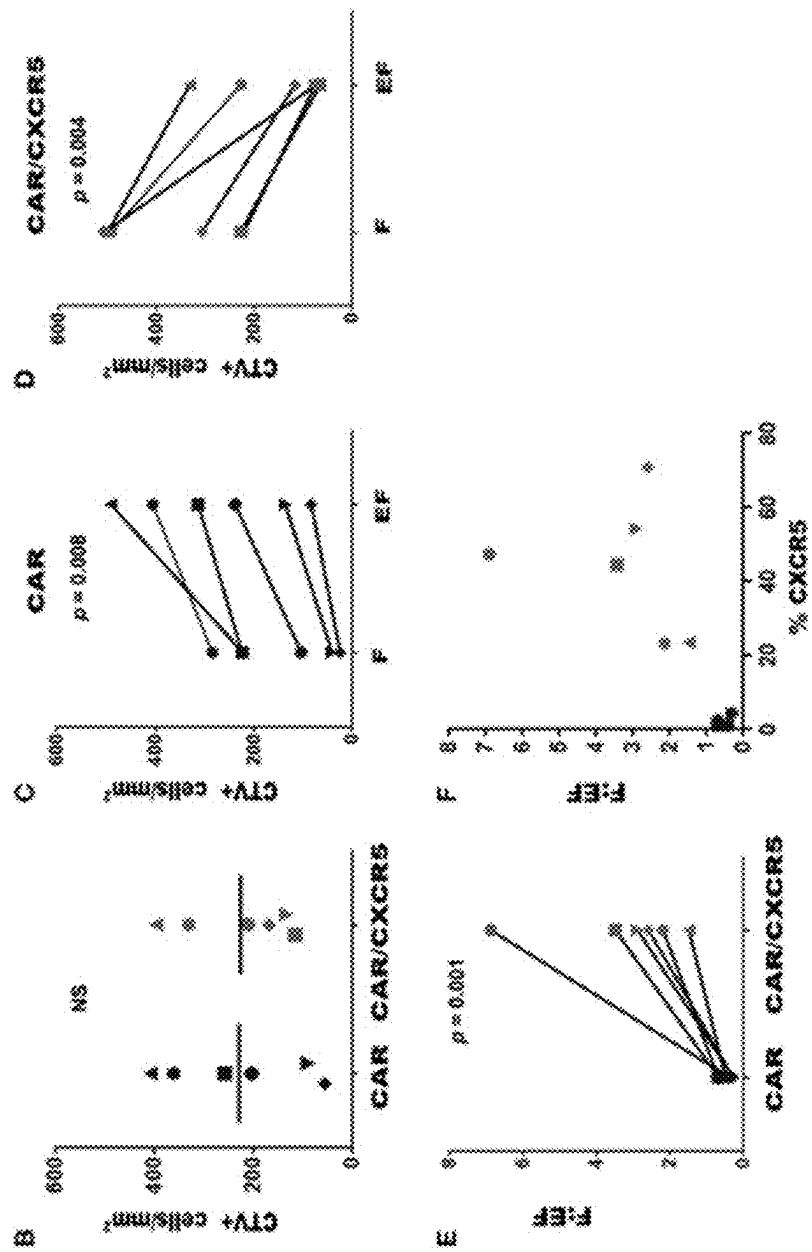


FIG. 7B – FIG. 7F

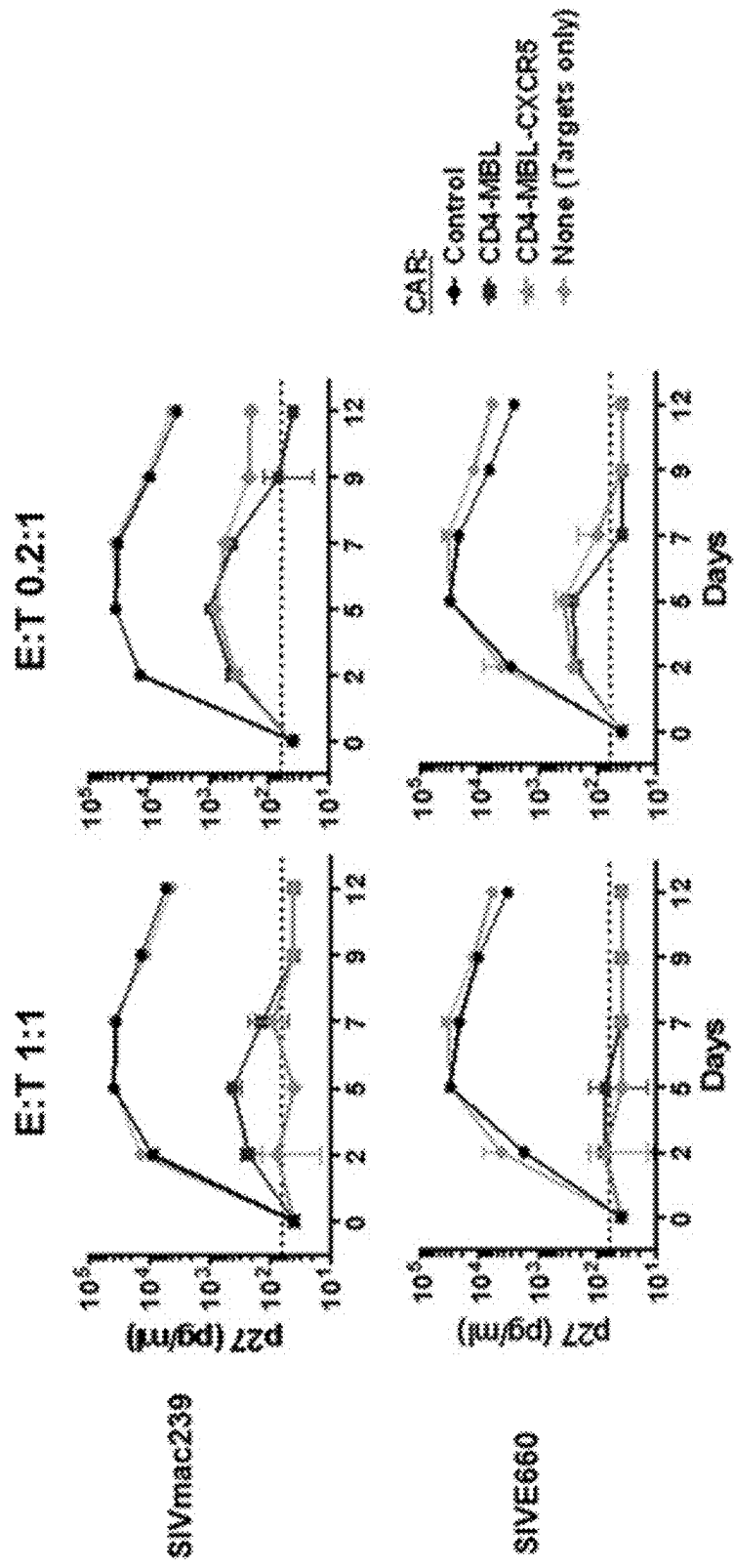


FIG. 8

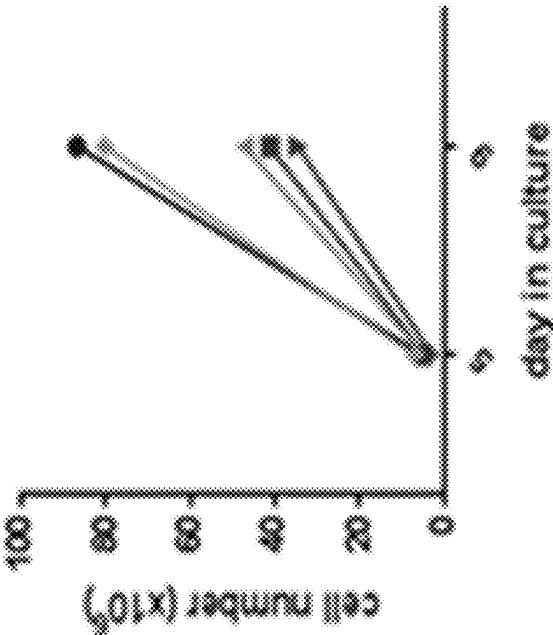


FIG. 10

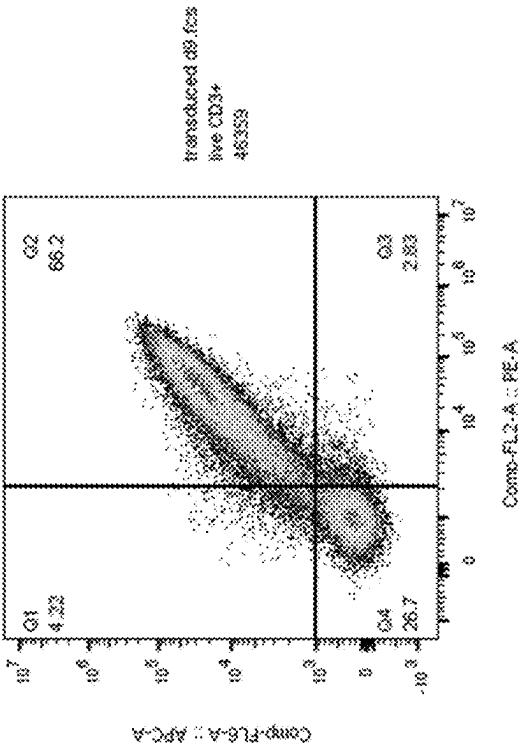


FIG. 9



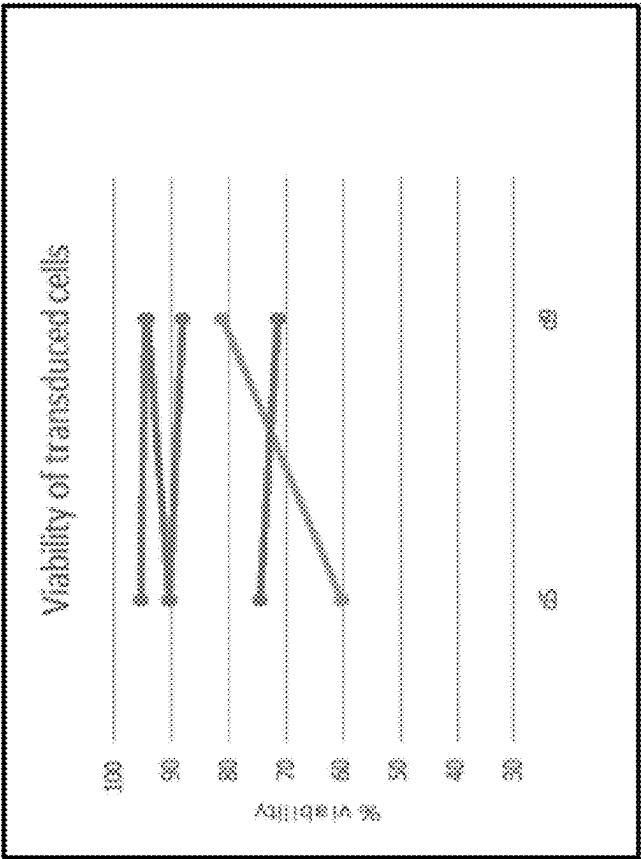


FIG. 11

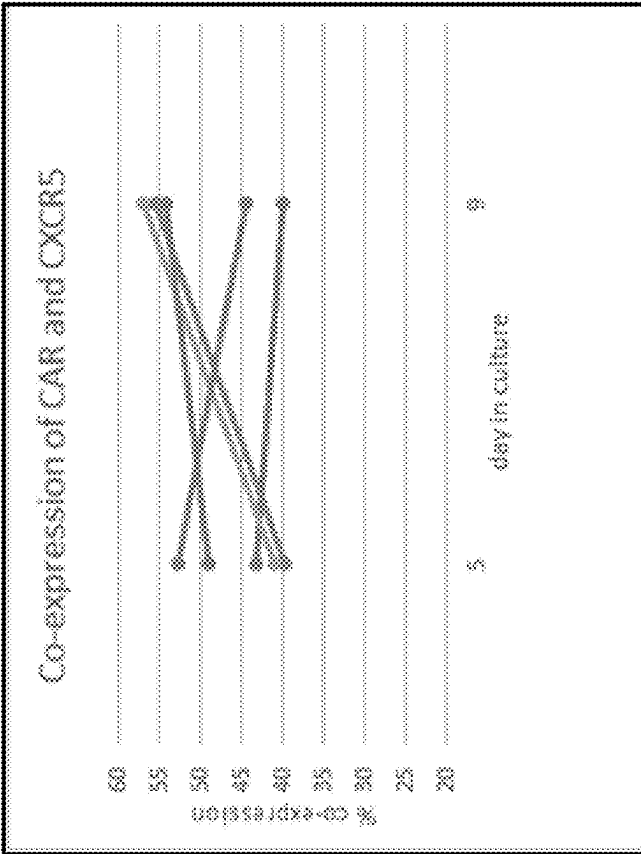


FIG. 12

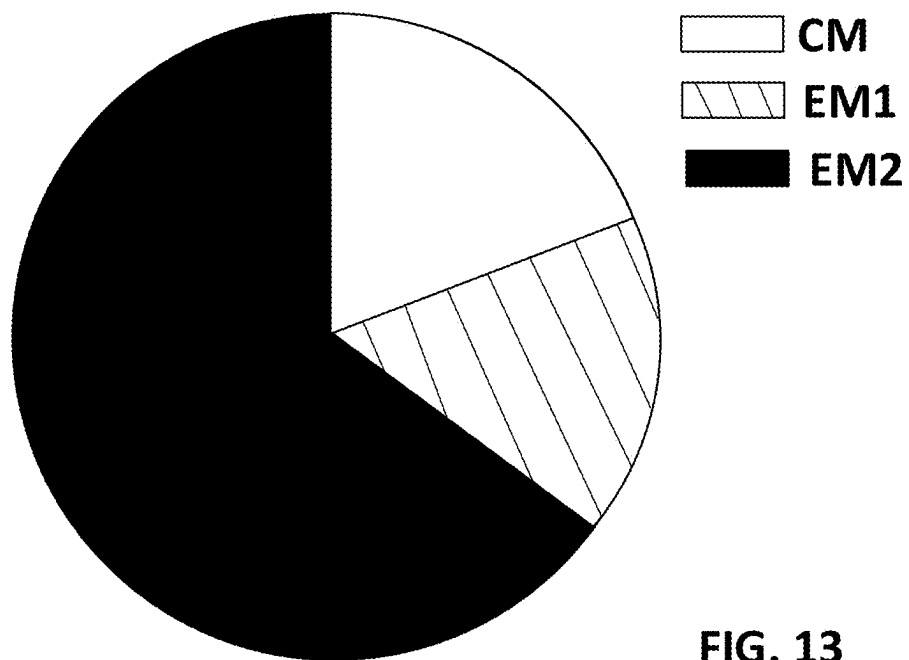


FIG. 13

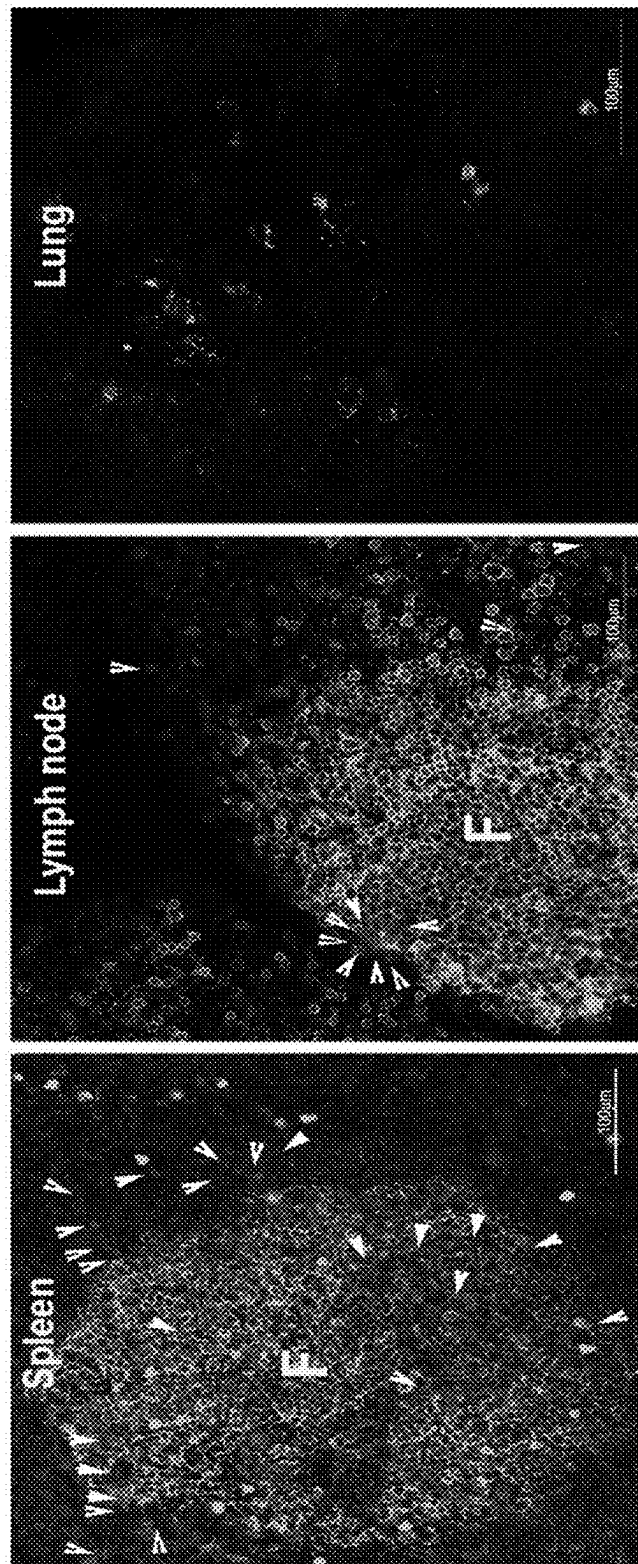


FIG. 14

1

**COMPOSITIONS AND METHODS FOR  
IMPROVING IMMUNOTHERAPY****CROSS REFERENCE TO RELATED  
APPLICATIONS**

This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Application No. 62/514,438 filed Jun. 2, 2017.

**FEDERALLY SPONSORED RESEARCH OR  
DEVELOPMENT**

This invention was made with government support under AI096966, HL127479 and AI026617 awarded by the National Institutes of Health. The government has certain rights in the invention.

**TECHNICAL FIELD**

This disclosure generally relates to compositions and methods for improving immunotherapy.

**BACKGROUND**

An estimated 37 million people are currently infected with HIV-1 including more than 1 million in the United States. Last year, approximately 2 million people became infected with HIV and 1.2 million people died of AIDS. No effective vaccine has yet been developed for HIV. This is largely due to the high mutation rate of the virus. At this time, antiretroviral medications are necessary to suppress HIV-1 replication and prevent disease progression. However, antiretroviral medications must be taken lifelong and on a daily basis to be effective. These drugs are costly, frequently induce unpleasant or toxic side effects, must be taken with strict adherence to be effective, and are often rendered ineffective due to the development of drug resistance by the virus. These drugs are also not a cure. When taken off drugs, virus levels rebound and disease progression resumes, sometimes at an increased rate. The estimated cost of treating an HIV infected individual over their lifetime is estimated to approach \$500,000. Thus, there is an urgent need to develop better treatment options and a cure for HIV.

**SUMMARY**

Compositions and methods for improving immunotherapy are described herein. For example, kits are provided that include vectors encoding a chimeric antigen receptor (CAR) construct and a CXCR5 polypeptide. The kits also can include any or all of the reagents needed to transduce cells. In addition, methods are provided that include transducing patient cells with a vector as described herein, followed by infusion back into the patient.

In one aspect, a CAR construct is provided that further includes a nucleic acid molecule encoding a CXCR5 polypeptide. In one embodiment, the CXCR5 polypeptide has the sequence shown in SEQ ID NO: 1 or 3. In one embodiment, the nucleic acid molecule encoding the CXCR5 polypeptide has the nucleic acid sequence shown in SEQ ID NO: 2 or 4. In some embodiments, the CAR construct is an HIV-based CAR construct. In some embodiments, the HIV-based CAR construct has the sequence shown in SEQ ID NO: 6, 8 or 10. In some embodiments, the HIV-based CAR construct is encoded by the nucleic acid sequence shown in SEQ ID NO: 7, 9 or 11.

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In another aspect, a vector including a CAR construct and a nucleic acid molecule encoding a CXCR5 polypeptide is provided. In some embodiments, the vector is a lentiviral vector.

5 In still another aspect, a kit including a vector is provided. Such a vector typically includes a CAR construct and a nucleic acid molecule encoding a CXCR5 polypeptide. In some embodiments, a kit can further include reagents for transducing cells (e.g., patient T cells).

10 In yet another aspect, a method of inhibiting replication of HIV in cells is provided. Such a method typically includes providing cells from a subject having HIV; transducing the cells with a vector comprising a CAR construct and a nucleic acid molecule encoding a CXCR5 polypeptide; and cultur-  
15 ing the cells. In some embodiments, the method can further include monitoring the cells for the presence of HIV. In some embodiments, the method can further include monitoring the cells for the amount of HIV (particle number).

20 In one aspect, a method of (functionally) curing HIV in a subject is provided. Such a method typically includes infusing cells into the subject, wherein the cells are autologous to the subject and comprise a vector, and wherein the vector comprises a CAR construct and a nucleic acid molecule encoding a CXCR5 polypeptide. In some embodiments, the  
25 method can further include monitoring the subject for the presence of HIV. In some embodiments, the method can further include monitoring the subject for the amount of HIV (viral load).

30 In some embodiments, any of the methods described herein can further include obtaining the cells from the subject. In some embodiments, any of the methods described herein can further include transducing the cells with the vector.

35 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods and compositions of matter belong. Although methods and materials similar or equivalent to those described  
40 herein can be used in the practice or testing of the methods and compositions of matter, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other  
45 references mentioned herein are incorporated by reference in their entirety.

**DESCRIPTION OF DRAWINGS****Part A—Preliminary Results**

FIG. 1A and FIG. 1B are schematics showing a representative genetic CAR construct composed of a targeting region consisting of an extracellular region of CD4 (domains 1+2) attached to the carbohydrate-recognition domain of mannose-binding lectin (MBL), followed by a transmembrane (TM) and intracellular signaling domains from CD28 and CD3 zeta chain. The CAR construct is followed by an internal ribosome entry site for translation of a second gene, the ZSGreen reporter (FIG. 1A) or the IRES-driven CXCR5 (FIG. 1).

FIG. 2A and FIG. 2B show the successful co-expression of an anti-HIV CAR and human CXCR5 in human PBMC. FIG. 2A are graphs showing the viability of the transduced cells (based on the mean fluorescent intensity of a live/dead cell stain that stains dead cells). FIG. 2B shows the transduction efficiency, with transduced cells detected using

antibodies against MBL (for the CAR construct) and CXCR5. The CAR construct also expresses the ZSGreen reporter (not shown).

FIG. 3A and FIG. 3B show the successful co-expression of an anti-SIV CAR and rhesus CXCR5 in rhesus PBMC. FIG. 3A are graphs showing the viability of the transduced cells (based on mean fluorescent intensity of a live/dead cell stain). FIG. 3B shows the transduction efficiency, with transduced cells detected using antibodies against MBL (for the CAR construct) and CXCR5. The CAR construct also induced expression of ZSGreen (not shown).

FIG. 4A-FIG. 4E shows that CXCR5 induced migration was detected in an ex vivo B cell follicle migration assay. Rhesus macaque PBMCs were transduced with CAR or CAR/CXCR5 vectors, labeled with CellTrace Violet (CTV), and layered onto freshly cut slabs of rhesus macaque lymph nodes. Panels A and B show tissues stained with anti-CD20 antibodies to delineate B cell follicles ("F") and anti-CD3 antibodies to delineate the T cell zone and extra-follicular areas ("EF"). Panel C shows that similar total levels of CFSE-labeled cells were detected in tissues treated with CAR or CAR/CXCR5 transduced cells. Panel D shows that, while CAR-transduced cells showed similar levels inside and outside of follicles, CAR/CXCR5-transduced cells primarily located within B cell follicles. Panel E shows that CAR/CXCR5-transduced cells exhibited much higher F:EF ratios compared to CAR-transduced cells.

Part B—Simian Immunodeficiency Virus (SIV)-Specific Chimeric Antigen Receptor-T Cells Engineered to Target B Cell Follicles and Suppress SIV Replication

FIG. 5A is a schematic of the construct design, and expression in rhesus macaque T cells. Schematics showing constructs encoding the chimeric antigen receptor (CAR) (rhCD4-MBL CAR), rhCXCR5, and the bi-cistronic CAR/CXCR5 encoding both proteins, with CXCR5 expression mediated by IRES or P2A. In all cases, the targeting domains are linked to domains from rhesus CD28 including a short extracellular hinge, transmembrane TM, and cytoplasmic signaling, followed by the CD3 activation domain.

FIG. 5B-FIG. 5F are graphs of flow cytometry of cells transduced with gamma-retroviral vectors encoding (Panel B) CAR; (Panel C) CXCR5; (Panel D) CAR/CXCR5 (IRES); (Panel E) CAR/CXCR5 (P2A); or (Panel F) mock-transduced. Cells were pre-gated sequentially on lymphocytes, singlets, live cells, and CD3+ cells (T cells) and evaluated for CAR and CXCR5 expression using antibodies against mannose binding lectin (MBL) and CXCR5, respectively.

FIG. 5G is a histogram depicting fluorescent intensities of CXCR5 expression from samples shown in Panels D-F.

FIG. 5H is a graph of the median percentage of T cells that expressed the CAR and CXCR5 in activated PBMCs transduced with CAR (n=3), CXCR5 (n=3), CAR/CXCR5 (IRES) (n=5), CAR/CXCR5 (P2A) (n=5) and mock-transduced (n=5).

FIG. 5I shows the percentage of T cells that co-expressed the CAR and CXCR5 in activated PBMCs transduced with either CAR/CXCR5 (IRES) or CAR/CXCR5 (P2A).

FIG. 6A-FIG. 6C are graphs showing that co-expression of CXCR5 promotes selective migration of chimeric antigen receptor (CAR)-T cells toward CXCL13 in vitro. The percentage of CAR- or CAR/CXCR5-transduced PBMC that migrated toward (FIG. 6A) CXCL12 (SDF-1) or (FIG. 6B) CXCL13 was measured in transwell plates. FIG. 6C shows the relationship between the percentage of cells expressing CXCR5 and the percentage of cells that migrated. In all panels, each data point symbol represents the mean value of

duplicate samples obtained with cells from individual animals, with colors indicating transduction with CAR (blue) or CAR/CXCR5 (red).

FIG. 7A are images showing that CXCR5 co-expression enhances CD8+ CAR-T cell migration to B cell follicles ex vivo. FIG. 7A shows chimeric antigen receptor (CAR; left) or CAR/CXCR5-transduced (right) rhesus macaque CD8+ T cells stained with cell trace violet dye (CTV) (pseudo-colored yellow), pipetted on to fresh rhesus macaque lymph node sections and incubated for 6 h at 37° C. Sections were then fixed and stained with anti-CD20 antibodies (green) to delineate B cell follicles ("F") and anti-CD3 antibodies (blue) to delineate the T cell zone and extrafollicular areas ("EF"). Arrowheads indicate CTV+ cells. Confocal images were collected with a 20× objective. Scale bars equal 100 μm.

FIG. 7B-FIG. 7F shows the experimental data demonstrating that CXCR5 co-expression enhances CD8+ CAR-T cell migration to B cell follicles ex vivo. Panel B shows similar total levels of CFSE-labeled CD8+ T cells were detected in tissues incubated with CAR- and CAR/CXCR5-transduced cells. Panel C demonstrates that CAR-transduced cells showed higher levels in the extrafollicular regions than in the follicles. Panel D demonstrates that, by contrast, CAR/CXCR5-transduced cells showed increased levels within B cell follicles. Panel E demonstrates that CAR/CXCR5-transduced cells showed higher F:EF ratios compared to CAR-transduced cells. Panel F shows the relationship between the percentage of transduced cells that expressed CXCR5 and F:EF ratios. Each symbol represents individual animals from which CD8+ T cells were derived.

FIG. 8 are graphs showing that chimeric antigen receptor (CAR)/CXCR5-transduced T cells suppress simian immunodeficiency virus (SIV) in vitro. PBMC target cells were infected with the indicated SIVmac239 (top row) and SIVE660 (bottom row) isolates for 24 h. The cells were then washed and mixed with the effector cells transduced as indicated, at effector-to-target ratios (E:T) of 1:1 (left panels) or 0.2:1 (right panels). Culture supernatants were collected on the indicated days, and the presence of virus was determined by p27 ELISA. The effector cells were transduced either with the CD4-mannose-binding lectin (MBL)-CAR alone or CD4-MBL-CAR plus CXCR5. As negative controls, no effector cells, or cells transduced with the negative control 139 CAR were used.

Part C—Nine Day Transduction and Expansion Protocol

FIG. 9 shows flow cytometric analysis of Rhesus macaque PBMCs transduced and expanded in 9 days sorted on live, singlet, CD3 T cells co-labeled with MBL antibodies (binds CD4-MBL-CAR; x-axis) and CXCR5 antibodies (y-axis).

FIG. 10 shows that the 9-day protocol described herein yields sufficient cell numbers for cellular immunotherapy.

FIG. 11 shows that co-expression was maintained after the 9-day protocol described herein.

FIG. 12 shows that viability was maintained after the 9-day protocol described herein.

FIG. 13 showing representative result central memory T cells (TCM) maintained after 9 day transduction and expansion. Phenotype definitions: CM defined as CD95+, CD28+, CCR7+; EM1 defined as CD95+, CD28+, CCR7-; EM2 defined as CD95+, CD28-, CCR7-.

FIG. 14 shows CTV-labeled CD4-CD3-CAR/CXCR5-transduced PBMCs (pseudo-colored red and indicated with arrow heads) inside B cell follicles of spleen (left) and LN (middle) but not in lung (right). Sections were stained with

either IgM antibodies or anti-CD20 antibodies to label B cells and identify follicles (F).

#### DETAILED DESCRIPTION

The methods described herein improve existing immunotherapy, particularly immunotherapy directed to B cells or B cell follicles. The methods described herein can be applied to any type of disease that includes B cell follicles, i.e., any type of disease in which the immunotherapy needs to reach B cell follicles, such as B cell lymphomas and other viral infections that include B cell follicles such as human immunodeficiency virus (HIV) or herpes viruses (e.g., HHV8, which causes Kaposi's sarcoma, or EBV, which can result in lymphomas).

The methods described herein are expected to result in a functional cure for HIV. As used herein, functional cure refers to suppressed viral replication in the absence of antiretroviral drugs. In some instances, functional cure refers to non-detectable levels of virus in the absence of antiretroviral drugs. Accordingly, the methods described herein can replace antiretroviral drug therapy to treat HIV. The methods described herein are expected to be substantially cheaper and have fewer side effects than antiretroviral drug therapy, and are expected to replace the need for conventional drugs. As used herein, HIV refers to both HIV type 1 (HIV-1) and HIV type 2 (HIV-2).

During HIV infection, virus-producing cells accumulate inside B cell follicles within lymphoid tissues; the same is true in the simian immunodeficiency virus (SIV)-infected rhesus macaque model of HIV B cell follicles have been shown to be, at least to some extent, immune-privileged sites, in that CD8 T cells are largely excluded from B cell follicles, which thereby permit ongoing viral replication (Connick et al., 2007, *J. Immunol.*, 178(11):6975-83)). The immune-privileged status of B cell follicles can explain the inability of HIV- and SIV-specific CD8 T cells to fully suppress virus replication.

Chimeric Antigen Receptor (CAR) T cell therapy is a type of immunotherapy that changes a subject's own T cells so they are better able to recognize and attack cancer cells or pathogens. It is demonstrated herein that engineering CAR-expressing CD8 T cells to also express CXCR5 (e.g., referred to herein as CAR/CXCR5 T cells) results in a substantial increase in the effectiveness of those immunotherapeutic T cells, to increase migration into B cell follicles. It has been shown that increasing levels of SIV-specific CD8 T cells in follicles is associated with a decrease in virus-producing cells in follicles, and also a decrease in plasma viral load. The same phenomena would be expected to occur in humans with HIV.

Thus, methods are provided that include collecting (e.g., obtaining, providing) cells from a subject having HIV, transducing those cells with both a CAR nucleic acid and a nucleic acid encoding a CXCR5 polypeptide, and returning those cells to the patient.

Cells collected from a subject can be any number of cells including, without limitation, a population of peripheral blood mononuclear cells (PBMCs; e.g., CD8 T cells, CD4 T cells and a few NK cells) or T cells (e.g., CD8 T cells). In addition or as an alternative to T cells, the methods described herein can be applied to NK cells (e.g., autologous or allogeneic NK cells) and/or hematopoietic stem cells (HSCs). This method also could be applied to engineered T cells that are not specific for a particular subject (e.g., universal T cells).

Cells can be collected from a subject using known methods. Typically, cells are collected in a biological sample. Biological samples can include, without limitation, blood, serum, cerebrospinal fluid, or tissues (e.g., tissue biopsy, e.g., muscle biopsy, skin biopsy). In some instances, apheresis is used to obtain the desired cells from the blood of a subject, which then can be returned back to the subject.

Following collection, cells can be transduced with one or more nucleic acid vectors or constructs (e.g., a CAR/CXCR5 construct, or a CAR construct and a construct encoding CXCR5). Methods of transducing cells are known in the art. For example, and without limitation, cells can be transduced with one or more nucleic acid molecules using, for example, mechanical means (e.g., electroporation), viral mechanisms (e.g., retroviruses carrying the CAR/CXCR5 construct), or gene editing (e.g., CRISPR or TALEN) (see, for example, Eyquem et al., 2017, *Nature*, 543:113-7).

CAR constructs are known in the art, and typically include an antigen-binding targeting portion, sometimes referred to as a ligand-binding targeting portion, and a signaling domain that results in T cell activation following binding by the antigen. The signaling domain can be a transmembrane domain, a cytoplasmic domain, or an intracellular domain.

CAR constructs and methods of making CAR constructs are known in the art. See, for example, Brentjens et al., 2010, *Mol. Ther.*, 18:4:666-8; Till et al., 2008, *Blood*, 12:2261-71; Park et al., 2011, *Trends Biotechnol.*, 29:550-7; Grupp et al., 2013, *N. Engl. J. Med.*, 368:1509-18; Han et al., 2013, *J. Hematol. Oncol.*, 6:47; Tumaini et al., 2013, *Cytotherapy*, 15:1406-17; Haso et al., 2013, *Blood*, 121:1165-74; WO 2012/079000; WO 2013/059593; US 2012/0213783; and U.S. Pat. No. 9,629,877, all of which are incorporated herein by reference in their entirety.

Specifically with respect to HIV, there are a number of CAR constructs that are known in the art. For example, and without limitation, see Liu et al., 2015, *J. Virology*, 89(13): 6685-94; Hale et al., 2017, *Mol. Ther.*, 25(3):570-9; Zhen et al., 2015, *Mol. Ther.*, 23(8):1358-67; U.S. Pat. Nos. 7,115,262; 7,151,087; and 8,420,099; US 2013/0189264; and US 2017/0267739, all of which are incorporated herein by reference in their entirety. A number of representative HIV CAR constructs are shown in SEQ ID NOs: 6, 8 and 10, which are encoded by the HIV CAR nucleic acid constructs shown in SEQ ID NOs: 7, 9 and 11, respectively. Representative HIV CAR constructs are described in more detail in WO 2015/077789.

A CAR construct (e.g., an existing CAR construct) can be genetically engineered to also include a nucleic acid molecule encoding a CXCR5 polypeptide, or a new vector can be generated that includes the necessary components of a CAR construct as well as a nucleic acid molecule encoding a CXCR5 polypeptide. Representative human CXCR5 polypeptides are shown in SEQ ID NO:1 (i.e., GenBank Accession No. NP\_001707.1) and SEQ ID NO:3 (i.e., GenBank Accession No. NP\_116743.1), which are encoded by the nucleic acid sequences shown in SEQ ID NO:2 (i.e., GenBank Accession No. NM\_001716.4) and SEQ ID NO:4 (i.e., GenBank Accession No. NM\_032966.2).

In some instances, it may be desirable to codon optimize the CXCR5 nucleic acid sequence and/or the CAR construct. Codon optimization is known in the art and can be performed using, for example, computer software. A representative computer software for codon optimizing a sequence can be obtained from IDT (see, for example, [idtdna.com/CodonOpt](http://idtdna.com/CodonOpt) on the World Wide Web), but others

are available. An example of a codon optimized human CXCR5 sequence is shown in SEQ ID NO: 5.

In some embodiments, a nucleic acid encoding a further polypeptide can be delivered to the T cells (e.g., CD4 T cells) such that the T cells are made resistant to infection by HIV. See, for example, Hale et al. (2017, *Mol. Ther.*, 25:570); and Younan et al. (2013, *Blood*, 122:179-87). A representative further polypeptide that can impart resistance to HIV infection by T cells is the mC46 polypeptide. A further polypeptide, whether it be to impart resistance to infection by HIV or for another purpose, can be included in the CAR/CXCR5 construct (e.g., under one of the existing promoters or under a different promoter) or, if CAR and CXCR5 are delivered on separate constructs, the further polypeptide can be included in one or the other or both of those constructs. Alternately, a further polypeptide can be included and delivered to the cells in its own construct.

Such a construct (e.g., CAR/CXCR5) or constructs (e.g., a CAR and a CXCR5) can be contained within a host cell or a virion for propagation outside of T cells and/or for maintenance in the T cells.

Methods of culturing and expanding the transduced cells are known in the art. In some instances, it may be desirable to include, without limitation, IL-15 or ALT-803, an IL-15 superagonist (NantKwest) in the culture. In some instances, it may be desirable to switch the cytokines that are present in the media during the culturing of the cells, which may lead to increased persistence of cells and fewer regulatory T cells (Treg cells). For example, various combinations of IL-15, IL-7, and/or IL-21, with or without IL-2, can be used in the culture media.

Following transduction of the patients' cells and appropriate culturing and expansion of the transduced cells, those cells are returned back to the same patient. Returning cells to a patient is oftentimes performed via infusion. Infusion is known in the art, and typically includes suspending the cells in a pharmaceutically acceptable carrier and introduced the composition back into the subject intravenously. Pharmaceutically acceptable carriers are known in the art and include, without limitation, saline or other physiological buffers, nutrients, preservatives, and the like.

In some instances, it may be desirable to lymphodeplete the subject prior to cellular immunotherapy to create space for the homeostatic proliferation of the infused cells. For example, lymphodepletion can be achieved using a chemotherapeutic such as cyclophosphamide (e.g., Cytosan) or, alternatively, an anti-CD20 antibody (e.g., Rituximab), which can temporarily deplete B cells and B cell follicles (i.e., the virus-producing cells).

As demonstrated herein, those T cells (e.g., CAR/CXCR5 T cells) migrate to B cell follicles and are able to significantly suppress the replication of HIV. As a part of the methods described herein, a subject can be monitored for the presence and/or amount (e.g., viral load) of HIV. In addition, because of the precise targeting provided by CXCR5, subjects receiving a CAR/CXCR5 construct as described herein would not require intravenous immunoglobulin (IVIG) treatments, as is needed for B cell depleting CARs like CD19-specific CARs, as they would not remain immunocompromised. For example, subjects (e.g., virally suppressed subjects on cART or infected subjects who have not received cART) can be infused with CAR/CXCR5 T cells (e.g., autologous CAR/CXCR5 T cells) or CAR/CXCR5 NK cells (e.g., autologous or allogeneic CAR/CXCR5 NK cells) as described herein. Upon receiving the CAR/CXCR5 T cells or CAR/CXCR5 NK cells, the subject typically will no longer receive antiviral therapy. The autologous CXCR5/

CAR T cells, or autologous or allogeneic CAR/CXCR5 NK cells, home to lymphoid B cell follicles, where HIV-producing cells are most concentrated, and suppress viral replication. In many subjects, immunotherapy, which is significantly improved by the presence of the CXCR5 polypeptide described herein, can lead to a functional cure of HIV.

In accordance with the present invention, there may be employed conventional molecular biology, microbiology, biochemical, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. The invention will be further described in the following examples, which do not limit the scope of the methods and compositions of matter described in the claims.

## EXAMPLES

### Part A—Preliminary Results

#### Example 1-CAR/CXCR5 Vectors and Transduced Cells

An HIV-1-specific CAR has been developed (Liu et al., 2015, *J. Virology*, 89(13):6685-94). In the field, CAR constructs have been generated in both a lentiviral vector background as well as a mouse gamma retroviral vector background. These vectors have been constructed with an internal ribosome entry site (IRES) to drive expression of CXCR5 with the CAR or with a protease cleavage site from which co-expressed proteins get cleaved by cellular proteases after expression. See, for example, Vagner et al., 2001, *EMBO Reports*, 2:893-8; Chinnasamy et al., 2006, *Virology J.*, 3:14.

Virus binding is primarily mediated by interaction of gp120, the external subunit of the HIV envelope glycoprotein (Env) with CD4 protein and various coreceptor molecules (one of several alternative chemokine receptors). These interactions then activate the gp41 transmembrane subunit of the envelope glycoprotein, to cause fusion between the virus and cell membranes.

Human CXCR5 (SEQ ID NO:2) was sub-cloned into the plasmid vectors containing the HIV-specific CAR, and rhesus CXCR5 (encoding the polypeptide shown in GenBank Accession No. XP\_001100017) was sub-cloned into the vectors containing the SIV-specific CAR to generate HIV- and SIV-based CXCR5/CAR vectors, respectively. FIG. 1 is a schematic of two of these vectors; FIG. 1A shows a SIV-based CAR vector (Rh CD4 MBL2 CAR), while FIG. 1B shows a SIV-based CXCR5/CAR vector (Rh CD4 MBL2 CAR+CXCR5). The plasmid vectors were verified by sequencing, used to generate pseudotyped retroviral vectors, and the virions were produced and titrated.

Primary CD8 T cells from humans and rhesus macaques then were transduced with each vector (or a CAR-only vector). Transduction efficiency was determined by staining transduced (and mock-transduced) cells with antibodies directed against CXCR5 and CAR. The viability of transduced cells was determined using a live-dead cell stain. FIG. 2 shows the co-expression of human CAR and CXCR5 in human PBMC, and FIG. 3 shows the co-expression of corresponding rhesus proteins in rhesus PBMC.

#### Example 2—Efficacy of CAR/CXCR5 Vector Transduced Cells

A comprehensive multicolor flow cytometric analysis of CXCR5-transduced cells is performed to determine the percentage and viability of transduced cells, as well as CD8

naïve, regulatory, memory, and memory stem cell subsets. Such flow cytometry strategies are well-established (Salusto et al., 2004, *Annu. Rev. Immunol.*, 22:745-63).

Efficacy of the CAR/CXCR5 T cells can be demonstrated by showing that the CAR/CXCR5-transduced T cells 1) migrate towards the ligand for CXCR5, CXCL13; 2) migrate to B cell follicles in tissues processed in the lab; and/or 3) migrate to B cell follicles in SIV-infected rhesus macaques.

The ability of transduced cells to migrate towards CXCL13 is assessed using a transwell chemotaxis assay as previously described (Meditz et al., 2008, *AIDS Res. Hum. Retrovir.*, 24(7):977-85).

Migration of CAR/CXCR5 transduced cells into B cell follicles was also demonstrated in an ex vivo migration assay. Here, rhesus macaque PBMCs were transduced with CAR or CAR/CXCR5 vectors, labeled with CTV, and dripped onto slabs of fresh rhesus macaque lymph nodes. Tissues were subsequently stained with anti-CD20 antibodies to delineate B cell follicles ("F") and anti-CD3 antibodies to delineate the T cell zone and extra-follicular areas ("EF") (FIGS. 4A and 4B). Similar total levels of CTV-labeled cells were detected in tissues treated with CAR and CAR/CXCR5 transduced cells (FIG. 4C). CAR-transduced cells showed similar levels in follicles and outside of follicles, whereas CAR/CXCR5-transduced cells primarily located within B cell follicles (FIG. 4D). CAR/CXCR5-transduced cells showed much higher F:EF ratios compared to CAR-transduced cells. Thus, these experiments demonstrated that CXCR5 induced migration in an ex vivo B cell follicle migration assay.

Example 3—Treatment of SIV-Infected Rhesus Macaque Chronically SIVmac239- or SIVmac251 infected rhesus macaque are treated with CXCR5/CAR transduced cells. CXCR5/CAR T cells are labeled with a live cell stain, such as CFSE or CTV, prior to infusion so that the cells can be tracked. CXCR5/CAR T cells, CD4 and CD8 T cell counts, and viral loads in blood are evaluated before treatment and, following treatment, are monitored over time. In addition to blood PBMC, CXCR5/CAR cells are tracked and quantified in lymphoid tissues (lymph node, spleen, GALT), and bronchoalveolar lavage (BAL) at multiple time points using flow cytometry (using CFSE+CD8+CXCR5+CD4+MBL+). On day 60 following the infusion, the animal is sacrificed and a necropsy performed in which multiple tissues are collected and examined. The tissues that are collected include lymph nodes (inguinal, axillary, iliac, mesenteric), spleen, gut associated lymphoid tissues, liver, brain (obex) and lung.

In tissues, transduced cells are visualized in situ using CFSE and immunostaining with antibodies (e.g., the antibodies described above for use in flow cytometry) in combination with anti-CD20 to define B cell follicles morphologically as previously described (Connick et al., 2007, *J. Immunol.*, 178(11):6975-83). In addition, anti-FITC antibodies may be used to amplify weak signal from CFSE-labeled cells that have gone through multiple cell divisions. As a secondary means to track transduced cells in tissues, in situ hybridization with a probe specific for the retroviral vector, combined with anti-CD20 staining, can be performed as described (Connick et al., 2007, *J. Immunol.*, 178(11):6975-83). Similarly, SIV producing cells are quantified in tissues using in situ hybridization as described (Connick et al., 2007, *J. Immunol.*, 178(11):6975-83).

Part B—Simian Immunodeficiency Virus (SIV)-Specific Chimeric Antigen Receptor-T Cells Engineered to Target B Cell Follicles and Suppress SIV Replication

#### Example 4—Plasmid Constructs and Retroviral Vectors Encoding CARs

All CAR targeting motifs were synthesized by GenScript, codon-optimized for expression in rhesus macaque cells, and sub-cloned into the plasmid pMSGV1 gamma-retrovirus vector backbone (Hughes et al., 2005, *Hum. Gene Ther.*, 16:457-72). The active antiviral CAR employed in this study was a rhesus variant of the human bispecific CAR designated CD4-MBL (Ghanem et al., 2017, *Cytother.*, doi: 10.1016/j.jcyt.2017.11.001). As a non-reactive negative control, the previously described 139 CAR was used, which does not react with cells in this system. The targeting domains were linked to extracellular hinge, transmembrane and cytoplasmic co-stimulatory domain of rhesus CD8 followed by the activation domain of rhesus CD3 zeta, as previously described (Liu et al., 2015, *J. Virol.*, 89:6685-94; Ghanem et al., 2017, *Cytother.*, doi:10.1016/j.jcyt.2017.11.001).

T cells were transduced to express either the rhCD4-MBL CAR, rhCXCR5, or the rhCD4-MBL CAR plus rhCXCR5. For co-expression, bi-cistronic plasmid constructs (produced by GenScript) were designed in which the rhCD4-MBL gene was linked to the downstream rhCXCR5 gene. CXCR5 expression was driven by either the ECMV internal ribosome entry site (IRES) or the self-cleaving P2A peptide from porcine teschovirus-1 with a GSG linker added at the N-terminus of the P2A peptide sequence (Kim et al., 2011, *PLoS One*, 6:e18556). The corresponding gamma-retroviruses were generated for expression of these genes in rhesus macaque T cells. In most experiments, these plasmids were co-transfected with the plasmid pBS-CMV-gagpol (Delang et al., 2016, *Sci. Rep.*, 6:31819) (a gift from Dr. Patrick Salmon, Addgene plasmid #35614), a plasmid encoding RD114 envelope glycoprotein (Porter et al., 1996, *Hum. Gene Ther.*, 7:913-9), and the plasmid pMD.G encoding VSV-G envelope (Gori et al., 2007, *J. Pharmacol. Exp. Ther.*, 322:989-97) (a gift from Dr. Scott McIvor) at ratios of 3:1:1:0.4, respectively. Retroviral vector supernatants were collected 48 h after transfection, and were titrated by transducing HEK293T cells. Retrovirus was snap frozen and stored at -80° C. In the SIV suppression studies, gamma-retrovirus vector production was carried out as previously described (Liu et al., 2015, *J. Virol.*, 89:6685-94).

#### Example 5—Transduction of Rhesus T Cells

Primary rhesus macaque PBMC, or CD8+ T cells enriched by negative selection (Miltenyi), were activated for 2 to 3 days in six-well plates with plate-bound anti-CD3 (FN18) and soluble anti-CD28.2 (both from NUP Reagent Resource) in either RPMI supplemented with 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 300 IU/ml IL-2, for early experiments, or in X-Vivo 15 completed with 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 50 IU/ml IL-2 for later experiments. RetroNectin (TaKaRa)-mediated transduction was carried out on the activated T cells. Retroviral vector supernatants, diluted in serum-free media, were added (eventual MOI of 0.5) to RetroNectin-coated six-well plates and centrifuged for 2 h at 2,000×g to facilitate binding of the retrovirus. After removal of the unbound retrovirus, activated PBMC or CD8+ T cells (1.5x



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10E6 cells/well) were added to the wells and centrifuged at 1,000×g for 10 min. Mock-transduced cells were subjected to exactly the same procedures without the addition of retrovirus to the RetroNectin-coated wells. Cells were cultivated in the media listed above for 5-6 days prior to analysis by flow cytometry.

## Example 6—Flow Cytometry

Cells were analyzed using an LSR Fortessa flow cytometer (BD Bioscience). The following antibodies were used: CD4 (M-T477, reactive with endogenous rhCD4 and the rhCD4-MBL CAR), CD3 (SP34-2), CD8 (RPA-T8) (all from BD Bioscience), CXCR5 (MU5UBEE) (eBioscience), MBL2 (3E7) (Invitrogen). Viability was assessed with the Live/Dead Fixable Near IR Dead Cell Stain Kit (Invitrogen). A minimum of 70,000 events were acquired for each sample. Data analysis utilized FlowJo v10 (FlowJo, LLC).

## Example 7—In Vitro Transwell Migration Assay

Rhesus macaque PBMCs were transduced with the CAR or CAR/CXCR5 vectors, or mock-transduced. Samples were run in duplicate. For each sample, one million cells in 100 µl X-Vivo-15 media containing 0.1% BSA were placed in the upper chamber of a 24-well plate, with a 5.0 µm transwell membrane (Costar). To the lower chamber containing 600 µl X-Vivo 15 and 0.1% BSA, either CXCL12 at 1 µg/ml or CXCL13 at 2.5 µg/ml (both from ProSpec) were added. No chemokine was added to control wells. After incubation for 4 h at 37° C., cells were collected from the lower chamber, fixed with 1% paraformaldehyde, and counted on a Cytotflex flow cytometer (Beckman). All samples were normalized with the addition of AccuCheck Counting Beads (Invitrogen). Specific cell migration was determined by first subtracting the number of cells that migrated to media alone from the number of cells that migrated to the chemokine and then dividing by the number of cells added to the upper chamber.

## Example 8—Ex Vivo B Cell Follicle Migration Assay

Chimeric antigen receptor- and CAR/CXCR5-transduced rhesus CD8+ T cells were used in conjunction with fresh lymph node tissue sections from allogeneic rhesus macaques. A gelatin sponge (7 mm Gel foam by Pfizer) was cut to fit and placed into a six-well plate containing 3-4 ml of RPMI with 20% heat inactivated FBS. The sponge was hydrated for 1 h at 37° C. Fresh rhesus macaque lymph nodes, collected at the Wisconsin National Primate Research Center, were shipped in chilled RPMI containing 100 µg/ml heparin overnight on ice blocks. Lymph nodes were cut into 0.5 cm×0.5 cm pieces and embedded in 40° C. PBS-buffered 4% low-melt agarose and cut into 300-µm thick slices using a Compressstone, as previously described (Abdelaal et al., 2015, Biol. Proced. Online, 17:2). Tissue sections and associated agarose were laid flat on the hydrated sponge without being submerged. Transduced CD8+ T cells were stained with a 5-µM solution of Cell Trace Violet Dye (CTV) (Molecular Probes). The dye was added at a 1:1 ratio to 1×10E7 cells/ml suspended in PBS/10% FBS, and cells were incubated for 15 min at 37° C., followed by two washes with complete RPMI supplemented with 10% heat inactivated FBS 100 U/ml penicillin, and 100 µg/ml streptomycin. For each fresh tissue section, one million CTV-stained transduced CD8+ T cells were re-suspended in 20-30 µl

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complete RPMI and were slowly pipetted onto the surface of the tissue. Tissue sections were incubated at 37° C. for 6 h followed fixation with 4% PBS-buffered paraformaldehyde for 2 hours at room temp. After fixation, sections were washed with chilled PBS containing 100 µg/ml heparin (PBS-H). Antigen retrieval was carried out by boiling tissues 3× in 0.01 M urea for 30 s. Tissues were permeabilized and blocked with PBS-H containing 0.3% Triton x-100 and 2% normal goat serum for 1 h, then incubated overnight with mouse-anti-human CD20 (0.19 µg/ml, clone L26, Novocasttra) to label B cells and rat-anti-human CD3 (2 µg/ml, CD3-12, Bio-Rad) to label T cells. After washing with PBS-H, secondary antibody staining was carried out by incubating tissues overnight with goat-anti-mouse-IgG/Alexa 488 (0.75 µg/ml Jackson ImmunoResearch Laboratories) and goat-anti-rat-IgG/Cy5 (0.3 µg/ml, Jackson ImmunoResearch Laboratories). All incubations were done at 4° C. on a rocking platform. Sections were imaged using a Leica confocal microscope. 512×512 pixel z-series were collected using a step size of 2 µm and with collection initiated at least 50 µm deep into each section. B cell follicles were identified morphologically as clusters of brightly stained closely aggregated CD20+ cells. Areas that showed loosely aggregated B cells that were ambiguous as to whether the area was a follicle were not included. Cell counts were done with individual z-scans. The total number of CTV-stained cells was counted inside follicles and the adjacent area outside of the follicles. For each sample, 2-3 tissue sections and a minimum of three follicles (range 3-8) were evaluated.

## Example 9-SIV Suppression Assay

To generate SIV-infected target cells, rhesus macaque PBMCs were re-suspended at 5×10E5/ml in complete medium, transferred to a T25 flask, and incubated at 37° C. in 5% CO2 for 2-3 days. The PBMCs were washed, adjusted to 3×10E6/ml in total of 4 ml volume in complete media containing 30 IU/ml IL-2, and incubated with 200-600 TCID50/ml of virus for 24 h at 37° C. in 5% CO2. Infected cells were washed three times using 20 ml of medium per wash and then re-suspended in complete medium at a density of 1.5×10E6 cells per ml in 96-well round bottom plates. To generate effector cells, T cells (derived from activated PBMCs) were transduced with the indicated gamma-retroviral vectors. In triplicate, 100 µl of SIV-infected targets were mixed with 100 µl of serially diluted effectors. Co-cultures were incubated at 37° C. in 5% CO2 for a total of 16 days. On the indicated days, supernatants were collected, and p27 content was determined by ELISA (ABL, Inc.).

## Example 10—Statistical Analysis

All statistical analyses assumed two-sided tests with P<0.05 considered significant. Paired t-tests with pooled variance were used to evaluate co-expression levels of the CAR and CXCR5 via IRES versus P2A constructs. An unpaired t-test with pooled variance was used to evaluate groups in the CXCL12 in vitro migration assays while an unpaired Welch's t-test of unequal variance was used to evaluate groups in the CXCL13 in vitro migration assays. Paired t-tests with pooled variance were used in all statistical analyses in the ex vivo migration assay. The F:EF ratios were log transformed before analysis. Statistical analyses

were conducted using GraphPad Prism (Version 6.01; GraphPad Software, Inc., La Jolla, CA, USA).

#### Example 11—Results

The goal of this work was to engineer rhesus macaque T cells to co-express a potent anti-SIV CAR along with CXCR5, in order to promote CAR-T cell trafficking to B cell follicles. To this end, constructs for expression of the CARs were designed, with or without co-expression of CXCR5. CAR-transduced T cells were analyzed using both an *in vitro* trans-well assay of chemokine-directed cell migration and a novel *ex vivo* B cell follicle migration assay. In addition, the ability of T cells expressing CAR and CXCR5 to suppress viral replication was tested *in vitro*.

#### Example 12—CAR and CXCR5 Expression in Transduced Primary Rhesus Macaque T Cells

Gamma-retroviral vectors encoding the rhCD4-MBL CAR and rhCXCR5 constructs were developed, and vectors encoding bicistronic constructs to express both proteins were developed. Two variations of bicistronic vectors were developed, one with CXCR5 co-expression driven by an internal ribosome entry site (IRES) and the other via a P2A self-cleavage site (Kim et al., 2011, PLoS One, 6:e18556). For simplicity, the constructs encoding the rhCD4-MBL CAR alone or the bicistronic rhCD4-MBL CAR plus rhCXCR5 are, respectively, referred to as CAR or CAR/CXCR5; for the latter, the use of either the IRES or P2A modalities is indicated. The constructs are shown schematically in FIG. 5A.

In FIGS. 5B-5F, T cells derived from activated rhesus PBMCs were transduced with gamma-retroviral vectors encoding the CAR or CXCR5 genes alone, or the bicistronic CAR/CXCR5 constructs (IRES or P2A). Cell viabilities post-transduction were 87-90% (data not shown). Antibodies directed against MBL or CXCR5 were used to detect surface expression of the CAR and CXCR5, respectively. Transduction with the CAR (FIG. 5B) or CXCR5 (FIG. 5C) vectors gave the expected surface expression of the corresponding individual proteins. For vectors encoding the bi-cistronic CAR/CXCR5, the P2A-based construct yielded a clear population of cells expressing both CAR and CXCR5, with only a small fraction of cells expressing only one of the proteins; by contrast, the IRES-based construct appeared less effective at co-expressing CXCR5 relative to CAR, since the fraction of cells expressing only the CAR was comparable to that expressing both proteins, with a minimal fraction expressing only CXCR5 only (FIGS. 5D, 5E). These results are consistent with the efficient P2A system producing equivalent amounts of the two post-cleavage components of a bi-cistronic construct, as contrasted with the relatively inefficient expression of the downstream component in the IRES system (Mizuguchi et al., 2000, Mol. Ther., 1:37-82; Ibrahimi et al., 2009, Hum. Gene Ther., 20:845-60). Moreover, as indicated in FIG. 5G, the P2A-based construct produced cells with nearly twofold higher surface expression levels of CXCR5 than obtained with the IRES-based construct (median 1.8-fold higher; range 1.4- to 2.2-fold).

The percentages of T cells that expressed the CAR and CXCR5 with each construct are shown in FIG. 1H. Transduction with the vectors encoding CAR-only or CXCR5-only yielded a median of 44.4% (range 40-47.6%) and 81.1% (range 51.8-84.2%) of cells expressing each protein, respectively. Cells transduced with the IRES-based bi-cis-

tronic CAR/CXCR5 vector showed higher number of cells expressing the CAR compared to CXCR5, with a median cell expression of 37.2% (range 5.6-50.2%) for the CAR and 19.4% (range 4.7-19.7%) for CXCR5. In contrast, cells transduced with the P2A-based CAR/CXCR5 vector showed similar expression of the two proteins, with a median of 40.2% (range 37.3-52.2%) for the CAR and 38.9% (range 27.5-50.2%) for CXCR5. Similar transduction efficiencies were found with enriched rhesus CD8 T cells transduced with these vectors (data not shown). The percentage of cells that co-expressed CAR and CXCR5 is shown in FIG. 5I. Cells transduced with the IRES-based construct showed a median co-expression efficiency of 18.1% (range 4.3-18.9%), whereas cells transduced with the P2A-based construct resulted in a significantly higher co-expression efficiency of 33.3% (range of 25.9-38.3). Thus, the data in FIG. 5 establish the suitability of the P2A-based bi-cistronic system for efficient co-expression of CAR and the B cell follicle-homing chemokine receptor CXCR5, and its superiority over the IRES-based system.

#### Example 13—CXCR5 Co-Expression Promotes CAR-T Cell Migration Selectively to CXCL13 *In Vitro*

The ability of CXCR5 co-expression to promote migration of CAR-T cells toward CXCL13, the chemokine ligand for CXCR5, was next tested. To this end, an *in vitro* transwell migration assay was utilized. Using this assay, it was found that both CAR-transduced and CAR/CXCR5-transduced PBMCs similarly migrated toward a positive control chemokine CXCL12 (SDF-1 alpha) that is strongly chemotactic for lymphocytes (Bleul et al., 1996, 184:1101-9), demonstrating the ability of both CAR and CAR/CXCR5-transduced cells to migrate to a chemotactic stimulus (FIG. 6A). In contrast, significantly more CAR/CXCR5-transduced than CAR-transduced PBMCs migrated toward CXCL13 (FIG. 6B). Furthermore, increasing specific migration to CXCL13 was seen with an increase in the percentage of cells expressing CXCR5 (FIG. 6C). For these studies, a median of 54% (range 12-64%) of CAR/CXCR5-transduced cells expressed CXCR5. By contrast, a median of only 2% (range 1-5%) of the CAR-transduced cells expressed CXCR5 and they showed minimal migration to the stimulus. These results demonstrate that co-expression of CXCR5 promotes selective migration of the CAR-T cells toward CXCL13 *in vitro*.

#### Example 14—CAR/CXCR5-Transduced CD8+ T Cells Selectively Migrate into B Cell Follicles *Ex Vivo*

As an additional means to evaluate the ability of CXCR5 to promote selective migration of CAR-T cells, a novel *ex vivo* B cell follicle migration assay was developed. This method was adapted from previously described *ex vivo* live tissue migration assays that tracked T cells in mouse thymus tissue using two-photon microscopy (Melichar et al., 2013, Sci. Signal, 6:ra92; Dzhagalov et al., 2013, PLoS Biol, 11:e1001566). For these studies, the migration of CTV-labeled CAR- and CAR/CXCR5-transduced primary rhesus macaque CD8+ T cells were evaluated in fresh lymph node tissue sections. FIG. 7A shows representative images of sections incubated with CTV-labeled CAR and CAR/CXCR5-transduced cells. Similar levels of total CTV+ cells were detected in lymph node sections incubated with CAR versus CAR/CXCR5-transduced cells (FIG. 7B). While total

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numbers of cells were similar, significant differences were observed in the levels of CAR- compared to CAR/CXCR5-transduced cells in follicular and extrafollicular compartments. Significantly lower levels of CTV+ cells were found in follicular compared to extrafollicular areas in sections incubated with CAR-transduced cells (FIG. 7C). In contrast, significantly higher levels of CTV+ cells were found in follicular compared to extrafollicular areas in sections incubated with CAR/CXCR5-transduced cells (FIG. 7D). As a result, significantly large increases in the follicular to extrafollicular ratios (F:EF) of CTV-labeled cells were detected in the tissue sections incubated with CAR/CXCR5-compared to CAR-transduced T cells. Sections incubated with CAR/CXCR5-transduced cells showed a median F:EF ratio of 2.8 (range of 1.5-6.9), whereas sections incubated with CAR-transduced T cells showed a median ratio of 0.4 (range 0.3-0.7) (FIG. 7E). An increased follicular to extrafollicular ratio was seen with an increase in the percentage of cells expressing CXCR5 (FIG. 7F). A median of 46% (range 23-71%) of CAR/CXCR5-transduced cells expressed CXCR5 and showed relatively high F:EF ratios. By contrast, a median of only 1.6% (range 0.2-4.1%) of the CAR-transduced cells expressed CXCR5 and showed correspondingly low F:EF ratios. Thus, in this novel ex vivo B cell follicle migration assay, CAR/CXCR5- but not CAR-transduced CD8+ T cells preferentially migrated to B cell follicles.

#### Example 15-CXCR5 Co-Expression Does Not Impair CAR-T Cell-Mediated Suppression of SIV Replication In Vitro

The all-rhesus CD4-MBL CAR (rhCD4-MBL) displayed potent suppression of multiple SIV strains. For this study, it was examined whether co-expression of CXCR5 affected the potency of SIV suppression by T cells expressing the rhesus CD4-MBL CAR. PBMCs transduced with the CAR or CAR/CXCR5 vectors, were co-cultured with rhesus PBMC targets infected with two different pathogenic SIV isolates, SIVmac239 and SIVE660. The negative controls employed included adding no effector T cells, and adding effector T cells that were transduced with the 139 CAR that recognizes an irrelevant epitope (a glioma-specific variant of the epidermal growth factor receptor (Morgan et al., 2012, Hum. Gene Ther., 23:1043-53)). Robust spreading of viral infection by both SIV strains was evident in the presence of the negative control effector cells (no effector T cells and 139 CAR-transduced T cells). In contrast, CAR-transduced and CAR/CXCR5-transduced effectors suppressed infection by both strains with equivalent high potency over the 12-day infection, at E:T ratios of 1:1 or 0.2:1 (FIG. 8). These data demonstrate that the antiviral activity of CAR-T cells is not altered by co-expression of the CXCR5 follicular trafficking chemokine receptor on the effector cell surface.

Part C—Nine Day Transduction and Expansion Protocol

#### Example 16—Retroviral Vector Production

Gamma-retroviruses were generated for expression of CAR and/or CXCR5 in rhesus macaque T cells. Lipofectamine 2000 (Invitrogen)-mediated transfection was carried out using CAR, CXCR5 or CAR P2A CXCR5 pMSGV plasmids as well as the plasmid pBS-CMV-gagpol (a gift from Dr. Patrick Salmon, Addgene plasmid #35614), a plasmid encoding RD114 envelope glycoprotein, and the plasmid pMD.G encoding VSV-G envelope (a gift from Dr. Scott McIvor) at ratios of 3:1:1:0.4, respectively. Retroviral

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vector supernatants were collected 48 hours after transfection and centrifuged to remove debris.

Lentivirus preparations were generated using pCL20cSLFR MSCV transfer plasmid as well as pCAG-SIVgprre (gag/pol), pMDG (VSV-G envelope) and pCAG4-RTR-SIV (tat/rev) at a ratio of 1:0.6:0.2:0.2 using lipofectamine-mediated transfection. Media was exchanged after 18 hr. Viral supernatants were collected 48 hr later and centrifuged to remove debris. All virus preparations were snap frozen and stored at -80° C. Functional titer was determined by transducing HEK293T cells with the virus.

#### Example 17—Activation and Transduction of Rhesus T Cells

Primary rhesus macaque PBMC were activated for 2 days in 6 well plates with plate-bound anti-CD3 (FN18) and soluble anti-CD28.2 (both from NHP Reagent Resource) in X-Vivo 15 completed with 10% heat inactivated FBS, 100 U/ml penicillin, 100 g/ml streptomycin, 2 mM glutamine, and 50 IU/ml IL-2. For PBMC activated with CD3 and CD28 antibodies, the population becomes highly enriched for T cells. RetroNectin (TaKaRa)-mediated transduction was carried out on the activated T cells with the gamma-retrovirus at an MOI of 0.5. After coating 6 well plates with RetroNectin, retroviral vector supernatants, diluted in serum-free media, were added and centrifuged for 2 hours at 2000xg to facilitate binding of the retrovirus. After removal of the unbound retrovirus, activated PBMC (1.5x10E6 cells/well) were added to the retrovirus-coated wells and centrifuged at 1000xg for 10 minutes. Mock-transduced cells were subjected to exactly the same procedures without the addition of retrovirus to the RetroNectin-coated wells. Cells were cultivated for 2 days prior to analysis by flow cytometry.

#### Example 18—Expansion of Transduced Rhesus T Cells

Two days post transduction, cells were seeded in a G-Rex 6 well plate (Wilson Wolf) at a density of 1x10E6 cells/cm<sup>2</sup> in 30 ml of X-Vivo 15 completed with 10% heat inactivated FBS, 100 U/ml penicillin, 100 g/ml streptomycin, 2 mM glutamine, and 50 M beta-mercaptoethanol, and 50 IU/ml IL-2. Cells were grown undisturbed for 4 days before collection and analysis by flow cytometry.

#### Example 19-CTV Staining of Transduced Cells

Cells were stained with the Cell Trace Violet Cell Proliferation kit (Invitrogen). Briefly, cells were placed in aliquots of 60x10E6 cells suspended in PBS containing 10% FBS. Cell Trace Violet, diluted in PBS, was added to a final concentration of 5.5 M and cells were incubated for 15 min at 37° C. The reaction was stopped with a 5 min incubation in 5 times the reaction volume of complete media. Cells were centrifuged and re-suspended in complete medium and allowed to rest for 10 minutes before use.

#### Example 20 Flow Cytometry

Cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter). For the 6 color standard panel, the following antibodies were used: CD4 (M-T477, reactive with endogenous rhCD4 and the rhCD4-MBL CAR), CD3 (SP34-2), CD8 (RPA-T8) (all from BD Bioscience), CXCR5 (MUSUBEE) (eBioscience), MBL2 (3E7) (Invitrogen).

Viability was assessed with the Live/Dead Fixable Near IR Dead Cell Stain Kit (Invitrogen). For the 11 color panel, the following antibodies were used in addition to CTV and the Live/Dead stain: CD4 (M-T477), CD3 (SP34-2), CD8 (RPA-T8), CCR7 (150503), CD28 (CD28.2), CD95(DX2) (all from BD Bioscience), CXCR5 (MUSUBEE) (eBioscience), MBL2 (3E7) (Invitrogen), CD159a (Z199) (Beckman Coulter). A minimum of 70,000 events were acquired for each sample. Data analysis utilized FlowJo v10 (FlowJo, LLC).

#### Example 21—Results

Methods have been developed that allow transduction (e.g., for CAR and CXCR5 co-expression) and expansion of cells in 9 days while maintaining viability and ~30% central memory T cells (TCM) (less differentiated memory cells). Most other methods of transducing and expanding cells take weeks, so the methods described herein represent a significant improvement in time without any apparent loss of quality. Rhesus macaque CD4-MBL-CAR/CXCR5 T cells were used herein, but the methods could be applied to cells from other species using similar but species-specific vectors.

Rhesus macaque PBMCs were transduced with CAR and CXCR5 and expanded in 9 days. Flow cytometric analysis of cells sorted on live, singlet, CD3 T cells co-labeled with MBL antibodies, which bind CD4-MBL-CAR, and CXCR5 antibodies is shown in FIG. 9.

The 9-day procedure described herein yields sufficient cell numbers for cellular immunotherapy (FIG. 10). This procedure was used to yield 10E8 cells/kg for treatment of an SIV-infected macaque. These experiments also demonstrated that co-expression (FIG. 11) and viability (FIG. 12) was maintained after expansion.

Central memory T cells (TCM) were maintained after 9 day transduction and expansion (FIG. 13). CD4-MBL-CAR/CXCR5 transduced cells were produced with the 9 day protocol. Briefly, rhesus PBMC were stimulated with anti CD3/anti CD28 beads or with plate bound anti CD3 and soluble anti CD28 for two days, subjected to one round of transduction with CAR/CXCR5 retrovirus and then expanded for 4 days in the GREX plate in 50 U/ml IL-2, 50  $\mu$ M beta mercaptoethanol. Cells were cryopreserved on d9, thawed and used immediately in flow cytometry. Gating strategy: Lymphocytes, singlets, live cells, CD3+, CD8+ for total cells or lymphocytes, singlets, live cells, CD3+, MBL+, CD8+ for transduced cells. Phenotype definitions: CM

defined as CD95+, CD28+, CCR7+; EM1 defined as CD95+, CD28+, CCR7-; EM2 defined as CD95+, CD28-, CCR7-.

An SIV infected animal was treated with CD4-MBL-CAR/CXCR5 T cells labeled with a live cell stain (CTV) and the animal was sacrificed two days later and the localization of the labeled cells evaluated. CTV-labeled cells were detected in spleen, lymph nodes, liver, and very few in lung. In the spleen and lymph nodes, CTV-labeled cells were detected in B cell follicles. Clusters of CTV+ cells were detected in the T cell zones, likely proliferating (a site that T cells normally undergo expansion). These data also indicate that 9 day transduction and expansion protocol described herein does not lead to transduced cells accumulating in the lung, which is an ongoing problem.

FIG. 14 shows that CTV-labeled CD4-CD3-CAR/CXCR5-transduced PBMCs (pseudo-colored red and indicated with arrow heads) were located inside B cell follicles of spleen (left) and LN (middle) but not in lung (right). Sections were stained with either IgM antibodies or anti-CD20 antibodies to label B cells and identify follicles (F).

It is to be understood that, while the methods and compositions of matter have been described herein in conjunction with a number of different aspects, the foregoing description of the various aspects is intended to illustrate and not limit the scope of the methods and compositions of matter. Other aspects, advantages, and modifications are within the scope of the following claims.

Disclosed are methods and compositions that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that combinations, subsets, interactions, groups, etc. of these methods and compositions are disclosed. That is, while specific reference to each various individual and collective combinations and permutations of these compositions and methods may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular composition of matter or a particular method is disclosed and discussed and a number of compositions or methods are discussed, each and every combination and permutation of the compositions and the methods are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed.

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Leu Phe Trp Glu Leu Asp Arg Leu Asp Asn Tyr Asn Asp Thr Ser Leu  
20 25 30

Val Glu Asn His Leu Cys Pro Ala Thr Glu Gly Pro Leu Met Ala Ser  
35 40 45

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Phe Lys Ala Val Phe Val Pro Val Ala Tyr Ser Leu Ile Phe Leu Leu  
 50 55 60  
 Gly Val Ile Gly Asn Val Leu Val Leu Val Ile Leu Glu Arg His Arg  
 65 70 75 80  
 Gln Thr Arg Ser Ser Thr Glu Thr Phe Leu Phe His Leu Ala Val Ala  
 85 90 95  
 Asp Leu Leu Leu Val Phe Ile Leu Pro Phe Ala Val Ala Glu Gly Ser  
 100 105 110  
 Val Gly Trp Val Leu Gly Thr Phe Leu Cys Lys Thr Val Ile Ala Leu  
 115 120 125  
 His Lys Val Asn Phe Tyr Cys Ser Ser Leu Leu Leu Ala Cys Ile Ala  
 130 135 140  
 Val Asp Arg Tyr Leu Ala Ile Val His Ala Val His Ala Tyr Arg His  
 145 150 155 160  
 Arg Arg Leu Leu Ser Ile His Ile Thr Cys Gly Thr Ile Trp Leu Val  
 165 170 175  
 Gly Phe Leu Leu Ala Leu Pro Glu Ile Leu Phe Ala Lys Val Ser Gln  
 180 185 190  
 Gly His His Asn Asn Ser Leu Pro Arg Cys Thr Phe Ser Gln Glu Asn  
 195 200 205  
 Gln Ala Glu Thr His Ala Trp Phe Thr Ser Arg Phe Leu Tyr His Val  
 210 215 220  
 Ala Gly Phe Leu Leu Pro Met Leu Val Met Gly Trp Cys Tyr Val Gly  
 225 230 235 240  
 Val Val His Arg Leu Arg Gln Ala Gln Arg Arg Pro Gln Arg Gln Lys  
 245 250 255  
 Ala Val Arg Val Ala Ile Leu Val Thr Ser Ile Phe Phe Leu Cys Trp  
 260 265 270  
 Ser Pro Tyr His Ile Val Ile Phe Leu Asp Thr Leu Ala Arg Leu Lys  
 275 280 285  
 Ala Val Asp Asn Thr Cys Lys Leu Asn Gly Ser Leu Pro Val Ala Ile  
 290 295 300  
 Thr Met Cys Glu Phe Leu Gly Leu Ala His Cys Cys Leu Asn Pro Met  
 305 310 315 320  
 Leu Tyr Thr Phe Ala Gly Val Lys Phe Arg Ser Asp Leu Ser Arg Leu  
 325 330 335  
 Leu Thr Lys Leu Gly Cys Thr Gly Pro Ala Ser Leu Cys Gln Leu Phe  
 340 345 350  
 Pro Ser Trp Arg Arg Ser Ser Leu Ser Glu Ser Glu Asn Ala Thr Ser  
 355 360 365  
 Leu Thr Thr Phe  
 370

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 2919

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 2

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aaaaaaaaa agt gatgagt tgtgaggcag gtcgcggccc tactgcctca ggagacgatg      60
cgcagctcat ttgcttaaat ttgcagctga cggtgccac ctctettagag gcacctggcg      120
gggagcctct caacataaga cagtgaccag tctggtgact cacagccggc acagccatga      180
actacccgct aacgctggaa atggacctcg agaacctgga ggacctgttc tgggaactgg      240

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acagattgga caactataac gacacctccc tgggtggaaaa tcatctctgc cctgccacag	300
aggggcccct catggcctcc ttcaaggccg tgttcgtgcc cgtggcctac agcctcatct	360
tcctcctggg cgtgatcggc aacgtcctgg tgcgtggtgat cctggagcgg caccggcaga	420
cacgcagttc cacggagacc ttctgttcc acctggccgt ggccgacctc ctgctgggtct	480
tcatcttgcc ctttgcctg gccgagggct ctgtgggctg ggctcctggg accttctct	540
gcaaaactgt gattgccctg cacaaagtca acttctactg cagcagcctg ctctggcct	600
gcctcgcctg ggaccgctac ctggccattg tccacgccgt ccatgcctac cgccaccgcc	660
gcctcctctc catccacatc acctgtggga ccatctggct ggtgggcttc ctcttgccct	720
tgccagagat tctcttcgcc aaagtccagc aaggccatca caacaactcc ctgccacgtt	780
gcaccttctc ccaagagaac caagcagaaa cgcctgcctg gttcacctcc cgattcctct	840
accatgtggc gggattcctg ctgcccatgc tgggtgatggg ctggtgctac gtgggggtag	900
tgcacagggt gcgccaggcc cagcggcgcc ctccaggcca gaaggcagtc aggggtggcca	960
tcctggtgac aagcatcttc ttctctgct ggtcacctca ccacatcgtc atcttcctgg	1020
acacctggc gaggtgaag gccgtggaca atacctgcaa gctgaatggc tctctccccg	1080
tggccatcac catgtgtgag ttctgggcc tggccactg ctgcctcaac cccatgctct	1140
acactttcgc cggcgtgaag ttccgcagtg acctgtcgcg gctcctgacg aagctgggct	1200
gtaccggccc tgcctccctg tgcagctct tccctagctg gcgcaggagc agtctctctg	1260
agtcagagaa tgccacctct ctccaccagt tctaggctcc agtgctccct tttattgctg	1320
cttttctctg gggcaggcag tgatgctgga tgcctctcc aacaggagct gggatcctaa	1380
gggctcaccg tggctaagag tgccttagga gtatcctcat ttggggtagc tagaggaacc	1440
aacccccatt tctagaacat ccttgccagc tcttctgcg gccctggggc taggctggag	1500
cccagggagc ggaaagcagc tcaaaggcac agtgaaggct gtccttacc atctgcacc	1560
ccctgggctg agagaacctc acgcacctcc catcctaate atccaatgct caagaaacaa	1620
cttctacttc tgcccttgcc aacggagagc gcctgccct cccagaacac actccatcag	1680
cttaggggct gctgacctcc acagcttccc ctctctctc ctgcccacct gtcaaacaaa	1740
gccagaagct gagcaccagg gcatgagtgg aggttaaggc tgaggaaagg ccagctggca	1800
gcagagtgtg gccttcggac aactcagtc ctaaaaacac agacattctg ccaggccccc	1860
aagcctgcag tcatcttgac caagcaggaa gctcagactg gttgagttca ggtagctgcc	1920
cctggctctg accgaaacag cgttgggtcc accccatgct accggatcct ggggtggtctg	1980
caggcagggc tgactctagg tgcccttgga gccagccag tgacctgagg aagcgtgaag	2040
gccgagaagc aagaaagaaa ccgcacagag ggaagaaaag agctttcttc ccgaaccca	2100
aggagggaga tggatcaatc aaaccggcg gtccctccg ccaggcgaga tgggggtggg	2160
tggagaactc ctagggtggc tgggtccagg ggatgggagg ttgtgggcat tgatgggaa	2220
ggaggtggc ttgtccctc ctactccct tcccataagc tatagaccg aggaaactca	2280
gagtcggaac ggagaaaggt ggaactggaag gggcccgtag gagtcatctc aaccatcccc	2340
tcctggcat caccttaggc agggaagtgt aagaaacaca ctgaggcagg gaagtcccca	2400
ggccccagga agcctgcccc tgccccgtg aggatgtcac tcagatggaa ccgcagggaag	2460
ctgctcctg cttgtttgct cacctggggt gtgggaggcc cgtccggcag ttctgggtgc	2520
tcctaccac ctccccagc tttgatcagg tggggagtca gggaccctg cccttgctcc	2580
actcaagcca agcagccaag ctcttgga gggccactg gggaaataac agctgtggct	2640

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cacgtgagag tgtcttcacg gcaggacaac gaggaagccc taagacgtcc cttttttctc 2700
tgagtatctc ctcgcaagct gggtaatcga tggggggagtc tgaagcagat gcaaagaggc 2760
aagaggctgg attttgaatt ttctttttaa taaaaaggca cctataaaac aggtcaatac 2820
agtacaggca gcacagagac ccccggaaca agcctaaaaa ttgtttcaaa ataaaaacca 2880
agaagatgtc ttcacatatt gtaaaaaaaaa aaaaaaaaaa 2919

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<210> SEQ ID NO 3
<211> LENGTH: 327
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 3

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Met Ala Ser Phe Lys Ala Val Phe Val Pro Val Ala Tyr Ser Leu Ile
 1             5             10            15

Phe Leu Leu Gly Val Ile Gly Asn Val Leu Val Leu Val Ile Leu Glu
 20            25            30

Arg His Arg Gln Thr Arg Ser Ser Thr Glu Thr Phe Leu Phe His Leu
 35            40            45

Ala Val Ala Asp Leu Leu Leu Val Phe Ile Leu Pro Phe Ala Val Ala
 50            55            60

Glu Gly Ser Val Gly Trp Val Leu Gly Thr Phe Leu Cys Lys Thr Val
 65            70            75            80

Ile Ala Leu His Lys Val Asn Phe Tyr Cys Ser Ser Leu Leu Leu Ala
 85            90            95

Cys Ile Ala Val Asp Arg Tyr Leu Ala Ile Val His Ala Val His Ala
100            105            110

Tyr Arg His Arg Arg Leu Leu Ser Ile His Ile Thr Cys Gly Thr Ile
115            120            125

Trp Leu Val Gly Phe Leu Leu Ala Leu Pro Glu Ile Leu Phe Ala Lys
130            135            140

Val Ser Gln Gly His His Asn Asn Ser Leu Pro Arg Cys Thr Phe Ser
145            150            155            160

Gln Glu Asn Gln Ala Glu Thr His Ala Trp Phe Thr Ser Arg Phe Leu
165            170            175

Tyr His Val Ala Gly Phe Leu Leu Pro Met Leu Val Met Gly Trp Cys
180            185            190

Tyr Val Gly Val Val His Arg Leu Arg Gln Ala Gln Arg Arg Pro Gln
195            200            205

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

Leu Cys Trp Ser Pro Tyr His Ile Val Ile Phe Leu Asp Thr Leu Ala
225            230            235            240

Arg Leu Lys Ala Val Asp Asn Thr Cys Lys Leu Asn Gly Ser Leu Pro
245            250            255

Val Ala Ile Thr Met Cys Glu Phe Leu Gly Leu Ala His Cys Cys Leu
260            265            270

Asn Pro Met Leu Tyr Thr Phe Ala Gly Val Lys Phe Arg Ser Asp Leu
275            280            285

Ser Arg Leu Leu Thr Lys Leu Gly Cys Thr Gly Pro Ala Ser Leu Cys
290            295            300

Gln Leu Phe Pro Ser Trp Arg Arg Ser Ser Leu Ser Glu Ser Glu Asn
305            310            315            320

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Ala Thr Ser Leu Thr Thr Phe  
325

<210> SEQ ID NO 4  
<211> LENGTH: 2896  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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ccactctaag gaatgcggtc cctttgacag gcgaaaaact gaagttggaa aagacaaagt    60
gatttggtta aaattgaaat ttgaaacttg acatttggtc agtgggccct atgtaggaaa    120
aaacctccaa gagagctagg gtctctctca gagaggaaaag acaggtcctt aggtctctac    180
cctcccgctc ccttgccctt gcagttcttg gaactggaca gattggacaa ctataacgac    240
acctccctgg tggaaaatca tctctgccct gccacagagg ggccctcat ggctccttc    300
aaggccgtgt tcgtgccctg ggctacagc ctcctcttcc tctgggctg gatcggaac    360
gtcctgggtc tgggtgacct ggagcggcac cggcagacac gcagttccac ggagaccttc    420
ctgttccacc tggcgtgggc cgacctctg ctggtcttca tcttgccctt tggcgtggcc    480
gagggctctg tgggctgggt cctggggacc ttcctctgca aaactgtgat tgccctgcac    540
aaagtcacct tctactgcag cagcctgctc ctggcctgca tcgccgtgga ccgctacctg    600
gccattgtcc acgcgttcca tgcctaccgc caccgccgcc tctctccat ccacatcacc    660
tgtgggacca tctggctggt gggcttcttc cttgccttgc cagagattct ctctgccaaa    720
gtcagccaag gccatcacia caactccctg ccacgttgca ccttctccca agagaaccaa    780
gcagaaacgc atgcctggtt cacctccga ttcctctacc atgtggcggg attcctgctg    840
cccatgctgg tgatgggctg gtgctacgtg ggggtagtgc acaggttgcg ccaggcccag    900
cggcgccctc agcggcagaa ggcagtcagg gtggccatcc tggtgacaag catcttcttc    960
ctctgctggt caccctacca catgctcacc ttcctggaca ccctggcgag gctgaaggcc   1020
gtggacaata cctgcaagct gaattgctct cctcccgctg ccataccat gtgtgagttc   1080
ctgggcctgg cccactgctg cctcaacccc atgctctaca ctttcgccgg cgtgaagttc   1140
cgcagtgaac tgtcggggt cctgacgaag ctgggctgta ccggccctgc ctccctgtgc   1200
cagctcttcc ctactggcg caggagcagt ctctctgagt cagagaatgc cacctctctc   1260
accacgttct aggtccagct gtcccctttt attgctgctt ttccttgggg caggcagtga   1320
tgctggatgc tcttccaaac aggagctggg atcctaaggg ctacccgtgg ctaagagtgt   1380
cctaggagta tctcatttg gggtagctag aggaaccaac cccatttct agaacatccc   1440
tgccagctct tctgccggcc ctggggctag gctggagccc agggagcgga aagcagctca   1500
aaggcacagt gaaggctgtc cttaccatc tgcaccccc tgggctgaga gaacctcag   1560
cacctcccat cctaatacgc caatgctcaa gaaacaactt ctacttctgc ccttgccaac   1620
ggagagcgcc tgcctctccc agaacacact ccatcagctt aggggctgct gacctccaca   1680
gcttccctc tctctctctg cccacctgtc aaacaaagcc agaagctgag caccagggga   1740
tgagtggagg ttaaggctga ggaaaggcca gctggcagca gagtgtggcc ttgggacaac   1800
tcagtcctta aaaacacaga cattctgcca ggcacccaag cctgcagtca tcttgaccaa   1860
gcaggaagct cagactgggt gagttcaggt agctgccctt ggctctgacc gaaacagcgc   1920
tgggtccacc ccatgtcacc ggatcctggg tggctctgag gcagggctga ctctaggtgc   1980
ccttggaggc cagccagtga cctgaggaag cgtgaaggcc gagaagcaag aaagaaaccc   2040

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gacagaggga agaaaagagc tttcttcccg aaccccaagg agggagatgg atcaatcaaa	2100
cccggcggtc cctcccgcca ggcgagatgg ggtgggggtg agaactccta ggggtggtgg	2160
gtccagggga tgggaggttg tgggcattga tggggaagga ggctggcttg tcccctctc	2220
actcccttcc cataagctat agacccgagg aaactcagag tcggaacgga gaaaggtgga	2280
ctggaagggg cccgtgggag tcactctaac catccctccc gtggcatcac cttaggcagg	2340
gaagtgtaa aaacacactg aggcaggga gtccccaggc ccaggaagc cgtgccctgc	2400
ccccgtgagg atgtcactca gatggaaccg caggaagctg ctccgtgctt gtttgcac	2460
ctgggggtgtg ggaggcccg cggcaggttc tgggtgctcc ctaccacctc ccagccttt	2520
gatcaggtgg ggagtcaggg acccctgccc ttgtccact caagccaagc agccaagctc	2580
cttgggaggc cccactgggg aaataacagc tgtggctcac gtgagagtgt cttcacggca	2640
ggacaacgag gaagccctaa gacgtccctt tttctctga gtatctctc gcaagctggg	2700
taatcgatgg gggagtctga agcagatgca aagaggcaag aggctggatt ttgaatttc	2760
tttttaataa aaaggcacct ataaaacagg tcaatacagt acaggcagca cagagacccc	2820
cggacaagc ctaaaaattg tttcaaaata aaaaccaaga agatgtcttc acatattgta	2880
aaaaaaaaaaaa	2896

<210> SEQ ID NO 5  
 <211> LENGTH: 1119  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically generated coding sequence  
 <400> SEQUENCE: 5

atgaactacc cctgacct ggagatggac ctggagaacc tggaggacct gttctgggag	60
ctggacagac tggacaacta caatgatacc tccctggtgg agaaccatct gtgcccgcgc	120
accgagggcc cctgatggc cagcttcaag gccgtgttcg tgcccgtggc ctacagcctg	180
atcttctcgc tgggctgat cggcaacgtg ctgggtgctg tgatcctgga gagacacaga	240
cagaccagaa gcagaccga gaccttctg ttccacctgg ccgtggccga cctgctgctg	300
gtgttcaccc tgccttctgc cgtggccgag ggcagcgtgg gctgggtgct gggcaccttc	360
ctgtgcaaga ccgtgatcgc cctgcacaag gtgaacttct actgcagcag cctgctgctg	420
gcctgcacgc ccgtggacag atacctggcc atcgtgcacg ccgtgcacgc ctacagacac	480
agaagactgc tgagcatcca catcacctgc ggcaccatct ggctgggtgg cttcctgctg	540
gcccctgccg agatcctgtt cgccaagggt agccagggcc accacaaca cagcctgcc	600
agatgcacct tcagccagga gaaccaggcc gagaccacg cctgggtcac cagcagattc	660
ctgtaccacg tggccgctt cctgctgccc atgctggtga tgggctgggt ctacgtgggc	720
gtggtgcaca gactgagaca ggcccagaga agacccaga gacagaaggc cgtgagagt	780
gccatcctgg tgaccagcat cttcttctg tgetggagcc cctaccacat cgtgatcttc	840
ctggacaccc tggccagact gaaggccgtg gacaacacct gcaagctgaa cggcagcctg	900
cccgtggcca tcaccatgtg cgagtctctg gccctggccc actgctgccc gaaccccatg	960
ctgtacacct tcgccgaggt gaagtgcaga agcgacctga gcagactgct gaccaagctg	1020
ggctgcacgc gccccgccc cctgtgccag ctgttcccca gctggagaag aagcagcctg	1080
agcgagagcg agaaccgccc cagcctgacc accttctga	1119

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<210> SEQ ID NO 6  
 <211> LENGTH: 717  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Protein encoded by synthetically generated  
 expression cassette

<400> SEQUENCE: 6

Met Val Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu  
 1 5 10 15  
 Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys  
 20 25 30  
 Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser  
 35 40 45  
 Ile Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Asn  
 50 55 60  
 Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala  
 65 70 75 80  
 Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Pro Leu Ile Ile  
 85 90 95  
 Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu  
 100 105 110  
 Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn  
 115 120 125  
 Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu  
 130 135 140  
 Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly  
 145 150 155 160  
 Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu  
 165 170 175  
 Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys  
 180 185 190  
 Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser  
 195 200 205  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
 210 215 220  
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly  
 225 230 235 240  
 Gly Gly Ser Gln Val Gln Leu Leu Glu Ser Gly Ala Glu Val Lys Lys  
 245 250 255  
 Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe  
 260 265 270  
 Ile Arg Tyr Ser Phe Thr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu  
 275 280 285  
 Glu Trp Met Gly Arg Ile Ile Thr Ile Leu Asp Val Ala His Tyr Ala  
 290 295 300  
 Pro His Leu Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser  
 305 310 315 320  
 Thr Val Tyr Leu Glu Leu Arg Asn Leu Arg Ser Asp Asp Thr Ala Val  
 325 330 335  
 Tyr Phe Cys Ala Gly Val Tyr Glu Gly Glu Ala Asp Glu Gly Glu Tyr  
 340 345 350  
 Asp Asn Asn Gly Phe Leu Lys His Trp Gly Gln Gly Thr Leu Val Thr  
 355 360 365

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Val Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly	
370 375 380	
Gly Ser Glu Leu Glu Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Ser	
385 390 395 400	
Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Ser Val Ser	
405 410 415	
Ser Asp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu	
420 425 430	
Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gly Val Pro Ala Arg Phe	
435 440 445	
Ser Gly Ser Gly Ser Gly Ala Glu Phe Thr Leu Thr Ile Ser Ser Leu	
450 455 460	
Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp	
465 470 475 480	
Pro Pro Arg Tyr Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Ala	
485 490 495	
Ala Ala Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys	
500 505 510	
Ser Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser	
515 520 525	
Pro Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val	
530 535 540	
Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile	
545 550 555 560	
Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr	
565 570 575	
Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln	
580 585 590	
Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys	
595 600 605	
Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln	
610 615 620	
Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu	
625 630 635 640	
Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg	
645 650 655	
Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met	
660 665 670	
Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly	
675 680 685	
Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp	
690 695 700	
Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg	
705 710 715	

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 2154

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Synthetically generated expression cassette

&lt;400&gt; SEQUENCE: 7

atggttcgag gcgtgcctt ccggcatctg ctgctggtgc tgcagctggc tctcctgcct 60

gccgccccc agggcaagaa agtggtgctg ggcaaaaagg gcgacaccgt ggaactgacc 120

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tgcaccgccca gccagaagaa gtccatccag ttccactgga agaacagcaa ccagatcaag	180
atcctggggca accagggcag ctctctgacc aaggggccca gcaagctgaa cgaccggggc	240
gatagccggc ggagcctgtg ggaccagggc aatttccac tgatcatcaa gaacctgaag	300
atcgaggaca gcgacaccta catctgcgag gtcgaagatc agaaagaaga ggtgcagctg	360
ctggtgttcg gcctgaccgc caactccgac acccatctgc tgcagggcca gagcctgacc	420
ctcaccctgg aaagccccc tggcagcagc cccagcgtgc agtgcagaag ccccagaggc	480
aagaacatcc agggcggcaa gacctgagc gtgtcccagc tggaactgca ggactccggc	540
acctggacct gtaccgtgct gcagaaccag aaaaaggctc agttcaagat cgacatcgtg	600
gtgctggcct tccagaaggc ctctggcggc ggaggatctg gcggagggtg aagtggcggg	660
ggaggtagtg gcggaggcgg atcagggtgc ggaggttcag gcggtggcgg aagcggaggc	720
ggtggatctc aggtccagct gctcgaatct ggccgcaag tgaagaaacc cggcagcagc	780
gtgaaagtgt cctgcaaggc cagcggcgac accttcaccc ggtacagctt cacatgggtc	840
cgacaggccc ctgggcaggc cctggaatgg atgggcccga tcatcaccat cctggacgtg	900
gcccactacg ccccatctct gcagggcaga gtgaccatca ccgccgacaa gagcaccagc	960
accgtgtacc tggaactgcg gaacctgcgg agcgacgata ccgccgtcta cttctgtgcc	1020
ggcgtgtacg agggcgaggc cgatgagggc gagtacgaca acaacggctt cctgaagcac	1080
tggggccagg gcacctcgt caccgtgacc agcggcggcg gaggatctgg cggagggtga	1140
agtggcgggg gaggtagtga gctggaactc acccagagcc ccgccacct gtccgtgtct	1200
ccaggcgaga gagccacct gagctgcaga gccagcgaga gcgtgtccag cgacctggcc	1260
tggtatcagc agaagcccg ccaggccccc agactgctga tctacggcg cagcaccaga	1320
gccacaggcg tgccagccag attcagcggc agcggtagcg gagccgagtt cacctgacc	1380
atcagcagcc tgcagagcga ggactttgcc gtgtactact gccagcagta caacaactgg	1440
ccccccagat acaccttcgg ccagggaacc cggctggaaa tcaaggcggc cgcaattgaa	1500
gttatgtatc ctctctcta cctagacaat gagaagagca atggaacct tatccatgtg	1560
aaagggaaac acctttgtcc aagtcccta tttcccgac cttctaagcc cttttgggtg	1620
ctggtggtgg ttggtggagt cctggcttgc tataccttgc tagtaacagt ggcctttatt	1680
attttctggg tgaggagtaa gaggagcagg ctccctgcaca gtgactacat gaacatgact	1740
ccccgccgcc cggggccac ccgcaagcat taccagccct atgcccacc acgcgacttc	1800
gcagcctatc gctccagagt gaagttcagc aggagcgcag acgccccgc gtaccagcag	1860
ggccagaacc agctctataa cgagctcaat ctaggacgaa gagaggagta cgatgttttg	1920
gacaagagac gtggccggga cctgagatg gggggaaaag cgagaaggaa gaacctcag	1980
gaaggcctgt acaatgaact gcagaaagat aagatggcgg aggcctacag tgagattggg	2040
atgaaaggcg agcgccggag gggcaagggg cacgatggcc tttaccaggg tctcagtaca	2100
gccaccaagg acacctacga cgccttcac atgcaggccc tgccccctcg ctaa	2154

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 692

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Protein encoded by synthetically generated expression cassette

&lt;400&gt; SEQUENCE: 8

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Ala	Leu	Leu	Pro 20	Ala	Ala	Thr	Gln	Gly 25	Lys	Lys	Val	Val	Leu 30	Gly	Lys
Lys	Gly	Asp 35	Thr	Val	Glu	Leu	Thr 40	Cys	Thr	Ala	Ser	Gln 45	Lys	Lys	Ser
Ile	Gln 50	Phe	His	Trp	Lys	Asn 55	Ser	Asn	Gln	Ile	Lys 60	Ile	Leu	Gly	Asn
Gln 65	Gly	Ser	Phe	Leu	Thr 70	Lys	Gly	Pro	Ser	Lys 75	Leu	Asn	Asp	Arg	Ala 80
Asp	Ser	Arg	Arg	Ser 85	Leu	Trp	Asp	Gln	Gly 90	Asn	Phe	Pro	Leu 95	Ile	Ile
Lys	Asn	Leu	Lys 100	Ile	Glu	Asp	Ser	Asp 105	Thr	Tyr	Ile	Cys	Glu 110	Val	Glu
Asp	Gln	Lys 115	Glu	Glu	Val	Gln	Leu 120	Leu	Val	Phe	Gly	Leu 125	Thr	Ala	Asn
Ser	Asp 130	Thr	His	Leu	Leu	Gln 135	Gly	Gln	Ser	Leu	Thr 140	Leu	Thr	Leu	Glu
Ser 145	Pro	Pro	Gly	Ser	Ser 150	Pro	Ser	Val	Gln	Cys 155	Arg	Ser	Pro	Arg	Gly 160
Lys	Asn	Ile	Gln 165	Gly	Gly	Lys	Thr	Leu	Ser 170	Val	Ser	Gln	Leu 175	Glu	Leu
Gln	Asp	Ser	Gly 180	Thr	Trp	Thr	Cys	Thr 185	Val	Leu	Gln	Asn	Gln 190	Lys	Lys
Val	Glu 195	Phe	Lys	Ile	Asp	Ile	Val 200	Val	Leu	Ala	Phe	Gln 205	Lys	Ala	Ser
Gly 210	Gly	Gly	Gly	Ser	Gly	Gly 215	Gly	Gly	Ser	Gln	Val 220	Gln	Leu	Leu	Glu
Ser 225	Gly	Ala	Glu	Val	Lys 230	Lys	Pro	Gly	Ser	Ser 235	Val	Lys	Val	Ser	Cys 240
Lys	Ala	Ser	Gly 245	Asp	Thr	Phe	Ile	Arg	Tyr 250	Ser	Phe	Thr	Trp 255	Val	Arg
Gln	Ala	Pro	Gly 260	Gln	Gly	Leu	Glu	Trp 265	Met	Gly	Arg	Ile 270	Ile	Thr	Ile
Leu	Asp 275	Val	Ala	His	Tyr	Ala	Pro 280	His	Leu	Gln	Gly 285	Arg	Val	Thr	Ile
Thr 290	Ala	Asp	Lys	Ser	Thr	Ser 295	Thr	Val	Tyr	Leu	Glu 300	Leu	Arg	Asn	Leu
Arg 305	Ser	Asp	Asp	Thr	Ala 310	Val	Tyr	Phe	Cys 315	Ala	Gly	Val	Tyr	Glu	Gly 320
Glu	Ala	Asp	Glu 325	Gly	Glu	Tyr	Asp	Asn 330	Asn	Gly	Phe	Leu	Lys 335	His	Trp
Gly	Gln	Gly	Thr 340	Leu	Val	Thr	Val	Thr 345	Ser	Gly	Gly	Gly 350	Gly	Ser	Gly
Gly	Gly 355	Gly	Ser	Gly	Gly	Gly	Gly 360	Ser	Glu	Leu	Glu	Leu 365	Thr	Gln	Ser
Pro 370	Ala	Thr	Leu	Ser	Val	Ser 375	Pro	Gly	Glu	Arg 380	Ala	Thr	Leu	Ser	Cys
Arg 385	Ala	Ser	Glu	Ser	Val 390	Ser	Ser	Asp	Leu	Ala 395	Trp	Tyr	Gln	Gln	Lys 400
Pro	Gly	Gln	Ala 405	Pro	Arg	Leu	Leu	Ile 410	Tyr	Gly	Ala	Ser	Thr	Arg	Ala 415

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Thr Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Ala Glu Phe  
                   420                  425                  430  
 Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr  
                   435                  440                  445  
 Cys Gln Gln Tyr Asn Asn Trp Pro Pro Arg Tyr Thr Phe Gly Gln Gly  
                   450                  455                  460  
 Thr Arg Leu Glu Ile Lys Ala Ala Ala Ile Glu Val Met Tyr Pro Pro  
                   465                  470                  475                  480  
 Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val Lys  
                   485                  490                  495  
 Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys Pro  
                   500                  505                  510  
 Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu  
                   515                  520                  525  
 Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser  
                   530                  535                  540  
 Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly  
                   545                  550                  555                  560  
 Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala  
                   565                  570                  575  
 Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala  
                   580                  585                  590  
 Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg  
                   595                  600                  605  
 Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu  
                   610                  615                  620  
 Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn  
                   625                  630                  635                  640  
 Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met  
                   645                  650                  655  
 Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly  
                   660                  665                  670  
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 Leu Pro Pro Arg  
                   690

<210> SEQ ID NO 9  
 <211> LENGTH: 2079  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically generated expression cassette

<400> SEQUENCE: 9

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tgcaccgccca gccagaagaa gtccatccag ttccactgga agaacagcaa ccagatcaag	180
atcctggggca accagggcgag cttcctgacc aaggggcccca gcaagctgaa cgaccggggc	240
gatagccggc ggagcctgtg ggaccagggc aatttccac tgatcatcaa gaacctgaag	300
atcgaggaca gcgacaccta catctgcgag gtcgaagatc agaaagaaga ggtgcagctg	360
ctggtgttcg gcctgaccgc caactccgac acccatctgc tgcagggccca gagcctgacc	420
ctcaccttgg aaagccccc tggcagcagc cccagcgtgc agtgcagaag ccccagaggc	480

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aagaacatcc agggcggcaa gaccctgagc gtgtcccagc tggaactgca ggactccggc 540
acctggacct gtaccgtgct gcagaaccag aaaaaggctc agttcaagat cgacatcgtg 600
gtgctggcct tccagaaggc ctctggcggc ggcggaagcg gagcggtgg atctcaggtc 660
cagctgctcg aatctggcgc cgaagtgaag aaaccggca gcagcgtgaa agtgtcctgc 720
aaggccagcg gcgacacctt catccggtac agcttcacat gggtcgcaca ggcccctggg 780
cagggcctgg aatggatggg ccggatcatc accatcctgg acgtggccca ctacgcccc 840
catctgcagg gcagagtgc catcacgcc gacaagagca ccagcacgt gtacctggaa 900
ctgcggaacc tgcggagcga cgataccgcc gtctacttct gtgcggcgt gtacgagggc 960
gagggcgtat agggcgagta cgacaacaac ggcttctga agcactgggg ccagggcacc 1020
ctcgtcaccc tgaccagcgg cggcgaggga tctggcgagg gtggaagtgg cgggggaggt 1080
agtgaagtgg aactaccca gagccccgcc accctgtccg tgtctccagg cgagagagcc 1140
accctgagct gcagagccag cgagagcgtg tccagcgacc tggcctggta tcagcagaag 1200
ccccgccagg ccccccagact gctgatctac ggcgccagca ccagagccac aggcgtgcca 1260
gccagattca gcggcagcgg tagcggagcc gagttcacc tgaccatcag cagcctgcag 1320
agcgaggact ttgcgtgta ctactgccag cagtacaaca actggcccc cagatacacc 1380
ttcgccagg gaaccggct ggaatcaag gcggcgcaa ttgaagttat gtatcctcct 1440
ccttacctag acaatgagaa gagcaatgga accattatcc atgtgaaagg gaaacacctt 1500
tgtccaagtc ccctatttcc cggaccttct aagccctttt ggggtgctgt ggtggttgg 1560
ggagtctcgg cttgctatag cttgctagta acagtggcct ttattatttt ctgggtgagg 1620
agtaagagga gcaggctcct gcacagtgc tacatgaaca tgactcccc cgccccggg 1680
ccccccgca agcattacca gcctatgcc ccaccacgcg acttcgcagc ctatcgctcc 1740
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tataacgagc tcaatctagg acgaagagag gactacgatg ttttgacaa gagacgtggc 1860
cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat 1920
gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc 1980
cggaggggca aggggcacga tggcctttac caggggtctc gtacagccac caaggacacc 2040
tacgacgccc ttcacatgca ggcctgccc cctcgctaa 2079

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<210> SEQ ID NO 10
<211> LENGTH: 708
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Protein encoded by synthetically generated
expression cassette

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<400> SEQUENCE: 10

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Met Val Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
1           5           10          15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
20          25          30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
35          40          45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Asn
50          55          60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala

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65				70				75				80			
Asp	Ser	Arg	Arg	Ser 85	Leu	Trp	Asp	Gln	Gly 90	Asn	Phe	Pro	Leu	Ile 95	Ile
Lys	Asn	Leu	Lys 100	Ile	Glu	Asp	Ser	Asp 105	Thr	Tyr	Ile	Cys	Glu 110	Val	Glu
Asp	Gln	Lys 115	Glu	Glu	Val	Gln	Leu 120	Leu	Val	Phe	Gly	Leu 125	Thr	Ala	Asn
Ser	Asp 130	Thr	His	Leu	Leu	Gln 135	Gly	Gln	Ser	Leu	Thr 140	Leu	Thr	Leu	Glu
Ser 145	Pro	Pro	Gly	Ser	Ser 150	Pro	Ser	Val	Gln	Cys 155	Arg	Ser	Pro	Arg	Gly 160
Lys	Asn	Ile	Gln	Gly 165	Gly	Lys	Thr	Leu	Ser 170	Val	Ser	Gln	Leu	Glu 175	Leu
Gln	Asp	Ser	Gly 180	Thr	Trp	Thr	Cys	Thr 185	Val	Leu	Gln	Asn	Gln 190	Lys	Lys
Val	Glu	Phe 195	Lys	Ile	Asp	Ile	Val 200	Val	Leu	Ala	Phe	Gln 205	Lys	Ala	Ser
Gly	Gly 210	Gly	Gly	Ser	Gly	Gly 215	Gly	Gly	Ser	Gly	Gly 220	Gly	Gly	Ser	Gly
Gly 225	Gly	Gly	Ser	Gly	Gly 230	Gly	Gly	Ser	Gly	Gly 235	Gly	Gly	Ser	Gly	Gly 240
Gly	Gly	Ser	Glu	Val 245	Gln	Leu	Val	Gln	Ser 250	Gly	Ala	Glu	Val	Lys 255	Lys
Pro	Gly	Ala	Thr 260	Val	Lys	Ile	Ser	Cys 265	Lys	Val	Ser	Gly	Tyr 270	Thr	Phe
Thr	Asp	Tyr 275	Tyr	Met	His	Trp	Val 280	Gln	Gln	Ala	Pro	Gly 285	Lys	Gly	Leu
Glu	Trp 290	Met	Gly	Leu	Val	Asp 295	Pro	Glu	Asp	Gly	Glu 300	Thr	Ile	Tyr	Ala
Glu 305	Lys	Phe	Gln	Gly	Arg 310	Val	Thr	Ile	Thr	Ala 315	Asp	Thr	Ser	Thr	Asp 320
Thr	Ala	Tyr	Met	Glu 325	Leu	Ser	Ser	Leu	Arg 330	Ser	Glu	Asp	Thr	Ala 335	Val
Tyr	Tyr	Cys	Ala 340	Thr	Glu	Arg	Thr	Asp 345	Tyr	Trp	Gly	Gln	Gly 350	Thr	Leu
Val	Thr 355	Val	Ser	Ser	Gly	Gly	Gly 360	Gly	Ser	Gly	Gly	Gly 365	Gly	Ser	Gly
Gly 370	Gly	Gly	Ser	Glu	Ile	Val 375	Leu	Thr	Gln	Ser	Pro 380	Leu	Ser	Leu	Ser
Val 385	Thr	Pro	Gly	Glu	Pro 390	Ala	Ser	Ile	Ser	Cys 395	Arg	Ser	Ser	Gln	Ser 400
Leu	Leu	Asp	Ser	Asp 405	Asp	Gly	Asn	Thr	Tyr 410	Leu	Asp	Trp	Tyr	Leu 415	Gln
Lys	Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Glu	Val	Ser	Asn	Arg
			420					425					430		
Phe	Ser	Gly 435	Val	Pro	Asp	Arg	Phe 440	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp
							445					445			
Phe	Thr 450	Leu	Lys	Ile	Ser	Arg 455	Val	Glu	Ala	Glu	Asp 460	Val	Gly	Val	Tyr
Tyr 465	Cys	Met	Gln	Ser	Ile	Gln 470	Leu	Pro	Trp	Thr 475	Phe	Gly	Gln	Gly	Thr 480
Lys	Leu	Glu	Ile	Lys 485	Arg	Ala	Ala	Ala	Ile 490	Glu	Val	Met	Tyr	Pro	Pro
				485					490					495	



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Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val Lys  
500 505 510

Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys Pro  
515 520 525

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu  
530 535 540

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser  
545 550 555 560

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly  
565 570 575

Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala  
580 585 590

Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala  
595 600 605

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg  
610 615 620

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu  
625 630 635 640

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn  
645 650 655

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met  
660 665 670

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly  
675 680 685

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala  
690 695 700

Leu Pro Pro Arg  
705

<210> SEQ ID NO 11  
<211> LENGTH: 2127  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically generated expression cassette

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tgcaccgccca gccagaagaa gtccatccag ttccactgga agaacagcaa ccagatcaag	180
atcctggggca accagggcag cttcctgacc aaggggcccca gcaagctgaa cgaccggggcc	240
gatagccggc ggagcctgtg ggaccagggc aatttccac tgatcatcaa gaacctgaag	300
atcgaggaca gcgacaccta catctgcgag gtcgaagatc agaaagaaga ggtgcagctg	360
ctgggtgttcg gcctgaccgc caactccgac acccatctgc tgcagggcca gagcctgacc	420
ctcaccctgg aaagccccc tggcagcagc ccagcgtgc agtgagaag ccccagaggc	480
aagaacatcc agggcggcaa gaccctgagc gtgtcccagc tggaactgca ggactccggc	540
acctggacct gtaccgtgct gcagaaccag aaaaaggtcg agttcaagat cgacatcgtg	600
gtgctggcct tccagaaggc ctctggcggc ggaggatctg gcggaggtgg aagtggcggg	660
ggaggtagtg gcggaggcgg atcaggtggc ggaggttcag gcggtggcgg aagcggaggc	720
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gtgaagatca gctgcaaggt gtcgggctac accttcaccg actactacat gcactgggtg	840
cagcaggccc ctggcaaggg cctggaatgg atgggactgg tggaccccga ggacggcgag	900
acaatctacg cggagaagtt ccagggcaga gtgaccatca ccgccgatac cagcaccgac	960
accgcctaca tggaaactgag cagcctgcgg agcgaggaca ccgccgtgta ctactgtgcc	1020
accgagcgga ccgattactg gggccaggga acaactcgta ccgtgtcaag tggcgggcga	1080
ggatctggcg gaggtggaag tggcggggga ggtagtga tctgtctgac ccagagcccc	1140
ctgtccctgt ctgtgacacc tggcgagcct gccagcatct cctgcagaag cagccagagc	1200
ctgtctggact ccgacgacgg caacacctac ctggactggt atctgcagaa acccggccag	1260
tccccccagc tgctgatcta cgagggtgcc aaccggttca gcggcgtgcc cgatagattt	1320
tccggctctg gcagcgccac cgacttcacc ctgaagatta gccgggtgga agccgaggac	1380
gtgggcgtgt actattgcat gcagagcatt cagctgcctt ggaccttcgg ccagggcacc	1440
aagctggaat tcaagagagc ggcggcaatt gaagttatgt atctctctcc ttacctagac	1500
aatgagaaga gcaatggaac cattatccat gtgaaaggga aacacctttg tccaagtccc	1560
ctatttcccc gaccttctaa gcccttttgg gtgctgggtg tgggtggtgg agtcctggct	1620
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aggctctctg acagtgacta catgaacatg actccccgcc gccccggggc caccgcgaag	1740
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atggggggaa agccgagaag gaagaaccct caggaaggcc tgtacaatga actgcagaaa	1980
gataagatgg cggaggccta cagttagatt gggatgaaag gcgagcgccg gaggggcaag	2040
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cacatgcagg ccctgcccc tcgctaa	2127

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What is claimed is:

1. A method of preparing a cellular composition for suppressing replication of HIV in B cell follicles in a subject having HIV, comprising:

providing peripheral blood mononuclear cells from the subject having HIV;

transducing the cells with a vector comprising a HIV-based CAR construct and a nucleic acid molecule encoding a CXCR5 polypeptide as shown in SEQ ID NO: 5;

culturing the transduced cells, and

infusing the cultured transduced cells into the subject, wherein the cultured transduced cells migrate to B cell follicles and suppress replication of HIV in B cell follicles to reduce levels of HIV in the subject in the absence of anti-viral HIV drugs relative to HIV levels in the subject prior to the infusing step.

2. The method of claim 1, further comprising monitoring the subject for the presence of HIV.

3. The method of claim 1, further comprising monitoring the subject for the amount of HIV.

4. The method of claim 1, further comprising obtaining the cells from the subject using apheresis.

5. The method of claim 1, wherein the HIV-based CAR construct comprises the sequence shown in SEQ ID NO: 6, 8, or 10.

6. The method of claim 1, wherein the HIV-based CAR construct is encoded by a nucleic acid having the sequence shown in SEQ ID NO: 7, 9, or 11.

7. The method of claim 1, wherein the vector is a lentiviral vector or a gamma retroviral vector.

8. The method of claim 1, wherein the transducing step comprises gene editing.

9. The method of claim 8, wherein the gene editing comprises CRISPR or TALEN.

10. A method of suppressing replication of HIV in B cell follicles in a subject having HIV, comprising:

providing peripheral blood mononuclear cells from the subject having HIV;

transducing the cells with a vector comprising a HIV-based CAR construct and a nucleic acid molecule encoding a CXCR5 polypeptide as shown in SEQ ID NO: 5, wherein the HIV-based CAR construct comprises an antigen-binding targeting portion that recognizes gp120 and/or gp41;

culturing the transduced cells, and

infusing the cultured transduced cells into the subject, wherein the cultured transduced cells migrate to B cell follicles, thereby suppressing replication of HIV in B cell follicles to reduce levels of HIV in the subject in the absence of anti-viral HIV drugs relative to HIV levels in the subject prior to the infusing step.

11. A method of suppressing replication of HIV in B cell  
follicles in a subject having HIV, comprising:  
providing peripheral blood mononuclear cells from the  
subject having HIV;  
transducing the cells with a vector comprising a HIV- 5  
based CAR construct and a codon-optimized nucleic  
acid molecule encoding a CXCR5 polypeptide as  
shown in SEQ ID NO: 5; wherein the HIV-based CAR  
construct comprises an antigen-binding targeting por-  
tion that recognizes gp120 and/or gp41; and 10  
culturing the transduced cells,  
infusing the cultured transduced cells into the subject,  
wherein the cultured transduced cells migrate to B cell  
follicles, thereby suppressing replication of HIV in B  
cell follicles to reduce levels of HIV in the subject in 15  
the absence of anti-viral HIV drugs relative to HIV  
levels in the subject prior to the infusing step.

\* \* \* \* \*