

(51) International Patent Classification:

A61K 35/17 (2025.01) A61K 48/00 (2006.01)

(21) International Application Number:

PCT/US2024/033096

(22) International Filing Date:

07 June 2024 (07.06.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/506,634 07 June 2023 (07.06.2023) US

(71) Applicant: OREGON HEALTH & SCIENCE UNIVERSITY [US/US]; 3181 SW Sam Jackson Park Road, Mail Code L106TT, Portland, Oregon 97239 (US).

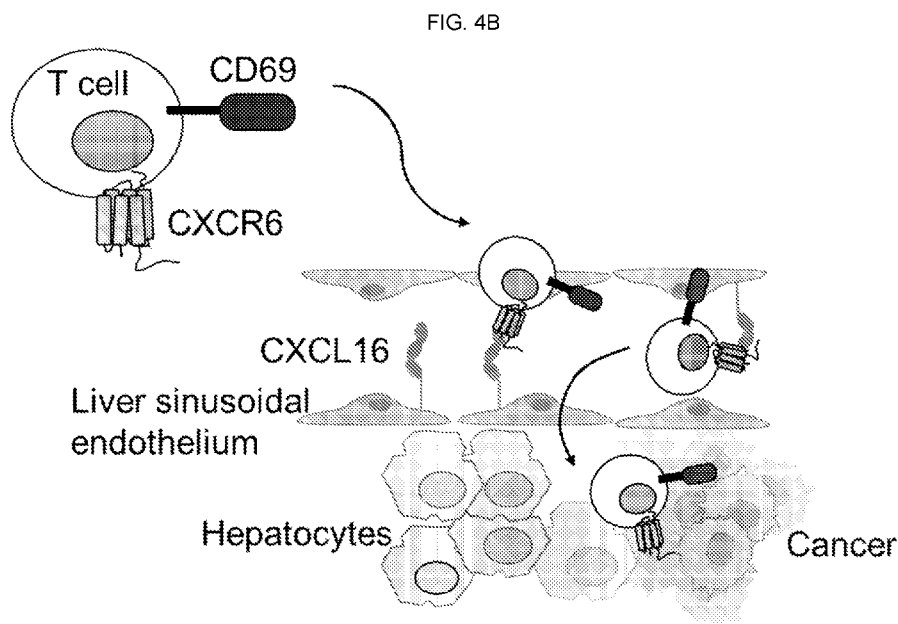
(72) Inventors: EIL, Robert; 3181 SW SAM JACKSON PARK RD, L106TT, Portland, Oregon 97239 (US). BARTLETT, Alexandra; 3181 SW SAM JACKSON PARK RD, L106TT, Portland, Oregon 97239 (US).

(74) Agent: QUISENBERRY, Chrystal et al.; Lee & Hayes PC, 601 W. Riverside Avenue Suite 1400, Spokane, Washington 99201 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: SYSTEMS AND METHODS TO DIRECT CELLULAR THERAPIES IN VIVO TO AN ORGAN



(57) Abstract: Systems and methods to direct cellular therapies *in vivo* are provided. Systems and methods described herein include cells genetically modified to achieve at least two of: expression of a recombinant receptor, modulated expression of a trafficking signal, or modulated expression of a retention signal. The genetic constructs can be used to facilitate localized action of cellular therapies.

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
- *with sequence listing part of description (Rule 5.2(a))*

SYSTEMS AND METHODS TO DIRECT CELLULAR THERAPIES IN VIVO TO AN ORGAN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/506,634 filed June 7, 2023, which is incorporated herein by reference in its entirety as if fully set forth herein.

REFERENCE TO SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in XML format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the file containing the Sequence Listing is 38V2645.xml. The file is 296,538 bytes, was created on June 6, 2024, and is being submitted electronically via Patent Center.

FIELD OF THE DISCLOSURE

[0003] The current disclosure provides systems and methods to direct cellular therapies *in vivo* to an organ. The organ can be a secondary treatment location, such as an organ to which a primary tumor has metastasized. The systems and methods disclosed herein can be used to facilitate localized action of cellular therapies at secondary treatment locations and to avoid action at primary treatment locations and/or healthy, non-cancerous tissues. The systems and methods disclosed herein also can be used to facilitate localized action of cellular therapies at a primary treatment location and to avoid action at secondary treatment locations and/or healthy, non-cancerous tissues. The systems and methods disclosed herein also can be used to facilitate localized action of cellular therapies at a primary treatment location and secondary treatment locations and to avoid action at healthy, non-cancerous tissues.

BACKGROUND OF THE DISCLOSURE

[0004] According to the World Health Organization, cancer is a leading cause of death globally, and was responsible for nearly 10 million deaths in 2020.

[0005] For many years, the chosen treatments for cancer have been surgery, chemotherapy, and/or radiation therapy. In recent years, more targeted therapies have emerged to specifically target cancer cells by identifying and exploiting specific molecular and/or immunophenotypic changes seen primarily in those cells. For example, many cancer cells preferentially express particular markers on their cellular surfaces and these markers have provided targets for antibody-based therapeutics.

[0006] Significant progress has been made in genetically engineering cells of the immune system

to target and kill unwanted cell types, such as cancer cells. Many of these immune cells are T cells that have been genetically engineered to express a recombinant receptor such as a chimeric antigen receptor (CAR). CAR are proteins including several distinct subcomponents that allow the genetically modified T cells to recognize and kill cancer cells. The subcomponents include at least an extracellular component and an intracellular component. The extracellular component includes a binding domain that specifically binds a marker (e.g., an antigen) that is preferentially present on the surface of unwanted cells. When the binding domain binds such markers, the intracellular component signals the T cell to destroy the bound cell. CAR can additionally include a transmembrane domain that can link the extracellular component to the intracellular component.

[0007] Although CAR T cells have had substantial success in treating various cancers, challenges remain. For example, CAR T cell-based therapies can be limited in some cancers due to toxicity resulting from on-target, off-tumor killing. This toxicity can occur when a targeted antigen is expressed by cancer cells as well as cells within healthy tissues. The CAR T cells will attack and destroy all cells expressing the antigen. Thus, strategies to improve CAR T cell efficacy, while reducing off-tumor toxicities are needed.

SUMMARY OF THE DISCLOSURE

[0008] The current disclosure provides cells genetically modified to achieve at least two of: expression of a recombinant receptor, modulated expression of a trafficking signal, or modulated expression of a retention signal. Genetic constructs described herein can be used to facilitate localized action of cellular therapies, for example at a selected organ. Expression of a trafficking signal and/or retention signal by genetically modified cells can induce the genetically modified cells to infiltrate a treatment site (e.g., organ or tissue) and/or prevent the genetically modified cells from leaving a treatment site, respectively. Concurrent expression of i) a recombinant receptor that binds a target antigen and ii) a surface molecule (e.g., trafficking signal and/or retention signal), by genetically modified cells, instructs the genetically modified cells to traffic to a select tissue (or away from a non-selected tissue), kill cells expressing the target antigen, and/or remain within the select tissue.

[0009] In particular embodiments, systems and methods disclosed herein are used to direct a treatment to an organ that represents a secondary treatment location. A secondary treatment location refers to a location in the body that is different from where a condition to be treated emerged. For example, in some embodiments, a secondary treatment location is an organ to which a primary tumor has metastasized. In particular embodiments, systems and methods disclosed herein are used to direct a treatment to a secondary treatment location and to avoid,

reduce, or prevent direction of the treatment to a primary treatment location. A primary treatment location is a location in the body where a condition to be treated emerged.

[0010] Particular embodiments of the systems and methods disclosed herein can be used to facilitate localized action of cellular therapies at secondary treatment locations and to avoid action at primary treatment locations and/or healthy, non-cancerous tissues. The systems and methods disclosed herein also can be used to facilitate localized action of cellular therapies at a primary treatment location and to avoid action at secondary treatment locations and/or healthy, non-cancerous tissues. The systems and methods disclosed herein also can be used to facilitate localized action of cellular therapies at a primary treatment location and secondary treatment locations and to avoid action at healthy, non-cancerous tissues.

[0011] In particular embodiments, a recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a hybrid thereof. In particular embodiments, the recombinant receptor includes a binding domain. In particular embodiments, the binding domain binds a target antigen. In particular embodiments, the target antigen is expressed by a cancer cell. In particular embodiments, the cancer includes metastases of colorectal cancer, pancreatic cancer, breast cancer, colon cancer, gastric cancer, prostate cancer, hepatocellular carcinoma, ovarian cancer, endometrial cancer, breast cancer, or uterine cancer. In particular embodiments, the target antigen includes a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), a mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9). In particular embodiments, the binding domain is an AHB7 scFv. In particular embodiments, the binding domain is a G8.8 scFv.

[0012] In particular embodiments, the recombinant receptor includes a CAR. In particular embodiments, the CAR includes a binding domain, a CD28 transmembrane domain, a CD28 intracellular signaling domain, and a CD3 ζ intracellular signaling domain.

[0013] In particular embodiments, a trafficking signal includes up- or down-regulated expression of CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3. In particular embodiments, a trafficking signal includes expression of CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, or CXCR4 to provide a positive directional signal (referred to as a “go here” trafficking signal). In particular embodiments, a trafficking signal includes down-regulated expression (e.g., by gene knockout) of β 7 integrin, CCR4, CXCR3, or CCR3 to provide a negative directional signal (referred to as a “don’t go here” inhibitory signal).

[0014] In particular embodiments, a retention signal includes expression of CD69, Runx3, Hobit, or CD103. In particular embodiments, a retention signal includes down-regulated expression of

CD69, Runx3, Hobit, or CD103.

[0015] In some embodiments, administration of genetically modified cells expressing a recombinant receptor (e.g., CAR) can cause toxicity at a primary treatment location (e.g., an organ where a primary tumor developed) because cancer cells and healthy tissue cells of the same organ can both express the antigen that the recombinant receptor targets (i.e., target antigen). Furthermore, if delivered systemically, toxicity can occur at an organ not involved with the cancer but happens to also express the target antigen. Therefore, in particular embodiments, the genetically modified cells are administered to secondary treatment locations (e.g., sites of metastasis). In particular embodiments, the genetically modified cells are delivered locally to a desired site of activity. In particular embodiments, the genetically modified cells are delivered systemically. In particular embodiments, the genetically modified cells include a trafficking signal and/or retention signal which causes the genetically modified cells to go to, stay away from, and/or stay at the desired secondary treatment location.

[0016] In particular embodiments, a sequence encoding a trafficking signal (e.g., CXCR6), a retention signal (e.g., CD69), and an anti-CEA CAR includes the sequence as set forth in SEQ ID NOs: 59, 60, 61, or 62. In particular embodiments, a sequence encoding a trafficking signal (e.g., CXCR6), a retention signal (e.g., CD69), and an anti-EpCAM CAR includes the sequence as set forth in SEQ ID NO: 199 or encoded by the sequence as set forth in SEQ ID NO: 202.

[0017] In particular embodiments, cells are engineered to knockout or reduce expression of a trafficking signal. In particular embodiments, knockout or reduction of expression includes delivering a nuclease and single guide RNA (sgRNA) targeting the trafficking signal. In particular embodiments, the trafficking signal includes CXCR3 or $\beta 7$ integrin. In particular embodiments, sgRNA targeting CXCR3 includes SEQ ID NO: 242, SEQ ID NO: 243, and/or SEQ ID NO: 244. In particular embodiments, sgRNA targeting $\beta 7$ integrin includes SEQ ID NO: 245, SEQ ID NO: 246, and/or SEQ ID NO: 247. In particular embodiments, the nuclease includes a Cas9 nuclease. Additionally, cells can be engineered to express a recombinant receptor (e.g., CAR). In particular embodiments, the recombinant receptor includes the sequence as set forth in SEQ ID NO: 199 or encoded by the sequence as set forth in SEQ ID NO: 202. In particular embodiments, the recombinant receptor includes the sequence as set forth in SEQ ID NO: 200 or encoded by the sequence as set forth in SEQ ID NO: 201.

[0018] Thus, in particular embodiments, the current disclosure provides a system to improve cancer treatments by directing cellular therapies *in vivo*. Genetic modification to alter expression of trafficking signals and/or retention signals by a cell, for example, induces trafficking of genetically modified cells to a desired site of activity (e.g., target organ or tissue). Alternatively,

down-regulated expression of a trafficking signal and/or retention signal within genetically modified cells can instruct the genetically modified cells to avoid a tissue, restricting access to healthy tissues, thereby reducing on-target, off-tumor toxicity.

[0019] The current disclosure differs significantly from other approaches that have been developed to direct the migration of CAR T cells. Previous approaches have directed the migration of CAR T cells to the sites of cancer cells regardless of the location of the cancer cells. In the current disclosure, cells are directed not to cancer cells, but to specific organs. In certain embodiments, cells are only directed to sites of metastasis and are not directed to a site of a primary tumor. This distinction provides an important advance in the ability to provide targeted therapies with reduced toxicity and side effects.

BRIEF DESCRIPTION OF THE FIGURES

[0020] Some of the drawings submitted herewith may be better understood in color. Applicant considers the color versions of the drawings as part of the original submission and reserves the right to present color images of the drawings in later proceedings.

[0021] FIGs. 1A-1F. Development and validation of carcinoembryonic antigen (CEA)-targeting chimeric antigen receptor (CAR)-T in human and mouse T cells. (1A) Modular structure of human CAR targeting human protein CEA (*CEACAM5*). SRP: signal recognition particle. scFV: single chain fragment variable. hCD28: human CD28. TMD: transmembrane domain. ICD: intracellular domain. (1B) Representative flow cytometry plots and (1C) summary bar graphs from *in vitro* validation of human T cells transduced with control retrovirus or hCEA hCAR-encoding virus in presence of tumor cells with expression of hCEA (LS174T) or not (Kasumi 2). (1D) Modular structure of mouse CAR targeting human protein CEA. mCD28: mouse CD28. mCD3z: mouse CD3zeta. (1E) Representative flow cytometry plots and (1F) summary bar graphs from *in vitro* validation of murine CD8 T cells transduced with control retrovirus or hCEA mCAR-encoding virus in presence of KPC cells with ectopic expression of hCEA or not (parental).

[0022] FIGs. 2A-2H. Targeting epithelial cell adhesion molecule (EpCAM) to model CAR-T cell targeting of a tumor associated antigen in solid cancer. (2A) EPCAM gene expression in selected human cancers (source: TCGA). (2B) EPCAM gene expression in selected normal human tissues (source: gtexportal.org/). (2C) Modular structure of mouse EpCAM CAR-T. (2D) Flow cytometric evaluation of surface expression of EpCAM on KPC cancer cells with knockout (KO), endogenous (parental), or expression (OE) of murine EpCAM. (2E-2H) Representative flow cytometry plots and summary bar graphs from *in vitro* validation of murine CD8⁺ EpCAM CAR-T activation in presence of KPC cells with knockout (KO), endogenous, or expression (OE) of EpCAM, or

stimulated with PMA and ionomycin to induce activation.

[0023] FIGs. 3A-3F. EpCAM CAR-T cells have some anti-tumor activity against subcutaneous KPC tumors, but exert lethal toxicity at higher doses. (3A-3C) Tumor growth curves from C57B6 mice that were subcutaneously implanted with KPC parental (3A), EpCAM KO (3B), or EpCAM OE (3C) tumor cells. 10 days after tumor implantation, mice received 5Gy total body irradiation (control) or 5Gy total body irradiation with subsequent retroorbital transfer of 1.5e6 EpCAM CAR-T cells. (3D) Change in body mice in C57B6 mice that received 4Gy total body irradiation followed by retro-orbital transfer of CD8⁺ T cells transduced with control or EpCAM CAR-T encoding retrovirus, n=3-6 per group. (3E) Kaplan-meier survival plot of mice from FIG. 3D. (3F) Representative hematoxylin and eosin images from the lung and pancreas of mice that received 1e6 or 3e6 EpCAM CAR-T cells (from experiment depicted in FIG. 3D). Statistical test: Simple linear Regression

[0024] FIGs. 4A-4G. Liver tissue residency programming induces liver tropism in OT1 transgenic T cells. (4A) Model schema depicting goal of locoregional strategy to improve safety of CAR-T cells targeting tumor associated antigens in solid tumors. Here, CAR T cells are directed to migrate to and remain in the liver. Created with BioRender. (4B) Schematic hypothesis for liver tissue residency in T cells through expression of surface proteins CXCR6 and CD69. Created with BioRender. (4C) Representative flow cytometry plots from murine CD8⁺ T cells transduced with a control retrovirus (CD90.1 alone) or the liver TRP (tissue residency programming) retrovirus. Liver TRP transduced cells have high co-expression of CXCR6 and CD69. (4D) Summary graphs from transwell migration assay where CD8⁺ T cells transduced with the control retrovirus or Liver TRP retrovirus are placed on top of a transwell filter within a well containing CXCL16 or not. CXCL16 is the specific ligand for CXCR6. Data depicts the amount of migration through the transwell filter toward the bottom of the well containing CXCL16 or not. (4E) Pre-transfer flow cytometry analyses of OT-1 CD8 T cells transduced with control retrovirus (CD45.1/2) or Liver TRP retrovirus (CD45.1/1) mixed at a 1:1 ratio prior to transfer to C57BL/6 (CD45.2/2) hosts. Prior to transfer, hosts received 5Gy radiotherapy (RT) total body irradiation. At time of transfer, hosts received T cell mix along with vaccinia virus-OVA. (4F) Summary analysis of flow cytometry on whole organs at day 6 after co-transfer of control and Liver TRP OT1 CD8 T cells (n=6 mice). (4G) Ratio of Liver TRP to control OT1 CD8 T cells in liver, lung, and spleen. Two-way ANOVA (for FIGs. 4D, 4F). One-way ANOVA (for FIGs. 4G, 4H). **P < 0.01, ***P < 0.001, ****P < 0.0001.

[0025] FIGs. 5A-5E. Liver tissue residency programmed (TRP) EpCAM CAR-T cells have enhanced safety and are effective against intrahepatic tumors. (5A) Summary data from mice who received 5Gy total body irradiation and co-transfer of EpCAM CAR-T cells with or without

Liver TRP. Data depict ratio of Liver TRP EpCAM CAR-T cells to control EpCAM CAR-T cells in liver, lung, and spleen. (5B) Change in body mass of mice that received 5Gy total body irradiation followed by retroorbital transfer of 3e6 control EpCAM CAR-T cells or Liver TRP EpCAM CAR-T cells. (5C) Representative IVIS images of mice with intrahepatic KPC-luciferase tumors, 7 days after receiving 5Gy total body irradiation alone (control, left) or 5Gy RT and Liver TRP EpCAM CAR-T cells (right). (5D) Normalized luminescence counts of mice with intrahepatic tumors treated with 5Gy total body irradiation alone (control) or 5Gy RT (n=6) and Liver TRP EpCAM CAR-T cells (n=7). (5E) Intrahepatic tumor weights from mice 7 days after treatment. Simple linear regression (for FIG. 5A). Two-way Student's T test (for FIG. 5E) **P < 0.01, ***P < 0.001

[0026] FIG. 6. Schematic of CEA CAR construct with CXCR6 and CD69, a cell expressing the construct, and schematic of the administration of said cell and its migration to the liver.

[0027] FIGs. 7A, 7B. CEA is a tumor associated antigen present in cancers and the healthy lung and intestine. (7A) mRNA expression data of CEA in the indicated cancers (cbioportal.org). (7B) Relative abundance of CEA in the indicated organs (gtexportal.org).

[0028] FIGs. 8A-8C. hCEA transgenic C57Bl/6 mice express CEA in a representative distribution and suffer CEA CAR-T cell off-tumor toxicity. To appropriately capture off-tumor toxicities in a mouse model, mouse embryos transgenic for human CEA were generated. (8A) Expression of hCEA in transgenic (hCEAtg) and wildtype (WT) C57BL/6 mice in various sites. (8B) Weight over time of hCEA CAR-T cell recipients. (8C) Colonic length measured at autopsy after CEA CAR T cell transfer.

[0029] FIGs. 9A, 9B. CEA CAR T cells slow growth of the mouse implantable KPC cell line engineered to express human CEA. (9A) Expression of hCEA in parental KPC and KPC cells engineered to stably express human CEA. (9B) Subcutaneous tumor size over time following treatment with hCEA CAR T cells (murine), control transduced cells, or phosphate buffered saline (PBS).

[0030] FIGs. 10A, 10B. Liver tissue residency programmed CEA CAR T cells preferentially traffic to liver and reduce growth of intrahepatic (IH) tumors. (10A) Summary data from mice who received 5Gy total body irradiation and co-transfer of CEA CAR-T cells with or without Liver tissue residency programming (TRP). Data depict ratio of Liver TRP CEA CAR-T cells to control CEA CAR-T cells in liver, lung, spleen, and gut. (10B) Intrahepatic tumor weights from mice 9 days after treatment with 5Gy RT, and control PBS or Liver TRP CEA CAR T Cells.

[0031] FIGs. 11A, 11B. Validation of Cxcr3 and Itgb7 CRISPR disruption followed by retroviral CAR transduction. Representative flow cytometry of CD8 T cells electroporated with Cas9 sgRNA complexes targeting lung (11A) or gut (11B) homing receptors, stimulated, and then transduced

with retroviral (RV) particles encoding EpCAM-CAR-T2A-CD90.1.

[0032] FIGS. 12A-12C. Genetic ablation of CXCR3 in EpCAM CAR T cells limits trafficking to lung and pancreas Flow cytometric evaluation of relative frequency of EpCAM CAR T cells in lung (12A) and pancreas (12B) with or without (KO) intact CXCR3; evaluated 6 days after cell transfer. (12C) Ratio of CXCR3 knockout to scramble (Scr) EpCAM CAR T cells in IH tumor, lung, pancreas, and spleen.

[0033] FIGS. 13A, 13B. Genetic ablation of Itgb7 in CEA CAR T cells limits trafficking to intestine. (13A) Flow cytometric evaluation of relative frequency of CEA CAR T cells in gut (Colon + Small intestine) with or without (KO) intact $\alpha 4\beta 7$ integrin; evaluated 6 days after cell transfer. (13B) Ratio of $\alpha 4\beta 7$ knockout to scramble (Scr) CEA CAR T cells in IH tumor, gut, lung, and spleen.

[0034] FIG. 14. Table of cancer antigen target, desired site of activity (secondary treatment location), and the signals used to traffic and/or retain genetically modified cells to the desired site of activity. Trafficking and inhibitory signals can be combinatorial or used individually. Acronyms: Exp (expression of provided genetic construct); KO (knockout). Alternative retention signals include Runx3 and Hobit expression (Crowl *et al.* Nat. Immunol. 23, 1121–1131 (2022); Christo *et al.* Nat. Immunol. 22, 1140–1151 (2021); and Milner and Goldrath. Curr. Opin. Immunol. 51, 162–169 (2018)).

[0035] FIG. 15. Sequences supporting the disclosure including CXCR6 Coding Sequence (SEQ ID NO: 1), RAKR Coding Sequence (SEQ ID NO: 2), T2A Coding Sequence (SEQ ID NO: 3), CD69 Coding Sequence (SEQ ID NO: 4), 2A Coding Sequence (SEQ ID NO: 5), Myc Coding Sequence (SEQ ID NO: 6), Extracellular Domain Spacer Coding Sequence (SEQ ID NO: 7), CD28 Transmembrane Domain Coding Sequence (SEQ ID NO: 8), CD28 Intracellular Signaling Domain Coding Sequence (SEQ ID NO: 9), CD3z Intracellular Signaling Domain Coding Sequence (SEQ ID NO: 10), IgG4 hinge (SEQ ID NO: 11) and coding sequence (SEQ ID NOs: 12-14), IgG4-CH2 domain (SEQ ID NOs: 15 and 16) and coding sequence (SEQ ID NOs: 17 and 18), IgG4-CH3 domain (SEQ ID NOs: 19 and 20) and coding sequence (SEQ ID NOs: 21 and 22), Intermediate Spacer (SEQ ID NO: 23) and coding sequence (SEQ ID NO: 24), Long Spacer (SEQ ID NO: 25) and coding sequence (SEQ ID NO: 26), CD28 Transmembrane Domain (SEQ ID NOs: 27-29) and coding sequence (SEQ ID NOs: 30-33), 41BB costimulatory domain (SEQ ID NOs: 34-36) and coding sequence (SEQ ID NOs: 37-39), CD28 signaling domain (SEQ ID NO: 40) and coding sequence (SEQ ID NO: 41), CD28/4-1BB signaling domain (SEQ ID NO: 42) and coding sequence (SEQ ID NO: 43), CD3 ζ signaling domain (SEQ ID NOs: 44-46) and coding sequence (SEQ ID NOs: 47 and 48), Thosaasigna Virus 2A (T2A) Peptide (SEQ ID NOs: 49 and 50), Porcine Teschovirus-1 2a (P2A) Peptide (SEQ ID NO: 51), Equine Rhinitis A Virus (ERAV) 2A

(E2A) Peptide (SEQ ID NO: 52), Foot-And-Mouth Disease Virus 2A (F2A) Peptide (SEQ ID NO: 53), T2A coding sequence (SEQ ID NOs: 54 and 55), EGFRt (SEQ ID NO: 56) and coding sequence (SEQ ID NO: 57), tCD19 coding sequence (SEQ ID NO: 58), CD90.1 (SEQ ID NO: 198), Retro_CXCR6_CD69_myc-hCEACAR (SEQ ID NO: 59), Retro_CXCR6_CD69_hCEACAR (SEQ ID NO: 60), Lenti_MND_hs_CXCR6_CD69_myc-hCEACAR (SEQ ID NO: 61), Lenti_MND_hs_CXCR6_CD69_hCEACAR (SEQ ID NO: 62), CEA CAR (SEQ ID NO: 200) and coding sequence (SEQ ID NO: 201), Example anti-EpCAM CAR (SEQ ID NO: 199) and coding sequence (SEQ ID NO: 202), and EpCAM CAR + Liver tissue residency program (TRP) (SEQ ID NO: 203).

DETAILED DESCRIPTION

[0036] For many years, the chosen treatments for cancer were surgery, chemotherapy, and/or radiation therapy. In recent years, more targeted therapies have emerged to specifically target cancer cells by identifying and exploiting specific molecular and/or immunophenotypic changes seen primarily in those cells. For example, many cancer cells preferentially express particular antigens on their cellular surfaces and these antigens have provided targets for successful therapeutics.

[0037] The shortcomings of chimeric antigen receptor (CAR) T cell therapy in solid tumors necessitates the need to reduce on-target, off-tumor toxicity.

[0038] The current disclosure provides cells genetically modified to achieve at least two of: express a recombinant receptor, modulate expression of a trafficking signal, or modulate expression of a retention signal. The genetic constructs described herein can be used to facilitate localized action of cellular therapies. Expression of a trafficking signal and/or retention signal by genetically modified cells can induce the genetically modified cells to infiltrate a treatment site (e.g., organ or tissue) and/or prevent the genetically modified cells from leaving a treatment site, respectively. Alternatively, down-regulated expression of a trafficking signal can keep genetically modified cells from entering a particular tissue. Concurrent expression of a i) recombinant receptor that binds a target antigen and ii) a surface molecule (e.g., trafficking signal and/or retention signal), by genetically modified cells, instructs the genetically modified cells to traffic to a select tissue (or away from a non-selected tissue), kill cells expressing the target antigen, and/or remain within the select tissue.

[0039] In particular embodiments, systems and methods disclosed herein are used to direct a treatment to an organ that represents a secondary treatment location. A secondary treatment location refers to a location in the body that is different from where a condition to be treated

emerged. For example, in some embodiments, a secondary treatment location is an organ to which a primary tumor has metastasized. In particular embodiments, systems and methods disclosed herein are used to direct a treatment to a secondary treatment location and to avoid, reduce, or prevent direction of the treatment to a primary treatment location. A primary treatment location is a location in the body where a condition to be treated emerged.

[0040] Particular embodiments of the systems and methods disclosed herein can be used to facilitate localized action of cellular therapies at secondary treatment locations and to avoid action at primary treatment locations and/or healthy, non-cancerous tissues. The systems and methods disclosed herein also can be used to facilitate localized action of cellular therapies at a primary treatment location and to avoid action at secondary treatment locations and/or healthy, non-cancerous tissues. The systems and methods disclosed herein also can be used to facilitate localized action of cellular therapies at a primary treatment location and secondary treatment locations and to avoid action at healthy, non-cancerous tissues. In particular embodiments, healthy, non-cancerous tissues can be referred to as “off-target”.

[0041] In particular embodiments, a recombinant receptor includes a CAR, an engineered T cell receptor (eTCR), or a hybrid thereof. In particular embodiments, the recombinant receptor includes a binding domain. In particular embodiments, the binding domain binds a target antigen. In particular embodiments, the target antigen is expressed by a cancer cell. In particular embodiments, the cancer includes colorectal cancer, pancreatic cancer, breast cancer, colon cancer, gastric cancer, prostate cancer, hepatocellular carcinoma, ovarian cancer, endometrial cancer, breast cancer, or uterine cancer. In particular embodiments, the target antigen includes a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9). In particular embodiments, the binding domain binds human CEA and is an AHB7 scFv. In particular embodiments, the binding domain binds mouse EpCAM and is a G8.8 scFv.

[0042] In particular embodiments, the recombinant receptor includes a CAR. In particular embodiments, the CAR includes a binding domain, a CD28 transmembrane domain, a CD28 intracellular signaling domain, and a CD3 ζ intracellular signaling domain.

[0043] In particular embodiments, a trafficking signal includes up- or down regulated expression of CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3. In particular embodiments, a trafficking signal includes expression of CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, or CXCR4 to provide a positive directional signal (referred to as a “go here”

trafficking signal). In particular embodiments, a trafficking signal includes down regulated expression (e.g., by gene knockout) of β 7 integrin, CCR4, CXCR3, or CCR3 to provide a negative directional signal (referred to as a “don’t go here” inhibitory signal).

[0044] In particular embodiments, a retention signal includes expression of CD69, Runx3, Hobit, or CD103. In particular embodiments, a retention signal includes down regulated expression of CD69, Runx3, Hobit, or CD103.

[0045] In some embodiments, administration of genetically modified cells expressing a recombinant receptor (e.g., CAR) can cause toxicity at the organ where a primary tumor developed because cancer cells and healthy tissue cells of the same organ can both express the antigen that the recombinant receptor targets (i.e., target antigen). Furthermore, if delivered systemically, toxicity can occur at an organ not involved with the cancer but happens to also express the target antigen. Therefore, in particular embodiments, the genetically modified cells of the present disclosure are administered to tissue sites to target metastasis (e.g., at desired site of activity). In particular embodiments, the genetically modified cells are delivered locally to a desired site of activity. In particular embodiments, the genetically modified cells are delivered systemically. In particular embodiments, the genetically modified cells include a trafficking signal and/or a retention signal which causes the genetically modified cells to go to, stay away from, and/or stay at the desired site of activity.

[0046] In particular embodiments, a sequence encoding a trafficking signal (e.g., CXCR6), a retention signal (e.g., CD69), and an anti-CEA CAR includes the sequence as set forth in SEQ ID NOs: 59, 60, 61, or 62. In particular embodiments, a sequence encoding a trafficking signal (e.g., CXCR6), a retention signal (e.g., CD69), and an anti-EpCAM CAR includes the sequence as set forth in SEQ ID NO: 199 or encoded by the sequence as set forth in SEQ ID NO: 202.

[0047] In particular embodiments, cells are engineered to knockout or reduce expression of a trafficking signal. In particular embodiments, knockout or reduction of expression includes delivering a nuclease and single guide RNA (sgRNA) targeting the trafficking signal. In particular embodiments, the trafficking signal includes CXCR3 or β 7 integrin. In particular embodiments, sgRNA targeting CXCR3 includes SEQ ID NO: 242, SEQ ID NO: 243, and/or SEQ ID NO: 244. In particular embodiments, sgRNA targeting β 7 integrin includes SEQ ID NO: 245, SEQ ID NO: 246, and/or SEQ ID NO: 247. In particular embodiments, the nuclease includes a Cas9 nuclease. Additionally, cells can be engineered to express a recombinant receptor (e.g., CAR). In particular embodiments, the recombinant receptor includes the sequence as set forth in SEQ ID NO: 199 or encoded by the sequence as set forth in SEQ ID NO: 202. In particular embodiments, the recombinant receptor includes the sequence as set forth in SEQ ID NO: 200 or encoded by the

sequence as set forth in SEQ ID NO: 201.

[0048] Thus, the current disclosure provides a system to improve cancer treatments by directing cellular therapies *in vivo*. Expression of a trafficking signal and a retention signal by genetically modified cells, for example, induces trafficking of genetically modified cells to a select tissue and retains the genetically modified cells within the select tissue, thereby reducing on-target, off-tumor toxicity at both the tumor site and/or other off-tumor sites. Alternatively, down regulated expression of a trafficking signal and/or retention signal within genetically modified cells can instruct the genetically modified cells to avoid a select tissue.

[0049] The current disclosure differs significantly from other approaches that have been developed to direct the migration of CAR T cells. Previous approaches have directed the migration of CAR T cells to the sites of cancer cells regardless of the location of the cancer cells. In the current disclosure, cells are directed not to cancer cells, but to specific organs. In certain embodiments, cells are only directed to sites of metastasis and are not directed to a site of a primary tumor. This distinction provides an important advance in the ability to provide targeted therapies with reduced toxicity and side effects.

[0050] Aspects of the current disclosure are now described in more supporting detail as follows: (I) Cells; (II) Cell Sample Collection and Cell Enrichment; (III) Genetically Modifying Cell Populations; (III-A) Trafficking Signals; (III-B) Retention Signals; (III-C) Recombinant Receptors; (III-C-1) Binding Domains; (III-C-2) Transmembrane Domains; (III-C-3) Intracellular Components; (III-D) Linkers; (III-E) Control Features Including Tag Cassettes, Transduction Markers, and/or Suicide Switches; ; (IV) Characterization of Genetically Modified Cells; (V) Cell Activating Culture Conditions; (VI) *Ex Vivo* Manufactured Cell Formulations; (VII) Targeted Viral Vectors & Nanoparticles for *In Vivo* Cell Modification; (VIII) Methods of Use; (IX) Kits; (X) Exemplary Embodiments; (XI) Experimental Example; and (XII) Closing Paragraphs. These headings are provided for organizational purposes only and do not limit the scope or interpretation of the disclosure.

[0051] (I) Cells. The present disclosure describes cells genetically modified to achieve at least two of: express a recombinant receptor, modulate expression of a trafficking signal, or modulate expression of a retention signal. Cells to be genetically modified can include immune cells, stem or progenitor cells, or adult cells. Immune cells can include T-cells, B cells, natural killer (NK) cells, NK-T cells, monocytes/macrophages, and lymphocytes. Stem or progenitor cells can include hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPC), pluripotent stem cells, mesenchymal stem cells, neural stem cells, endothelial stem cells, and/or a mixture thereof. In particular embodiments, genetically modified cells include T-cells.

[0052] Several different subsets of T-cells have been discovered, each with a distinct function. For example, a majority of T-cells have a T-cell receptor (TCR) existing as a complex of several proteins. The actual T-cell receptor is composed of two separate peptide chains, which are produced from the independent T-cell receptor alpha and beta (TCR α and TCR β) genes and are called α - and β -TCR chains.

[0053] $\gamma\delta$ T-cells represent a small subset of T-cells that possess a distinct T-cell receptor (TCR) on their surface. In $\gamma\delta$ T-cells, the TCR is made up of one γ -chain and one δ -chain. This group of T-cells is much less common (2% of total T-cells) than the $\alpha\beta$ T-cells.

[0054] CD3 is expressed on all mature T cells. Activated T-cells express 4-1BB (CD137), CD69, and CD25. CD5 and transferrin receptor are also expressed on T-cells.

[0055] T-cells can further be classified into helper cells (CD4⁺ T-cells) and cytotoxic T-cells (CTLs, CD8⁺ T-cells), which include cytolytic T-cells. T helper cells assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and activation of cytotoxic T-cells and macrophages, among other functions. These cells are also known as CD4⁺ T-cells because they express the CD4 protein on their surface. Helper T-cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of antigen presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response.

[0056] Cytotoxic T-cells destroy virally infected cells and tumor cells and are also implicated in transplant rejection. These cells are also known as CD8⁺ T-cells because they express the CD8 glycoprotein on their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body.

[0057] "Central memory" T-cells (or "TCM") refers to an antigen experienced CTL that expresses CD62L or CCR7 and CD45RO on the surface thereof and does not express or has decreased expression of CD45RA as compared to naive cells. In particular embodiments, central memory cells are positive for expression of CD62L, CCR7, CD25, CD127, CD45RO, and CD95, and have decreased expression of CD45RA as compared to naive cells.

[0058] "Effector memory" T-cell (or "TEM") refers to an antigen experienced T-cell that does not express or has decreased expression of CD62L on the surface thereof as compared to central memory cells and does not express or has decreased expression of CD45RA as compared to a naive cell. In particular embodiments, effector memory cells are negative for expression of CD62L and CCR7, compared to naive cells or central memory cells, and have variable expression of CD28 and CD45RA. Effector T-cells are positive for granzyme B and perforin as compared to memory or naive T-cells.

[0059] "Naive" T-cells refers to a non-antigen experienced T cell that expresses CD62L and CD45RA and does not express CD45RO as compared to central or effector memory cells. In particular embodiments, naive CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naive T-cells including CD62L, CCR7, CD28, CD127, and CD45RA.

[0060] Natural killer cells (also known as NK cells, K cells, and killer cells) are activated in response to interferons or macrophage-derived cytokines. They serve to contain viral infections while the adaptive immune response is generating antigen-specific cytotoxic T cells that can clear the infection. NK cells express CD8, CD16 and CD56 but do not express CD3.

[0061] NK cells include NK-T cells. NK-T cells are a specialized population of T cells that express a semi invariant T cell receptor (TCR $\alpha\beta$) and surface antigens typically associated with natural killer cells. NK-T cells contribute to antibacterial and antiviral immune responses and promote tumor-related immunosurveillance or immunosuppression. Like natural killer cells, NK-T cells can also induce perforin-, Fas-, and TNF-related cytotoxicity. Activated NK-T cells are capable of producing IFN- γ and IL-4. In particular embodiments, NK-T cells are CD3⁺/CD56⁺.

[0062] Macrophages (and their precursors, monocytes) reside in every tissue of the body (in certain instances as microglia, Kupffer cells and osteoclasts) where they engulf apoptotic cells, pathogens and other non-self-components. Monocytes/macrophages express CD11b, F4/80; CD68; CD11c; IL-4R α ; and/or CD163.

[0063] Immature dendritic cells (i.e., pre-activation) engulf antigens and other non-self-components in the periphery and subsequently, in activated form, migrate to T-cell areas of lymphoid tissues where they provide antigen presentation to T cells. Dendritic cells express CD1a, CD1b, CD1c, CD1d, CD21, CD35, CD39, CD40, CD86, CD101, CD148, CD209, and DEC-205.

[0064] Stem cells are cells capable of differentiation into other cell types, including those having a particular, specialized function (e.g., tissue specific cells, parenchymal cells and progenitors thereof). There are various classes of stem cells, which can be characterized in their ability to differentiate into a desired cell/tissue type. For example, "progenitor cells" can be either multipotent or pluripotent. Progenitor cells are cells that can give rise to different terminally differentiated cell types, and cells that are capable of giving rise to various progenitor cells. The term "pluripotent" or "pluripotency" refers to cells with the ability to give rise to progeny cells that can undergo differentiation, under the appropriate conditions, into cell types that collectively demonstrate characteristics associated with cell lineages from all of the three germinal layers (endoderm, mesoderm, and ectoderm).

[0065] Hematopoietic Stem/Progenitor Cells or HSPC refer to a combination of hematopoietic stem cells and hematopoietic progenitor cells.

[0066] Hematopoietic stem cells refer to undifferentiated hematopoietic cells that are capable of self-renewal either *in vivo*, essentially unlimited propagation *in vitro*, and capable of differentiation to all other hematopoietic cell types.

[0067] A hematopoietic progenitor cell is a cell derived from hematopoietic stem cells or fetal tissue that is capable of further differentiation into mature cell types. In certain embodiments, hematopoietic progenitor cells are CD24^{lo} Lin⁻ CD117⁺ hematopoietic progenitor cells. HPC can differentiate into (i) myeloid progenitor cells which ultimately give rise to monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, or dendritic cells; or (ii) lymphoid progenitor cells which ultimately give rise to T-cells, B-cells, and NK-cells.

[0068] HSPC can be positive for a specific marker expressed in increased levels on HSPC relative to other types of hematopoietic cells. For example, such markers include CD34, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, HLA DR, or a combination thereof. Also, the HSPC can be negative for an expressed marker relative to other types of hematopoietic cells. For example, such markers include Lin, CD38, or a combination thereof. Preferably, the HSPC are CD34⁺ cells.

[0069] Pluripotent stem cells are a type of cell that undergoes self-renewal while maintaining an ability to give rise to all three germ layer-derived tissues and germ cell lineages. Pluripotent stem cells include induced pluripotent stem cells and embryonic stem cells.

[0070] Mesenchymal stem cells also known as mesenchymal stromal cells are multipotent stromal cells. Mesenchymal stem cells are more differentiated than pluripotent stem cells, but retain the ability to differentiate into a variety of cell types, including osteoblasts, chondrocytes, myocytes and adipocytes.

[0071] Neural stem cells are stem cells found in the adult brain. Endothelial stem cells are a type of multipotent stem cell found in the bone marrow. Endothelial stem cells differentiate into endothelial cells or cells that line blood vessels as well as lymphatic vessels.

[0072] Adult cells include any cell type that is terminally differentiated. In particular embodiments, adult cells include fibroblasts, hepatocytes, myoblasts, neurons, osteoblasts, osteoclasts, kidney cells, chondrocytes, or adipocytes. A statement that a cell or population of cells is "positive" for or expressing a particular marker refers to the detectable presence on or in the cell of the particular marker. When referring to a surface marker, the term can refer to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-

matched control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0073] A statement that a cell or population of cells is "negative" for a particular marker or lacks expression of a marker refers to the absence of substantial detectable presence on or in the cell of a particular marker. When referring to a surface marker, the term can refer to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

[0074] In particular embodiments, immune cells to be genetically modified according to the teachings of the current disclosure can be patient-derived cells (autologous) or allogeneic. In particular embodiments, immune cells can be genetically modified *in vivo* or *ex vivo*.

[0075] (II) Cell Sample Collection and Cell Enrichment. Methods of sample collection and enrichment are known by those skilled in the art. In particular embodiments, cells are derived from humans, for example a patient to be treated. Cells can be derived from cell lines.

[0076] In some embodiments, T cells are derived or isolated from samples such as whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. In particular embodiments, cells from the circulating blood of a subject are obtained, e.g., by apheresis or leukapheresis. The samples, in particular embodiments, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, HSC, HPC, HSPC, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets and further processing is necessary. In particular embodiments, T cells are derived from PBMCs.

[0077] In some embodiments, blood cells collected from a subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps.

[0078] Isolation can include one or more of various cell preparation and separation steps, including separation based on one or more properties, such as size, density, sensitivity or

resistance to particular reagents, and/or affinity, e.g., immunoaffinity, to antibodies or other binding partners. In particular embodiments, the isolation is carried out using the same apparatus or equipment sequentially in a single process stream and/or simultaneously. In particular embodiments, the isolation, culture, and/or engineering of the different populations is carried out from the same starting material, such as from the same sample.

[0079] In particular embodiments, a sample can be enriched for T cells by using density-based cell separation methods and related methods. For example, white blood cells can be separated from other cell types in the peripheral blood by lysing red blood cells and centrifuging the sample through a Percoll or Ficoll gradient.

[0080] In particular embodiments, a bulk T cell population can be used that has not been enriched for a particular T cell type. In particular embodiments, a selected T cell type can be enriched for and/or isolated based on cell-marker based positive and/or negative selection. In positive selection, cells having bound cellular markers are retained for further use. In negative selection, cells not bound by a capture agent, such as an antibody to a cellular marker are retained for further use. In some examples, both fractions can be retained for a further use. In particular embodiments, CD4+ and/or CD8+ T cells are enriched from PBMCs. In particular embodiments, CD8+ T cells are enriched from PBMCs.

[0081] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type refers to increasing the number or percentage of such cells but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type refers to decreasing the number or percentage of such cells but need not result in a complete removal of all such cells.

[0082] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection.

[0083] In some embodiments, an antibody or binding domain for a cellular marker is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection.

[0084] In some embodiments, affinity-based selection is via magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Auburn, CA). MACS systems are capable of high-purity selection of cells having magnetized particles attached thereto.

[0085] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in

a fluidic stream.

[0086] Cell-markers for different T cell subpopulations are described above. In particular embodiments, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, e.g., CCR7, CD45RO, CD8, CD27, CD28, CD62L, CD127, CD4, and/or CD45RA T cells, are isolated by positive or negative selection techniques.

[0087] CD3+, CD28+ T cells can be positively selected for and expanded using anti-CD3/anti-CD28 conjugated magnetic beads (e.g., DYNABEADS® M-450 CD3/CD28 T Cell Expander).

[0088] In particular embodiments, a CD8+ or CD4+ selection step is used to separate CD4+ helper and CD8+ cytotoxic T cells. Such CD8+ and CD4+ populations can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations.

[0089] In some embodiments, enrichment for central memory T (TCM) cells is carried out. In particular embodiments, memory T cells are present in both CD62L subsets of CD8+ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L, CD8 and/or CD62L+CD8+ fractions, such as by using anti-CD8 and anti-CD62L antibodies.

[0090] In some embodiments, the enrichment for central memory T (TCM) cells is based on positive or high surface expression of CCR7, CD45RO, CD27, CD62L, CD28, CD3, and/or CD127; in some aspects, it is based on negative selection for cells expressing or highly expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8+ population enriched for TCM cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CCR7, CD45RO, and/or CD62L. In one aspect, enrichment for central memory T (TCM) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L.

[0091] Other cell types can be enriched based on known marker profiles and techniques. For example, CD34+ HSC, HSP, and HSPC can be enriched using anti-CD34 antibodies directly or indirectly conjugated to magnetic particles in connection with a magnetic cell separator, for example, the CliniMACS® Cell Separation System (Miltenyi Biotec, Bergisch Gladbach, Germany).

[0092] (III) Genetically Modifying Cell Populations. Cell populations are genetically modified to include a genetic construct that results in two or more of: expression of a recombinant receptor, up- or down-regulated expression of a trafficking signal, or up- or down-regulated expression of a retention signal.

[0093] (III-A) Genetic Engineering Techniques. Genetic constructs encoding a molecule of

interest (e.g., CAR, eTCR, trafficking signal, retention signal) and/or resulting in down-regulated expression of a molecule of interest (e.g., trafficking signal, retention signal) can be introduced into cells by any method known in the art, including transfection, electroporation, microinjection, lipofection, calcium phosphate mediated transfection, infection with a viral or bacteriophage vector including the gene sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, *in vivo* nanoparticle-mediated delivery, etc. Numerous techniques are known in the art for the introduction of foreign genetic constructs into cells (see e.g., Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen, *et al.*, 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and may be used, provided that the necessary developmental and physiological functions of the recipient cells are not unduly disrupted. The technique can provide for the stable transfer of the genetic construct to the cell, so that the gene is expressible by the cell and, in certain instances, preferably heritable and expressible by its cell progeny.

[0094] Methods of genetic engineering described herein can be used to cause expression or down-regulate (knockdown/knockout) expression of molecules described herein.

[0095] The term “gene” refers to a nucleic acid sequence (used interchangeably with polynucleotide or nucleotide sequence) that encodes a molecule of interest. This definition includes various sequence polymorphisms, mutations, and/or sequence variants wherein such alterations do not substantially affect the function of the encoded molecule of interest. The term “gene” may include not only coding sequences but also regulatory regions such as promoters, enhancers, and termination regions. Gene sequences encoding the molecule can be DNA or RNA that directs the expression of the molecule of interest. These nucleic acid sequences may be a DNA strand sequence that is transcribed into RNA or an RNA sequence that is translated into protein. The sequences can also include degenerate codons of the native sequence or sequences that may be introduced to provide codon preference in a specific cell type (e.g., a mammalian cell). Portions of complete gene sequences are referenced throughout the disclosure as is understood by one of ordinary skill in the art.

[0096] Gene sequences encoding molecules of interest are provided herein and can also be readily prepared by synthetic or recombinant methods from the relevant amino acid sequences and other description provided herein. In embodiments, the gene sequence encoding any of these sequences can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the gene sequence encoding the sequence with another gene sequence encoding a different sequence.

[0097] “Encoding” refers to the property of specific sequences of nucleotides in a gene, such as

a cDNA, or an mRNA, to serve as templates for synthesis of other macromolecules such as a defined sequence of amino acids.

[0098] Polynucleotide gene sequences encoding more than one portion of an expressed genetic construct can be operably linked to each other and relevant regulatory sequences. For example, there can be a functional linkage between a regulatory sequence and an exogenous nucleic acid sequence resulting in expression of the latter. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence.

[0099] A "vector" is a nucleic acid molecule that is capable of transporting another nucleic acid. Vectors may be, e.g., plasmids (DNA plasmids or RNA plasmids), transposon-based systems, cosmids, bacterial artificial chromosomes, viruses, or phage. An "expression vector" is a vector that is capable of directing the expression of a protein encoded by one or more genes carried by the vector when it is present in the appropriate environment.

[0100] "Lentivirus" refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Several examples of lentiviruses include HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

[0101] A lentiviral vector is a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., *Mol. Ther.* 17(8): 1453-1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include: the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art. In particular embodiments, cells are genetically modified (or genetically engineered) to express a recombinant receptor or surface molecule using a lentivirus or lentiviral vector.

[0102] "Retroviruses" are viruses having an RNA genome. "Gammaretrovirus" refers to a genus of the retroviridae family. Exemplary gammaretroviruses include mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses.

[0103] Retroviral vectors (see Miller, *et al.*, 1993, *Meth. Enzymol.* 217:581-599) can be used. In such embodiments, the gene to be expressed is cloned into the retroviral vector for its delivery into cells. In particular embodiments, a retroviral vector includes all of the cis-acting sequences necessary for the packaging and integration of the viral genome, i.e., (a) a long terminal repeat (LTR), or portions thereof, at each end of the vector; (b) primer binding sites for negative and positive strand DNA synthesis; and (c) a packaging signal, necessary for the incorporation of

genomic RNA into virions. More detail about retroviral vectors can be found in Boesen, *et al.*, 1994, *Biotherapy* 6:291-302; Clowes, *et al.*, 1994, *J. Clin. Invest.* 93:644-651; Kiem, *et al.*, 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114. Adenoviruses, adeno-associated viruses (AAV) and alphaviruses can also be used. See Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503, Rosenfeld, *et al.*, 1991, *Science* 252:431-434; Rosenfeld, *et al.*, 1992, *Cell* 68:143-155; Mastrangeli, *et al.*, 1993, *J. Clin. Invest.* 91:225-234; Walsh, *et al.*, 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; and Lundstrom, 1999, *J. Recept. Signal Transduct. Res.* 19: 673-686. Other methods of gene delivery include use of mammalian artificial chromosomes (Vos, 1998, *Curr. Op. Genet. Dev.* 8:351-359); liposomes (Tarahovsky and Ivanitsky, 1998, *Biochemistry (Mosc)* 63:607-618); ribozymes (Branch and Klotman, 1998, *Exp. Nephrol.* 6:78-83); and triplex DNA (Chan and Glazer, 1997, *J. Mol. Med.* 75:267-282).

[0104] There are a large number of available viral vectors suitable within the current disclosure, including those identified for human gene therapy applications (see Pfeifer and Verma, 2001, *Ann. Rev. Genomics Hum. Genet.* 2:177). Methods of using retroviral and lentiviral viral vectors and packaging cells for transducing mammalian host cells with viral particles including transgenes are described in, *e.g.*, US 8,119,772; Walchli, *et al.*, 2011, *PLoS One* 6:327930; Zhao, *et al.*, 2005, *J. Immunol.* 174:4415; Engels, *et al.*, 2003, *Hum. Gene Ther.* 14:1155; Frecha, *et al.*, 2010, *Mol. Ther.* 18:1748; and Verhoeven, *et al.*, 2009, *Methods Mol. Biol.* 506:97. Retroviral and lentiviral vector constructs and expression systems are also commercially available.

[0105] Targeted genetic engineering approaches may also be utilized to insert or knockout gene sequences. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated protein) nuclease system is an engineered nuclease system used for genetic engineering that is based on a bacterial system. Information regarding CRISPR-Cas systems and components thereof are described in, for example, US8697359, US8771945, US8795965, US8865406, US8871445, US8889356, US8889418, US8895308, US8906616, US8932814, US8945839, US8993233 and US8999641 and applications related thereto; and WO2014/018423, WO2014/093595, WO2014/093622, WO2014/093635, WO2014/093655, WO2014/093661, WO2014/093694, WO2014/093701, WO2014/093709, WO2014/093712, WO2014/093718, WO2014/145599, WO2014/204723, WO2014/204724, WO2014/204725, WO2014/204726, WO2014/204727, WO2014/204728, WO2014/204729, WO2015/065964, WO2015/089351, WO2015/089354, WO2015/089364, WO2015/089419, WO2015/089427, WO2015/089462, WO2015/089465, WO2015/089473 and WO2015/089486, WO2016205711,

WO2017/106657, WO2017/127807 and applications related thereto. In particular embodiments, a nuclease includes Cas9 or Cpf1.

[0106] Particular embodiments utilize zinc finger nucleases (ZFNs) as gene editing agents. For information regarding ZFNs and ZFNs useful within the teachings of the current disclosure, see, e.g., US 6,534,261; US 6,607,882; US 6,746,838; US 6,794,136; US 6,824,978; 6,866,997; US 6,933,113; 6,979,539; US 7,013,219; US 7,030,215; US 7,220,719; US 7,241,573; US 7,241,574; US 7,585,849; US 7,595,376; US 6,903,185; US 6,479,626; US 2003/0232410 and US 2009/0203140 as well as Gaj *et al.*, Nat Methods, 2012, 9(8):805-7; Ramirez *et al.*, Nucl Acids Res, 2012, 40(12):5560-8; Kim *et al.*, Genome Res, 2012, 22(7): 1327-33; Urnov *et al.*, Nature Reviews Genetics, 2010, 11 :636-646; Miller, *et al.* Nature biotechnology 25, 778-785 (2007); Bibikova, *et al.* Science 300, 764 (2003); Bibikova, *et al.* Genetics 161, 1169-1175 (2002); Wolfe, *et al.* Annual review of biophysics and biomolecular structure 29, 183-212 (2000); Kim, *et al.* Proceedings of the National Academy of Sciences of the United States of America 93, 1156-1160 (1996); and Miller, *et al.* The EMBO journal 4, 1609-1614 (1985).

[0107] Particular embodiments can use transcription activator like effector nucleases (TALENs) as gene editing agents. For information regarding TALENs, see US 8,440,431; US 8,440,432; US 8,450,471; US 8,586,363; and US 8,697,853; as well as Joung and Sander, Nat Rev Mol Cell Biol, 2013, 14(l):49-55; Beurdeley *et al.*, Nat Commun, 2013, 4: 1762; Scharenberg *et al.*, Curr Gene Ther, 2013, 13(4):291-303; Gaj *et al.*, Nat Methods, 2012, 9(8):805-7; Miller, *et al.* Nature biotechnology 29, 143-148 (2011); Christian, *et al.* Genetics 186, 757-761 (2010); Boch, *et al.* Science 326, 1509-1512 (2009); and Moscou, & Bogdanove, Science 326, 1501 (2009).

[0108] Particular embodiments can utilize MegaTALs as gene editing agents. MegaTALs have a rare-cleaving nuclease structure in which a TALE is fused with the DNA cleavage domain of a meganuclease. Meganucleases, also known as homing endonucleases, are single peptide chains that have both DNA recognition and nuclease function in the same domain. In contrast to the TALEN, the megaTAL only requires the delivery of a single peptide chain for functional activity.

[0109] Particular embodiments can use transposon-based systems as gene editing agents to mediate the integration of a genetic construct into cells.

[0110] Several transposon/transposase systems have been adapted for genetic insertions of heterologous DNA sequences. Examples of such transposases include sleeping beauty ("SB", e.g., derived from the genome of salmonid fish); piggyback (e.g., derived from lepidopteran cells and/or the *Myotis lucifugus*); mariner (e.g., derived from *Drosophila*); frog prince (e.g., derived from *Rana pipiens*); Tol1; Tol2 (e.g., derived from medaka fish); TcBuster (e.g., derived from the red flour beetle *Tribolium castaneum*), Helraiser, Himar1, Passport, Minos, Ac/Ds, PIF, Harbinger,

Harbinger3-DR, HSmar1, and spinON. Transposases and transposon systems are further described in U.S. Pat. Nos. 6,489,458; 7,148,203; 8,227,432; and 9,228,180.

[0111] Nanoparticles that result in selective *in vivo* genetic modification of targeted cell types can be used within the teachings of the current disclosure. In particular embodiments, the nanoparticles can be those described in WO2014153114, WO2017181110, and WO201822672.

[0112] (III-A) Trafficking Signals. A trafficking signal refers to any molecule that impacts the location of a cell in the body via a positive directional signal or a negative directional signal. As shown in FIG. 14, a positive directional signal can provide a 'go here' signal and a negative directional signal can provide a 'don't go here' signal by either up- or down-regulated expression of the trafficking signal. In particular embodiments, cells can be engineered to traffic to a select tissue by introducing a genetic construct expressing a trafficking signal to the cell. In particular embodiments, a cell can be engineered to avoid a non-selected tissue by down-regulating expression of an endogenous trafficking signal.

[0113] In particular embodiments, the product of an introduced coding sequence may be expressed at low levels in non-targeted cells, for example at less than 1% or 1%, 2%, 3%, 5%, 10%, 15% or 20% of the levels at which the product is expressed in targeted cells.

[0114] A "select" or "target" tissue as it refers to genetically modified cells being trafficked to or residing in the select tissue means that the genetically modified cells are trafficked to or reside in the intended tissue and are not substantially trafficked to nor residing in other tissue types, as explained in additional detail below. In particular embodiments, not substantially trafficked to nor residing in other tissue types means that less than 50% are found in a reference tissue type as compared to a select tissue type; less than 40% found in a reference tissue type as compared to a select tissue type; less than 30% found in a reference tissue type as compared to a select tissue type; less than 20% found in a reference tissue type as compared to a select tissue type; or less than 10% found in a reference tissue type as compared to a select tissue type. In particular embodiments, a reference tissue type refers to non-selected tissues. In particular embodiments, a reference tissue type is a non-selected tissue from a different organ or location within the body or with a different function than the selected tissue. In particular embodiments, the select tissue is the site of desired activity.

[0115] A "non-targeted" or "non-selected" tissue refers to any tissue or multiple tissues in which the genetically modified cells are not intentionally trafficked to nor are intended to reside in. In particular embodiments, genetically modified cells are not substantially trafficked to nor substantially reside in a non-selected tissue. In particular embodiments, a trafficking signal results in trafficking of genetically modifieds cell to a selected tissue. In particular embodiments, down-

regulated expression of a trafficking signal results in trafficking such that genetically modified cells are not trafficked to a non-selected tissue.

[0116] In particular embodiments, a trafficking signal can be up-regulated or down-regulated in order to direct genetically modified cells to a select tissue or not toward a non-selected tissue. In particular embodiments, a trafficking signal includes CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3. In particular embodiments, a trafficking signal includes expression of or down-regulated expression of CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3. In particular embodiments, a trafficking signal includes expression of CXCR6, integrin β 2 (LFA-1), CCR1, CCR2, or CXCR4 to provide a positive directional signal (referred to as a “go here” trafficking signal). In particular embodiments, a trafficking signal includes down-regulated expression of β 7 integrin, CCR4, CXCR3, or CCR3 to provide a negative directional signal (referred to as a “don’t go here” inhibitory signal).

[0117] In particular embodiments, expression of CXCR6 by genetically modified cells results in trafficking to the liver. In particular embodiments, expression of integrin β 2 (LFA-1), CCR1, and/or CCR2 by genetically modified cells results in trafficking to the pancreas. In particular embodiments, expression of CXCR4 by genetically modified cells results in trafficking to the bone.

[0118] In particular embodiments, down-regulated expression of β 7 integrin by genetically modified cells results in trafficking away from the colon and/or small intestine. In particular embodiments, down-regulated expression of CCR4, CXCR3, and/or CCR3 by genetically modified cells results in trafficking away from the lung. In particular embodiments, down-regulated expression of CXCR3 by genetically modified cells results in trafficking away from the prostate. In particular embodiments, down-regulated expression of CCR4, CXCR3, CCR3, and/or β 7 integrin by genetically modified cells results in trafficking away from the lung, kidney, and/or stomach.

[0119] Down-regulated expression refers to cellular treatment that decreases expression of an endogenously expressed protein under comparable conditions to the same cell type that has not been exposed to the cellular treatment. In particular embodiments, down-regulated expression is achieved through gene knockout. In particular embodiments, down-regulated expression results from a genetic modification that disrupts expression of a gene or genetic sequence within a cell. Other methods to down-regulate endogenous gene expression is through interfering RNA, administration of compounds that de-stabilize mRNA or cDNA, or DNA modification.

[0120] CXCR6, or CXC motif chemokine receptor 6, increases the migration of cells expressing CXCR6 to tissues expressing the CXCR6 ligand, CXCL16. In particular embodiments, CXCR6 [Homo sapiens] (NCBI Reference Sequence: NP_001373364.1) includes the sequence:

MAEHDYHEDYGFSSFNDSQEEHQDFLQFSKVFLPCMYLVVFCGLVGNSLVLVISIFYHKLQS
 LTDVFLVNLPLADLVFVCTLPFWAYAGIHEWVFGQVMCKSLLGIYTINFYTSMLILTCITVDRFIVV
 VKATKAYNQQAQRMTWGVKVTSLLIWWISLLVSLPQIIYGNVFNLDKLCGYHDEAISTVVLATQMT
 LGFFLPLLTMIVCYSVIIKTLLHAGGFQKHRSLKIIFLVMVAVFLLTQMPFNLMKFIRSTHWEYYAMT
 SFHYTIMVTEAIAYLRACLNPLYAFVSLKFRKNFWKLVDIGCLPYLGVSHQWKSSSEDNSKTF
 SASHNVEATSMFQL (SEQ ID NO: 66). In particular embodiments, CXCR6 is encoded by a
 sequence as set forth in SEQ ID NO: 1.

[0121] Integrin $\beta 2$, also referred to as CD18 is the beta chain of an integrin known to bind with CD11a, CD11b, CD11c, or CD11d, resulting in the formation of the Lymphocyte Functions Associated Antigen 1 (LFA-1). LFA-1 is a protein expressed by B cells, T cells, macrophages, neutrophils, and NK cells. It is involved in the adhesion and binding to antigen presenting cells through interactions with ICAM-1. In particular embodiments, integrin $\beta 2$ [Homo sapiens] (UniProtKB/Swiss-Prot: P05107.2) includes the sequence:

MLGLRPPLLALVGLLSLGCVLSECTKFKVSSCRECIESGPGCTWCQKLNFTGPGDPDSIRCD
 TRPQLLMRGCAADDIMDPTSLAETQEDHNGGQKQLSPQKVTLYLRPGQAAAFNVTFERRAKGY
 PIDLYYLMDSYSMLDDLNRNVKKLGDDLRLALNEITESGRIGFGSFVDKTVLPFVNTHPDKLRNP
 CPNKEKECQPPFAFRHVLKLTNNSNQFQTEVGKQLISGNLDAPEGGLDAMMQVAACPEEIGW
 RNVTRLLVFATDDGFHFAGDGKLGAILTPNDGRCHLEDNLYKRSNEFDYPSVGQLAHKLAENNI
 QPIFAVTSRMVKTYEKLTEIIPKSAVGELSEDSSNVVQLIKNAYNKLSSRVFLDHNALPDTLKVTY
 DSFCSNGVTHRNQPRGDCDGVQINVPITFQVKVTATECIQECSFVIRALGFTDIVTVQVLPQCE
 CRCRDQSRDRSLCHGKGFLECGICRCDTGYIGKNCECQTQGRSSQELEGSCRKDNNNSIICSG
 LGDCVCGQCLCHTSDVPGKLIYGQYCECDTINCERYNGQVCGGPGRGLCFGKCRCHPGFE
 GSACQCERTTEGCLNPRRVECSGRGRRCNVCECHSGYQLPLCQCECPGCPSPCGKYISCAE
 CLKFEKGPF GKNC SAAC PGLQLSNNPVKGR TCKERDSEGCWWAYTLEQQDGM DRYLIYVDES
 RECVAGPNIAAIVGGTVAGIVLIGILLVWKALIHLSDLREYRRFEKEKLKSQWNNDNPLFKSAT
 TTVMNPKFAES (SEQ ID NO: 67).

[0122] CCR1 (C-C motif chemokine receptor 1) is a seven transmembrane protein similar to a G protein-coupled receptor. The ligands of this receptor include macrophage inflammatory protein 1 alpha (MIP-1 alpha), regulated on activation normal T expressed and secreted protein (RANTES), monocyte chemoattractant protein 3 (MCP-3), and myeloid progenitor inhibitory factor-1 (MPIF-1). Chemokines and their receptors mediated signal transduction are critical for the recruitment of effector immune cells to the site of inflammation. In particular embodiments, CCR1 [Homo sapiens] (NCBI Reference Sequence: NP_001286.1) includes the sequence:

METPNTTEDYDTTTEFDYGDATPCQKVNERAFGAQLLPPLYSLVVFVIGLVGNILVVLVLVQYKRL

KNMTSIYLLNLAISDLLFLFTLPFWIDYKLKDDWWFGDAMCKILSGFYTTGLYSEIFFIILLTIDRYL
AIVHAVFALRARTVTFGVITSIIWALAILASMPGLYFSKTQWEFTHHTCSLHFPHESLREWKLQ
ALKLNLFGLVLP LLVMIICYTGIIKILLRRPNEKKS KAVRLIFVIMIIFFLFWTPYNLTILISVFQDFLFT
HECEQSRHLDLAVQVTEVIAYTHCCVNPVIYAFVGERFRKYLRQLFHRRVAVHLVKWLPFLSVD
RLERVSSTSPSTGEHELSAGF (SEQ ID NO: 68).

[0123] CCR2 (C-C motif chemokine receptor 2) is a receptor for monocyte chemoattractant protein-1, a chemokine which specifically mediates monocyte chemotaxis. Monocyte chemoattractant protein-1 is involved in monocyte infiltration in inflammatory diseases such as rheumatoid arthritis as well as in the inflammatory response against tumors. In particular embodiments, CCR2 isoform A [Homo sapiens] (NCBI Reference Sequence: NP_001116513.2) includes the sequence:

MLSTSRSRFIRNTNESGEEVTTFFDYDYGAPCHKFDVKQIGAQLLPPLYSLVFIFGFVGNMLVVL
ILINCKKLKCLTDIYLLNLAISDLLFLITLPLWAHSAANEWWFGNAMCKLFTGLYHIGYFGGIFFIILL
TIDRYLAIVHAVFALKARTVTFGVVTSVITWLAVFASVPGIIFTKCQKEDSVYVCGPYFPRGWN
NFHTIMRNILGLVLP LLIMVICYSGILKTLLRCRNEKKRHRAVRVIFTIMIVYFLFWTPYNIVILLNTF
QEFFGLSNCESTS QLDQATQVTETLGMTHCCINPIIYAFVGEKFRSLFHIALGCRIAPLQKPVCG
GPGVRPGKNVKVTTQGLLDGRGKGKSIGRAPEASLQDKEGA (SEQ ID NO: 69). In particular
embodiments, CCR2 isoform B [Homo sapiens] (NCBI Reference Sequence: NP_001116868.1)
includes the sequence:

MLSTSRSRFIRNTNESGEEVTTFFDYDYGAPCHKFDVKQIGAQLLPPLYSLVFIFGFVGNMLVVL
ILINCKKLKCLTDIYLLNLAISDLLFLITLPLWAHSAANEWWFGNAMCKLFTGLYHIGYFGGIFFIILL
TIDRYLAIVHAVFALKARTVTFGVVTSVITWLAVFASVPGIIFTKCQKEDSVYVCGPYFPRGWN
NFHTIMRNILGLVLP LLIMVICYSGILKTLLRCRNEKKRHRAVRVIFTIMIVYFLFWTPYNIVILLNTF
QEFFGLSNCESTS QLDQATQVTETLGMTHCCINPIIYAFVGEKFRRYLSVFFRKHITKRFCQKCP
VFYRETVDGVTSTNTPSTGEQEVSAGL (SEQ ID NO: 70).

[0124] CXCR4 (C-X-C motif chemokine receptor 4) is a receptor specific for stromal cell-derived factor-1. The protein has 7 transmembrane regions and is located on the cell surface. In particular embodiments, CXCR4 [Homo sapiens] (GenBank: CAA12166.1) includes the sequence:
MEGISSIP LLLQIYTS DNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGNGLV
ILVMGYQKKLRSM TDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNFLCKAVHVIYTVNLYSS
VLILAFISLD RYLAIVHATNSQRPRKLLAEKVYVGVWIPALLLTIPDFIFANVSEADDRYICDRFY
PNDLWVVFQFQHIMVGLILPGIVILSCYCIISKLSHSGHQKRKALKTTVILILAFFACWLPYYIGI
SIDSFILLEI IKQGC EFENTVHKWISITEALAFFHCCLNPILYAF LGAKFKTSAQHALTSVSRGSSL
KILSKGKRGGHSSVSTESESSSFHSS (SEQ ID NO: 71).

[0125] Integrin $\alpha 4 \beta 7$ is expressed on memory T cells destined to home to the gut, where the ligands CCL25 and MadCAM1 are constitutively expressed (Mora *et al.*, 2003, Nature. 424:88-93). In particular embodiments, integrin $\alpha 4$ subunit [Homo sapiens] (GenBank: CAD53329.1) includes the sequence:

RVCALEQQKSAMQTLKGIVQFLSKTDKRLLYCIKADPHCLNFLCNFGKMESGKEASVHIQLEGR
PSILEMDETSALKFEIRATGFPEPNPRVIELNKDENVAHVLEGLHHQRPKRYFTIVISSSLLLGLI
VLLLISYVMWKAGFFKRQYKSILQEENRRDSWSYINSKSNDD (SEQ ID NO: 72).

[0126] In particular embodiments, integrin $\beta 7$ [Homo sapiens] (UniProtKB/Swiss-Prot: P26010.1) includes the sequence:

MVALPMVLVLLLVLRSGESELDKIPSTGDATEWRNPHLSMLGSCQPAPSCQKCILSHPSCAW
CKQLNFTASGEAEARRCARREELLARGCPLEEELEPRGQQEVLQDQPLSQGARGEGATQLAP
QVRVTLRPGEPQQQLQVRFLRAEGYPVDLYLMDLSYSMKDDLRLVRQLGHALLVRLQEVTH
SVRIGFGSFVDKTVLPFVSTVPSKLRHPCPTRLERCQSPFSFHHVLSLTGDAQAFEREVGRQS
VSGNLDSPGGFDAILQAALCQEQIGWRNVSRLLVFTSDDTFHTAGDGKLGIFMPSDGHCHL
DSNGLYSRSTEFDYPVGQVAQALSAANIQPIFAVTSAAALPVYQELSKLIPKSAVGELSEDSSNV
VQLIMDAYNSLSSTVTLEHSSLPPGVHISYESQCEGPEKREGKAEDRGQC�NHVRINQTVTFWV
SLQATHCLPEPHLLRLRALGFSEELIVELHTLDCNCSDTQPQAPHCSDGQGHLLQCGVCSCAP
GRLGRLCECSVAELSSPDLESGCRAPNGTGPLCSGKGHCQCGRCSCSGQSSGHLCECDDAS
CERHEGILCGGFGRCQCGVCHCHANRTGRACECSGDMDSISCPEGGLCSGHGRCKCNRCQ
CLDGYYGALCDQCPGCKTPCERHRDCAECGAFRTGPLATNCSTACAHTNVTALAPILDDGW
CKERTLDNQLFFFLVEDDARGTVVLRVRPQEKGADHTQAIVLGCVGGIVAVGLGLVLAYRLSVE
IYDRREYSRFEKEQQQLNWKQDSNPLYKSAITTTINPRFQEADSPTL (SEQ ID NO: 73).

[0127] CC chemokine receptor (CCR)4/CC chemokine ligand (CCL)17, CCR8/CCL1, and CCR10/CCL27 enable migration to the skin, and cutaneous lymphocyte antigen allows them to bind to local selectins (Campbell *et al.*, 1999, Nature. 400:776–780; Schaerli *et al.*, 2004, J. Exp. Med. 199:1265–1275; Sigmundsdottir *et al.*, 2007, Nat. Immunol. 8:285–293). In particular embodiments, CCR4 [Homo sapiens] (NCBI Reference Sequence: NP_005499.1) includes the sequence:

MNPTDIADTTLDESIYSNYLYYESIPKPCTKEGIKAFGELFLPPLYSLVFVFGLLGNSVVLVLFKY
KRLRSMTDVYLLNLAISDLLFVFSLPFWGYAADQWWFGLGLCKMISWMYLVGFYSGIFFVML
MSIDRYLAIVHAVFSRLRARTLTYGVITSLATWSVAVFASLPGFLFSTCYTERNHTYCKTKYSLNS
TTWKVLSSLEINILGLVIPLGIMLFCYSMIIRTLQHCKNEKKNKAVKMIFAVVVLFLGFWTPYNIVL
FLETLVELEVLDQCTFERYLDAIQATETLAFVHCCLNPIIYFFLGEKFRKYILQLFKTCRGLFVLC
QYCGLLQIYSADTPSSSYTQSTMDHDLHDAL (SEQ ID NO: 74).

[0128] CXCR3 (C-X-C Motif Chemokine Receptor 3) is a Gai protein-coupled receptor that promotes recruitment of Th17 cells from the blood into the liver after liver injury. In particular embodiments, CXCR3 [Homo sapiens] (UniProtKB/Swiss-Prot: P49682.2) includes the sequence:

MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQDFSLNFDRAFLPALYSLL
FLLGLLGNGAVAAVLLSRRTALSSTDFTLLHLAVADTLLVLTPLWAVDAAVQWVFGSGLCKVA
GALFNINFYAGALLACISFDRLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAH
HDERLNATHCQYNFPQVGRTALRVLQLVAGFLLPLLVMAYCYAHILAVLLVSRGQRRRLRAMRL
VVVVVAFALCWTPYHLVVLVDILMDLGALARNCGRESRVDVAKSVTSGLYMHCCNLNPLLYA
FVGVKFRERMWMLLLRLGCPNQRGLQRQPSSSRDSSWSETSEASYSGL (SEQ ID NO: 75).

[0129] CCR3 (C-C motif chemokine receptor 3) is important in leukocyte chemotaxis. In particular embodiments, CCR3 [Homo sapiens] (GenBank: AAL85154.1) includes the sequence:

MTTSLDTVETFGTTSYYDDVGLLCEKADTRALMAQFVPPLYSLVFTVGLLGNVVVMILIKYRRL
RIMTNIYLLNLAISDLLFLVTLFPWIHYVRGHNWVFGHGMCKLLSGFYHTGLYSEIFFIILLTIDRYL
AIVHAVFALRARTVTFGVITSIVTWGLAVLAALPEFIFYETEELFEETLCSALYPEDTVYSWRHFH
TLRMTIFCLVPLLVMAICYTGIIKTLLRCPSKKKYKAIRLIFVIMAVFFIFWTPYNVAILLSSYQSILF
GNDCERSKHLDLVMLVTEVIAYSHCCMNPVIYAFVGERFRKYLRFHFFHRHLLMHLGRYIPFLPS
EKLERTSSVSPSTAEPELSIVF (SEQ ID NO: 76).

[0130] Various gene editing methods can be used to inactivate a gene or inactivate a gene regulating the expression of the gene. For example, the gene editing techniques discussed earlier herein (e.g., CRISPR/Cas9, TALENs, transposon system) can be used. In particular embodiments, to genetically modify or knockout a trafficking signal, a guide RNA (gRNA) targeting the sequence encoding the trafficking signal can be used. In particular embodiments, a trafficking signal knockout includes a trafficking signal gRNA. Example gRNA sequences are presented in Table 1.

[0131] Table 1. gRNA Targeting Trafficking Signals.

Target	Sequence	SEQ ID NO:
CXCR6 in human	TCACCAGGAACACATCCGTC	77
CXCR6 in human	GCAGGCCCTCAGGTATGCGA	78
CXCR6 in human	GGTCATGTGCAAGAGCCTAC	79
Integrin β 2 in human	GCAGGCGGCGACCTGCATCA	80

Integrin β 2 in human	GAATGCATCGAGTCGGGGCC	81
Integrin β 2 in human	GCAGCTGATTTCCGGAAACC	82
CCR1 in human	GGTACCTGTCAATCGTCAGC	83
CCR1 in human	TCATCCTGCTGACGATTGAC	84
CCR1 in human	GTTGACACAGCAGTGCGTGT	85
CCR2 in human	CTCCTCGATGGTCGTGAAA	86
CCR2 in human	CTTCACGTCAAATTTATGAC	87
CCR2 in human	CTGTATCACATCGGTTATTT	88
CXCR4 in human	CACTTCAGATAACTACACCG	89
CXCR4 in human	ATGCTGATCCCAATGTAGTA	90
CXCR4 in human	TGTTGGCTGCCTTACTACAT	91
β 7 integrin in human	GGGACGCACAAGCCTTCGAG	92
β 7 integrin in human	AGCGGGTCCGGGTCACGCTG	93
β 7 integrin in human	CCGGGTATCCCTCAGCACGA	94
CCR4 in human	ACACCGCGTGCACAATTGCC	95
CCR4 in human	ACTCTCTCAACTCCACGACG	96
CCR4 in human	GTGGTGTCTGCTATATCCGT	97
CXCR3 in human	CCAAGTGCTAAATGACGCCG	98
CXCR3 in human	TTCAACATCAACTTCTACGC	99
CXCR3 in human	GAATCCCGGCGGGAAGACGA	100
CCR3 in human	CGTTCTCCCTCTGCTCGTTA	101
CCR3 in human	CACGTCATCATAGTAGGATG	102
CCR3 in human	CACATCCTACTATGATGACG	103

[0132] In particular embodiments, expression of CXCR6 results in trafficking to the liver. In particular embodiments, expression of integrin β 2 (LFA-1), CCR1, and/or CCR2 results in trafficking to the pancreas. In particular embodiments, expression of CXCR4 results in trafficking to the bone.

[0133] In particular embodiments, down-regulating expression of β 7 integrin results in trafficking

away from the colon. In particular embodiments, down-regulating expression of CCR4, CXCR3, and/or CCR3 results in trafficking away from the lung. In particular embodiments, down-regulating expression of CXCR3 results in trafficking away from the prostate. In particular embodiments, down-regulating expression of CCR4, CXCR3, CCR3, and/or $\beta 7$ integrin results in trafficking away from the lung and kidney. In particular embodiments, down-regulating expression of CCR4, CXCR3, CCR3, and/or $\beta 7$ integrin results in trafficking away from the stomach, lung, and/or kidney. In particular embodiments, down-regulating expression of $\beta 7$ integrin results in trafficking away from the small intestine and/or colon.

[0134] (III-B) Retention Signals. Cells can be engineered to remain within a select tissue by introducing a genetic construct expressing a retention signal to the cell. Alternatively, a cell can be engineered to not remain within a tissue by knocking out or down-regulating an endogenous retention signal.

[0135] In particular embodiments, a retention signal includes CD69, Runx3, Hobit, or CD103. In particular embodiments, CD69, Runt-related transcription factor 3 (Runx3), Hobit, or CD103 is expressed to create a retention signal. In particular embodiments, expression of a retention signal by genetically modified cells can induce the genetically modified cells to remain in a select tissue. In particular embodiments, down-regulated expression of a retention signal within genetically modified cells can prevent the genetically modified cells from remaining in a select tissue.

[0136] CD69 expression is considered a marker of tissue residency or retention in certain tissues. CD69 is a membrane-bound, type II C-lectin receptor and a marker for early lymphocyte activation and T cell differentiation. CD69 is commonly used as a marker for tissue-resident-memory CD8⁺ T cells (T_{RM}). T_{RM} are an example of non-circulating T cells that persist at a specific site. In particular embodiments, CD69 [Homo sapiens] (GenBank: CAA80298.1) includes the sequence: MSSENC FVAENSSLHPESGQENDATSPHFSTRHEGSFQVPVLCVAVMN VVFITILIALIALSVGQ YNCPGQYTF SMPSDSHVSSCEDWVG YQRKCYFISTVKRSWTS AQNACSEHGATLAVIDSEK DMNFLKRYAGREEHWVGLKKEPGHPWKWSNGKEFNW FNVGTGSDKCVFLKNTEVSSMECE KNLYWICNKPYK (SEQ ID NO: 104).

[0137] In particular embodiments, CD69 promotes retention in a tissue. In particular embodiments, CD69 promotes retention in liver, pancreas, or bone.

[0138] Runx3 expression enhances tumor-specific CD8⁺ T cell abundance whereas Runx3-deficiencies result in a failure of T cells to accumulate in tumors. In particular embodiments, Runx3 [Homo sapiens] (GenBank: AAH13362.1) includes the sequence: MASNSIFDSFPTYSPTFIRD PSTSRFTPPSPAFPCGGGGGKMGENSGALSAQA AVGPGGRA RPEVRSMVDVLADHAGELVRTDSPNFLCSVLPSHWRCNKTL PVAFKVVALGDVPDGT VVTVM

AGNDENYSAELRNASAVMKNQVARFNDLRFVGRSGRGKSFTLTITVFTNPTQVATYHRAIKVT
VDGPREPRRHRQKLEDQTKPFPDRFGDLERLRMRVTPSTPSPRGSLSTTSHFSSQPQTPIQG
TSELNPFSDPRQFDRSFPTLPTLTESRFPDPRMHYPGAMSAAFPYSATPSGTSISSLSVAGMP
ATSRFHHTYLPPYPGAPQNNQSGPFQANPSPYHLYYGTSSGSYQFSMVAGSSSGGDRSPTR
MLASCTSSAASVAAGNLMNPSLGGQSDGVEADGSHSNSPTALSTPGRMDEAVWRPY (SEQ
ID NO: 105).

[0139] Hobit (homolog of Blimp-1 in T cells), also referred to as zinc finger protein ZNF683, is almost exclusively expressed in effector T cells and contribute to residency in tissues. In particular embodiments, Hobit [Homo sapiens] (NP_001294854.1) includes the sequence: MKEESAAQLGCCHRPALGGTGGSLSPSLDFQLFRGDQVFSACRPLPDMVDAHGPSCASWL
CPLPLAPGRSALLACLQDLNLCTPQPAPLGTDLQGLQEDALSMKHEPPGLQASSTDDKKFT
VKYPQNKDKLGKQPERAGEGAPCPAFSSHNSSSPPLQNRKSPSPLAFPCPPVNSISKELPF
LLHAFYPGYPLLLPPPHLFTYGALPSDQCPHLLMLPQDPSYPTMAMPSSLMMVNELGHPSARW
ETLLPYPGAFAQSGQALPSQARNPGAGAAPTDSPGLERGGMASPAKRVLSSQTGTAAALPYP
LKKKNGKILYECNICGKSFGQLSNLKVHLRVHSGERPFQCALCQKSFTQLAHLQKHHLVHTGE
RPHKCSIPWWPGRNHWKSFQAWREREVCHKRFSSSSNLKTHLRLHSGARPFQCSVCRSRFT
QHIHLKLHHLHAPQPCGLVHTQLPLASLACLAQWHQGALDLMAVASEKHMGYDIDEVKVSST
SQGKARAVSLSSAGTPLVMGQDQNN (SEQ ID NO: 106).

[0140] CD103 is a marker for alloantigen-induced regulatory CD8+ T cells. CD103 is also known as integrin, alpha E. For additional information on CD103, see Szabo *et al.* (*Sci Immunol.*2019, 4(34):eaas9673). In particular embodiments, CD103 [Homo sapiens] (GenBank: AA113437.1) includes the sequence:

MWLFHTLLCIASLALLAAFNVDVARPWLT PKGGAPFVLSSLLHQPSTNQTWLLVTSPRTKRTP
GPLHRCSLVQDEILCHPVEHVPIPKGRHRGVTVVRSHHGVLCIQVLVRRPHSLSSSELTGTCSLL
GPDLRPQAQANFFDLENLLDPDARVDTGDCYSNKEGGGEDDVNTARQRRALEKEEEEEDKEEE
EDEEEEEAGTEIAIILDGSGSIDPPDFQRAKDFISNMMRNIFYEKCFCFNALVQYGGVIQTEFDL
RDSQDVMASLARVQNTQVGSVTKTASAMQHVLDSIFTSSHGSRRKASKVMVVLTDGGIFEDP
LNLTTVINSPKMQGVERFAIGVGEEFKSARTARELNLIASDPDETHAFKVTNYMALDGLLSKLRY
NIISMEGTVGDALHYQLAQIGFSAQILDERQVLLGAVGAFDWGGALLYDTRSRRGRFLNQTA
AAADAEAAQYSYLGAVAVLHKTCSLSYVAGAPQYKHHGAVFELQKEGREASFLPVLEGEQM
GSYFGSELCPVDIDMDGSTDFLLVAAPFYHVHGEGRVYVYRLSEQDGSFSLARILSGHPGFT
NARFGFAMAAMGDLSDKLTDAIGAPLEGFGADDGASFGSVYIYNHWDGLSASPSQRIRA
STVAPGLQYFGMSMAGGFDISGDGLADITVGTLGQAVVFRSRPVVRLKVSMAFTPSALPIGFN
GVVNVRLCFEISSVTTASESGLREALLNFTLDVDVGKQRRRLQCSDVRSCLGCLREWSSGSQ

CEDLLLMPTEGELCEEDCFSNASVKVSYQLQTPEGQTDHPQPILDRTYEPFAIFQLPYEKACKN
 KLFCVAELQLATTVSQQELVVGLTKELTLNINLTNSGEDSYMTSMALNYPRNLQLKRMQKPPSP
 NIQCDDPQPVASVLIMNCRIGHPVVKRSSHVSVVWQLEENAFPNRTADITVTVTNSNERRSLA
 NETHTLQFRHGFVAVLSKPSIMYVNTGQGLSHHKEFLFHVHGENLFGAEYQLQICVPTKLRGL
 QVAAVKKLTRTQASTVCTWSQERACAYSSVQHVEEWHSVSCVIASDKENVTVAEISWDHSE
 ELLKDVTELQILGEISFNKSLYEGLNAENHRTKITVVFLKDEKYHSLPIIKGSVGGLLVILVILVILFK
 CGFFKRKYQQLNLESIRKAQLKSENLLLEEN (SEQ ID NO: 191).

[0141] As described earlier, various gene editing methods can be used to inactivate a gene or inactivate a gene regulating the expression of the gene. In particular embodiments, to genetically modify or knockout a retention signal, a guide RNA (gRNA) targeting the sequence encoding the retention signal can be used. In particular embodiments, a retention signal knockout includes a retention signal gRNA. Example gRNA sequences are presented in Table 2.

[0142] Table 2. gRNA Targeting Retention Signals.

Target	Sequence	SEQ ID NO:
CD69 in human	CTTCATGACGTGTTGAGAAA	107
CD69 in human	CATTTCTCAACACGTCATGA	108
CD69 in human	ATGGCATTGAGAATGTGTAT	109
CD69 in human	GTGGGCCAATACAATTGTCC	110
CD69 in human	AACTTTCTAAACGATACGC	111
CD69 in human	TTTGACAGGTTCAACGTTAC	112
CD69 in mouse	CCACAAAAATGTTACCGCTG	113
CD69 in mouse	TCCAACCTTCTCGTACAAGCC	114
CD69 in mouse	ACCACTATTAACACAGCCCA	115
Runx3 in human	TACTCGCCGACCTTCATCCG	116
Runx3 in human	GGACGTGCCGGATGGTACGG	117
Runx3 in human	CTCGGTGGTAGGTGCGCCACT	118
Hobit in human	CCATAAGATCTAGTGCCCCC	119
Hobit in human	CACATATGTTGCACTCGTAC	120
Hobit in human	CCAGGGGGCACTAGATCTTA	121
CD103 in human	ACGCTGCCTTTAATGATGAT	192
CD103 in human	GGCCACATCCACATTGAAAG	193
CD103 in human	ACCGGGGAGTGACCGTTGTC	194

(III-C) Recombinant Receptor. In particular embodiments, a recombinant receptor is or includes a binding domain that binds a target antigen, wherein the recombinant receptor is expressed by a cell following the artificial introduction of nucleic acid encoding the recombinant receptor into the cell. The recombinant receptor can be, e.g., a CAR, an engineered T-cell receptor (eTCR), or a CAR/TCR hybrid.

[0143] CAR, for example, include several distinct subcomponents that allow genetically modified cells to recognize and kill unwanted cells, such as cancer cells. The subcomponents include at least an extracellular component and an intracellular component. The extracellular component includes a binding domain that specifically binds a marker that is preferentially present on the surface of unwanted cells. When the binding domain binds such markers, the intracellular component activates the cell to destroy the bound cell. CAR additionally include a transmembrane domain that links the extracellular component to the intracellular component, and other subcomponents that can increase the CAR's function. For example, the inclusion of a spacer and/or one or more linker sequences can allow the CAR to have additional conformational flexibility, often increasing the binding domain's ability to bind the target antigen.

[0144] Many considerations associated with CAR apply to eTCR as well. eTCR disclosed herein include a binding domain that binds a target antigen linked to the C α and/or C β chains of a TCR. A TCR is a heterodimeric fusion protein that typically includes an α and β chain. Each chain includes a variable region (V α and V β) and a constant region (C α and C β). In particular embodiments, an eTCR does not include the native TCR variable region but does include the native TCR constant region. In particular embodiments, the eTCR includes an scFv as the variable region of either the α or β chain. In particular embodiments, the eTCR includes an scFv as the variable region of both the α and β chain. In particular embodiments, eTCR include a C α and/or C β chain sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a known or identified TCR C α or C β .

(III-C-1) Binding Domains. In certain examples, recombinant receptors include a binding domain that binds a target antigen that is preferentially present on the surface of unwanted cells. Binding domains include any substance that binds to a cellular marker to form a complex. The choice of binding domain can depend upon the type and number of cellular markers that define the surface of a target cell. Examples of binding domains include cellular marker ligands, receptor ligands, antibodies, peptides, peptide aptamers, receptors (e.g., T cell receptors), or combinations and engineered fragments or formats thereof.

[0145] Antibodies are one example of binding domains and include whole antibodies or binding fragments of an antibody, e.g., Fv, Fab, Fab', F(ab')₂, and single chain (sc) forms and fragments thereof that bind specifically a cellular marker. Antibodies or antigen binding fragments can include all or a portion of polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, synthetic antibodies, non-human antibodies, recombinant antibodies, chimeric antibodies, bispecific antibodies, mini bodies, and linear antibodies.

[0146] Antibodies are produced from two genes, a heavy chain gene and a light chain gene. Generally, an antibody includes two identical copies of a heavy chain, and two identical copies of a light chain. Within a variable heavy chain and variable light chain, segments referred to as complementary determining regions (CDRs) dictate epitope binding. Each heavy chain has three CDRs (i.e., CDRH1, CDRH2, and CDRH3) and each light chain has three CDRs (i.e., CDRL1, CDRL2, and CDRL3). CDR regions are flanked by framework residues (FR).

[0147] CDR sets can be based on, for example, Kabat numbering (Kabat *et al.* (1991) "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. ("Kabat" numbering scheme)); Chothia (Al-Lazikani *et al.* (1997) JMB 273:927-948 ("Chothia" numbering scheme)); Martin (Abinandan *et al.* (2008) Mol Immunol. 45:3832-3839 ("Martin" numbering scheme)); Gelfand (Gelfand and Kister (1995) Proc Natl Acad Sci USA. 92:10884–10888; Gelfand *et al.* (1998) Protein Eng. 11:1015–1025; Gelfand *et al.* (1996) Proc Natl Acad Sci USA. 93:3675–3678; Gelfand *et al.* (1998) J Comput Biol. 5:467–477 ("Gelfand" numbering scheme)); Contact (MacCallum *et al.* (1996) J. Mol. Biol. 262:732-745 (Contact numbering scheme)); IMGT (Lefranc *et al.* (2003) Dev Comp Immunol 27(1):55-77 ("IMGT" numbering scheme)); AHo (Honegger and Plückthun (2001) J Mol Biol 309(3):657-670 ("AHo" numbering scheme)); North (North *et al.* (2011) J Mol Biol. 406(2):228-256 ("North" numbering scheme)); or other numbering schemes. Software programs and bioinformatical tools, such as ABodyBuilder. The boundaries of a given CDR or FR may vary depending on the scheme used for identification.

[0148] In some instances, scFvs based on the binding domains described herein and for use in a recombinant receptor can be prepared according to methods known in the art (see, for example, Bird *et al.*, (1988) Science 242:423-426 and Huston *et al.*, (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions of an antibody together using flexible polypeptide linkers. If a short polypeptide linker is employed (e.g., between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientations and sizes see, e.g., Hollinger *et al.* 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448,

US 2005/0100543, US 2005/0175606, US 2007/0014794, and WO2006/020258 and WO2007/024715. More particularly, linker sequences that are used to connect the VL and VH of an scFv are generally five to 35 amino acids in length. In particular embodiments, a VL-VH linker includes from five to 35, ten to 30 amino acids or from 15 to 25 amino acids. Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies. scFv are commonly used as the binding domains of recombinant receptors.

[0149] Other binding fragments, such as Fv, Fab, Fab', F(ab')₂, can also be used within the recombinant receptors disclosed herein. Additional examples of antibody-based binding domain formats for use in a recombinant receptor include scFv-based grababodies and soluble VH domain antibodies. These antibodies form binding regions using only heavy chain variable regions. See, for example, Jespers *et al.*, Nat. Biotechnol. 22:1161, 2004; Cortez-Retamozo *et al.*, Cancer Res. 64:2853, 2004; Baral *et al.*, Nature Med. 12:580, 2006; and Barthelemy *et al.*, J. Biol. Chem. 283:3639, 2008.

[0150] In particular embodiments, a VL region in a binding domain of the present disclosure is derived from or based on a VL of an antibody disclosed herein and contains one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the VL of the antibody disclosed herein. An insertion, deletion or substitution may be anywhere in the VL region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided a binding domain containing the modified VL region can still specifically bind its target with an affinity similar to the wild type binding domain.

[0151] In particular embodiments, a binding domain VH region of the present disclosure can be derived from or based on a VH of an antibody disclosed herein and can contain one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the VH of the antibody disclosed herein. An insertion, deletion or substitution may be anywhere in the VH region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided a binding domain containing the modified VH region can still specifically bind its target with an affinity similar to the wild type binding domain.

[0152] In particular embodiments, the recombinant receptor includes a binding domain that binds

a target antigen. In particular embodiments, the target antigen is a cancer antigen. In particular embodiments, target antigens are expressed by cancer cells or tumors.

[0153] In particular embodiments, target antigens are preferentially expressed by target cells (e.g., cancer cells). “Preferentially expressed” means that a target antigen is found at higher levels on target cells as compared to other cell types. In some instances, a target antigen is only expressed by the targeted cell type. In other instances, the target antigen is expressed on the targeted cell type at least 25%, 35%, 45%, 55%, 65%, 75%, 85%, 95%, 96%, 97%, 98%, 99%, or 100% more than on non-targeted cells. In particular embodiments, the target antigen is expressed on the target cell and healthy tissue cell.

[0154] In particular embodiments, target antigens can be significantly expressed on cancer cells and on healthy tissue cells. This means that there may be negative side effects due to on-target/off-tumor toxicities. For example, Cetuximab is an anti-EGFR antibody associated with severe skin rashes thought to be caused by EGFR expression in the skin. Another example is Herceptin (trastuzumab), which is an anti-HER2 (ERBB2) antibody. Herceptin is associated with cardiotoxicity due to target expression in the heart. Moreover, targeting HER2 with a CAR-T cell was lethal in a patient due to on-target, off-cancer expression in the lung. Table 3 provides examples of cancer antigens that are more likely to be expressed in particular primary tumor types. These target antigens can be expressed by cancer cells that have metastasized and by primary tumors. Despite this, healthy tissues in other parts of the body may express the target antigens and methods disclosed herein describe how to avoid these on-target/off-tumor scenarios. These methods rely on trafficking cells to particular organs rather than to sites of cancer generally.

[0155] Table 3. Target Antigens for Targeting Primary Tumors.

Cancer Antigen	Primary Tumor
Carcinoembryonic antigen (CEA)	Colorectal, pancreatic
Epithelial cell adhesion molecule (EpCAM)	Colorectal
Human epidermal growth factor receptor 2 (HER2)	Breast, colon, gastric
Prostate-Specific Antigen (PSA)	Prostate
Glypican-3 (GPC3)	Hepatocellular carcinoma
Mesothelin	Pancreatic
Folate receptor alpha (FOLR1)	Ovarian, endometrial
Mucin short variant S1 (MUC1)	Breast, uterine, ovarian, endometrial
Carbohydrate antigen 19-9 (CA19-9)	Pancreatic, cholangiocarcinoma

[0156] In particular embodiments, CEA is a protein found in various malignancies including colorectal cancer, medullary thyroid cancer, breast cancer, mucinous ovarian cancer, and pancreatic adenocarcinoma. In particular embodiments, CEA includes the sequence referenced in GenBank: CAE75559.1, Accession: AAB64011.1, or Accession: AAA40910.1. In particular

(SEQ ID NO: 209), and a variable heavy chain including a CDRH1 including the sequence DYYMN (SEQ ID NO: 210), CDRH2 including the sequence FIGNKANGYTTEYSASVKG (SEQ ID NO: 211), and CDRH3 including the sequence DRGLRFYFDY (SEQ ID NO: 212), according to Kabat. CDR predictions according to other CDR definitions can also be determined.

[0160] In particular embodiments, EpCAM is a protein found various malignancies. In particular embodiments, EpCAM includes the sequence referenced in Accession: P16422.2, Accession: AAH05618.1, Accession: O55159.1, Accession: KAF6429286.1, or Accession: XP_016803985.1. In particular embodiments, a binding domain that binds EpCAM (i.e., an EpCAM binding domain) includes G8.8, ab71916, AUA-1, Ber-EP4, HEA125, EpMab-16, MOC31, or VU-1D9. In particular embodiments, a binding domain that binds mouse EpCAM includes G8.8. In particular embodiments, the anti-EpCAM antibody includes a variable light chain including the sequence: EVQLLEQSGAELVRPGTSVKISCKASGYAFTNYWLGWVKQRPGHGLEWIGDIFPGSGNIHYNE KFKGKATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDEPMDYWGQGTITVTVSSGGGG SGGGGSGGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQP PKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPLTFGAGTKLEIK GGGGSDIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNGNTYLSWYLQKPGQSPQSLIYKVSN RFGSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGSGTKVEIKGEGTSTGSG AIPVSLRGSGSGGGADQVQLVESGGGVVQPGRLRLSCAASGFTFSSYGMHWWRQAPGKQL EWWAQISFDGSNKYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCASERGHYYDSSA FDYWGQGTITVTVSSHHHHHH (SEQ ID NO: 122) and a variable heavy chain including the sequence:

ELVMTQSPSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKGGGGSGGGGSG GGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFTNYWLGWVKQRPGHGLEWIGDIFPGSGN IHYNEKFKGKATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDEPMDYWGQGTITVTVSS GGGGSQVQLVESGGGVVQPGRLRLSCAASGFTFTKAWMHWRQAPGKQLEWWAQIKDKS NSYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGQGTITVTVSSGEGTSTGSGAIPVSLRGSGSGGADIVMTQTPLSLSVTPGQPASISCKSSQSIHSSGNT YLSWYLQKPGQSPQSLIYKVSNRFGSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGS HVGPTFGSGTKVEIKHHHHHH (SEQ ID NO: 123).

[0161] In particular embodiments, the anti-EpCAM antibody includes a variable light chain including a CDR1 including the sequence RASQSVSSNLA (SEQ ID NO: 127), CDR2 including the sequence GASTTAS (SEQ ID NO: 128), and CDR3 including the sequence QQYNNWPPAYT (SEQ ID NO: 129) and a variable heavy chain including a CDR1 including the sequence SYAIS

(SEQ ID NO: 124), a CDR2 including the sequence SYAIS (SEQ ID NO: 125), and CDR3 including the sequence SYAIS (SEQ ID NO: 126).

[0162] In particular embodiments, a G8.8 antibody includes a variable light chain including the sequence:

DIQMTQSPASLSASLGTVSIECLASEGISNDLAWYQQKSGKSPQLLIYATSR LQDGVPSRFSG
SGSGTRYSLKISGMQPEDEADYFCQQSYKYPWTFGGGTKLELK (SEQ ID NO: 195) and a
variable heavy chain including the sequence:

EVQLAESGGGLVQPGRSMKLSAASGFTFSNFPMAWVRQAPTKGLEWWATISTSGGSTYYRD
SVKGRFTISRDNASTLYLQMNSLRSEDATYYCTRTLYILRVFYFDYWGQGMVTVSS (SEQ
ID NO: 196). In particular embodiments, the variable light chain and variable heavy chain are
connected with a GlySer linker including the sequence: GSGGGSGGGGS (SEQ ID NO: 197).

[0163] In particular embodiments, the anti-EpCAM binding domain includes a variable light chain including a CDRL1 including the sequence LASEGISNDLA (SEQ ID NO: 206), a CDRL2 including the sequence ATSR LQD (SEQ ID NO: 213), and CDRL3 including the sequence QQSYKYPWT (SEQ ID NO: 214), and a variable heavy chain including a CDRH1 including the sequence NFPMA (SEQ ID NO: 215), CDRH2 including the sequence TISTSGGSTYYRDSVKG (SEQ ID NO: 216), and CDRH3 including the sequence TLYILRVFYFDY (SEQ ID NO: 217), according to Kabat. CDR predictions according to other CDR definitions can also be determined.

[0164] In particular embodiments, HER2 is a protein found on the surface of all breast cells. It can help breast cancer cells grow more quickly. In particular embodiments, HER2 includes the sequence referenced in Accession: P04626.1. In particular embodiments, a binding domain that binds HER2 includes trastuzumab or pertuzumab. In particular embodiments, the trastuzumab includes a variable light chain includes the sequence:

DIQMTQSPILLSASVGDRVTITCRASQDVNTAVAWYQQR TNGSPRLLIYSASFLYSGVPSRFSG
SRSGTDFTLTISSLQPEDIADYYCQQHYTTPPTFGAGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 130) and a variable heavy chain includes the
sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQSPGKGLEWWARIYPTNGYTRYADS
VKGRFTISADTSKNTAYLQMNSLRAEDTAIYYCSRWGGDGFYAMDYWGQGT LVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 131).

[0165] In particular embodiments, the anti-HER2 binding domain includes a variable light chain including a CDRL1 including the sequence RASQDVNTAVA (SEQ ID NO: 218), a CDRL2

including the sequence SASFLYS (SEQ ID NO: 219), and CDRL3 including the sequence QQHYTTPPT (SEQ ID NO: 220), and a variable heavy chain including a CDRH1 including the sequence DTYIH (SEQ ID NO: 221), CDRH2 including the sequence RIYPTNGYTRYADSVKG (SEQ ID NO: 222), and CDRH3 including the sequence WGGDGFYAMDY (SEQ ID NO: 223), according to Kabat. CDR predictions according to other CDR definitions can also be determined.

[0166] In particular embodiments, the pertuzumab includes a variable light chain includes the sequence:

DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSTRFSG
SGSGTDFTLTISLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYSLSSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 132) and a variable heavy chain includes the sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWADVNPNSGGSIYN
QRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGGQGLTVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH (SEQ ID NO: 133).

[0167] In particular embodiments, the anti-HER2 binding domain includes a variable light chain including a CDRL1 including the sequence KASQDVSIGVA (SEQ ID NO: 224), a CDRL2 including the sequence SASYRYT (SEQ ID NO: 225), and CDRL3 including the sequence QQYYIYPYT (SEQ ID NO: 226), and a variable heavy chain including a CDRH1 including the sequence DYTMD (SEQ ID NO: 227), CDRH2 including the sequence DVNPNSGGSIYNQRFKG (SEQ ID NO: 228), and CDRH3 including the sequence NLGPSFYFDY (SEQ ID NO: 229), according to Kabat. CDR predictions according to other CDR definitions can also be determined.

[0168] In particular embodiments, PSA is a protein produced by normal and malignant cells of the prostate gland. PSA is a member of the kallikrein-related peptidase family and is secreted by the epithelial cells. In particular embodiments, PSA includes the sequence referenced in Accession: NP_001639.1, Accession: NP_001025218.1, or Accession: NP_001025219.1. In particular embodiments, a binding domain that binds PSA includes MSVA-603R, AS10 1352, 8A9B8, or A94916. In particular embodiments, an anti-PSA antibody includes a variable light chain including a CDRL1 including the sequence RASQNVNTDVA (SEQ ID NO: 134), a CDRL2 including the sequence STSYLQS (SEQ ID NO: 135), and a CDRL3 including the sequence QQYSNYPLT (SEQ ID NO: 136); and a variable heavy chain including a CDRH1 including the sequence TTGMGVS (SEQ ID NO: 137), a CDRH2 including the sequence HIYWDDDKRYSTSLK (SEQ ID NO: 138), and a CDRH3 including the sequence KGYGYFDY

(SEQ ID NO: 139).

[0169] In particular embodiments, GPC3 is a heparan sulfate proteoglycan that is highly expressed in hepatocellular carcinoma. In particular embodiments, GPC3 includes the sequence referenced in Accession: P51654.1, Accession: NP_001158090.1, Accession: NP_001158089.1, Accession: NP_004475.1, or Accession: NP_001158091.1. In particular embodiments, the anti-GPC3 antibody includes a variable light chain including a CDRL1 including the sequence RASQSISSYLN (SEQ ID NO: 140), a CDRL2 including the sequence AASSLQS (SEQ ID NO: 141), and a CDRL3 including the sequence QQSYSTPLT (SEQ ID NO: 142); and a variable heavy chain including a CDRH1 including the sequence GYTFTSYFLH (SEQ ID NO: 143), a CDRH2 including the sequence IIDPPTGRTTYAQKFQG (SEQ ID NO: 144), and a CDRH3 including the sequence GNYGGRYFDY (SEQ ID NO: 145).

[0170] In particular embodiments, mesothelin is a tumor-associated antigen broadly expressed on various malignant tumor cells, while its expression is generally limited to normal mesothelial cells. In particular embodiments, mesothelin includes the sequence referenced in Accession: AAH09272.1, Accession: AAH03512.1, Accession: KAI4052676.1, Accession: KAI4052675.1, or Accession: AAV87530.1. In particular embodiments, the anti-mesothelin antibody includes a variable light chain including a CDRL1 including the sequence RASQSVSSYLA (SEQ ID NO: 146), a CDRL2 including the sequence DASNRAT (SEQ ID NO: 147), and a CDRL3 including the sequence QQRSNWPLT (SEQ ID NO: 148); and a variable heavy chain including a CDRH1 including the sequence IYGMH (SEQ ID NO: 149), a CDRH2 including the sequence VIWYDGSHEYADSVKG (SEQ ID NO: 150), and a CDRH3 including the sequence DGDYYDSGSPLDY (SEQ ID NO: 151).

[0171] In particular embodiments, FOLR1 expression is tumor specific and is expressed in various epithelial tumors such as ovarian, breast, endometrial, and lung cancers. In particular embodiments, FOLR1 includes the sequence referenced in Accession: P15328.3, Accession: NP_057937.1, or Accession: CAG46816.1. In particular embodiments, a binding domain that binds FOLR1 includes Farletuzumab. In particular embodiments, the Farletuzumab includes a variable light chain includes the sequence:

DIQLTQSPSSLSASVGDRVITITCSVSSSISSNNLHWYQQKPGKAPKRWIYGTSNLSGVPSRFS
GSGSGTDYFTISSLQPEDATYYCQQWSSYPYMYTFGQGTKVEIK (SEQ ID NO: 152) and a variable heavy chain includes the sequence:

EVQLVESGGGVVQPGRSLRLSCSASGFTFSGYGLSWVRQAPGKGLEWAMISSGGSYTYYA
DSVKGRFAISRDNKNTLFLQMDSLRPEDTGVYFCARHGDDPAWFAYWGQGTPVTVSS (SEQ ID NO: 153).

[0172] In particular embodiments, the FOLR1-binding domain includes the Farletuzumab antibody (MorAb-003). In particular embodiments, the FOLR1-binding domain is a human or humanized binding domain including a variable heavy chain including a CDRH1 sequence including GYGLS (SEQ ID NO: 154), a CDRH2 sequence including MISSGGSYTTYADSVKG (SEQ ID NO: 155), and a CDRH3 sequence including HGDDPAWFAY (SEQ ID NO: 156), and a variable light chain including a CDRL1 sequence including SVSSSISSNNLH (SEQ ID NO: 157), a CDRL2 sequence including GTSNLAS (SEQ ID NO: 158), and a CDRL3 sequence including QQWSSYPYMYT (SEQ ID NO: 159), according to Kabat numbering scheme.

[0173] In particular embodiments, MUC1 is an oncoprotein found in 40% of breast cancers. In particular embodiments, MUC1 includes the sequence referenced in Accession: CAA56734.1 or Accession: P15941.3. In particular embodiments, a binding domain that binds MUC1 includes GP1.4 or SM3. In particular embodiments, the SM3 includes a variable light chain includes the sequence:

DIVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWWQEKPDHLFTGLIGGTNNRAPGVPARF
SGSLIGDKAALTITGAQTEDEAIYFCALWYSNHHWWFGGGTKLTVLGSEKSSPSVTLPFPSSSEEL
ETNKATLVCTITDFYPGVVTVDWKVDGTPVTQGMETTQPSKQSNNKYMSSYLTLTARAWER
HSSYSCQVTHEGHTVEKSLSRADCS (SEQ ID NO: 160) and a variable heavy chain includes the sequence:

QVQLQESGGGLVQPGGSMKLSCLVASGFTFSNYWMNWRQSPKGLEWVAEIRLKSNNYATH
YAESVKGRFISRDDSKSSVYLQMNRLRAEDTGIIYCTGVGQFAYWGQGTITVTVSSAKTTPPTV
YPLAPGSNAASQSMVTLGCLVKGYFPEPVTVTWNSGSLASGVHTFPAVLQSDLYTLSSSVTVP
SSTWPSETVTCNVAHPASSTKVDKIVPRD (SEQ ID NO: 161).

[0174] In particular embodiments, the MUC1 binding domain includes a variable light chain including a CDRL1 including the sequence RSSTGAVTTSNYAN (SEQ ID NO: 230), a CDRL2 including the sequence GTNNRAP (SEQ ID NO: 231), and CDRL3 including the sequence ALWYSNHHWV (SEQ ID NO: 232), and a variable heavy chain including a CDRH1 including the sequence NYWMN (SEQ ID NO: 233), CDRH2 including the sequence EIRLKSNNYATHYAESVKG (SEQ ID NO: 234), and CDRH3 including the sequence VGQFAY (SEQ ID NO: 235), according to Kabat. CDR predictions according to other CDR definitions can also be determined.

[0175] In particular embodiments, CA 19-9 expression is often a sign of pancreatic cancer or other types of cancers. CA 19-9 is also known as sialyl Le^A, SLe^A, or as the IUPAC name: (12S,14S,15R,16R,32R,33R,34S,35S,36R,52R,53S,54R,55R,56E,72S,73S,74R,75S,76S)-15,55-Diacetamido-14,33,35,56,73,74,75-heptahydroxy-36,52-bis(hydroxymethyl)-76-methyl-

16-[(1R,2R)-1,2,3-trihydroxypropyl]-2,4,6-trioxa-1,7(2),3(4,2),5(4,3)-tetraoxanaheptaphane-12-carboxylic acid. In particular embodiments, a binding domain that binds CA 19-9 includes 5B1. In particular embodiments, the 5B1 antibody includes a variable light chain including the sequence: MAGFPLLLTLLTHCAGSWAQSFLTQPPSASGTPGQRVTISCSGSSSNIGSNFVYWYQQLPGTA PKLLIYRNNQRPSGVPDRFSGSRSGTSASLAISGLRSEDEADYCAAWDDSLGGHYVFGTGTK VTVL (SEQ ID NO: 162) and a variable heavy chain includes the sequence: MEFGLSWLFLVAILKGVQCQVQLVESGGGSVQPGRSLRLSCEASGFTFEAYAMHWRQPPG KGLEWWSSINWNSGRIAYADSVKGRFTISRDNARNLSLYLQMNSLRLEDTAFFYYCAKDIRRFSTG GAEFEYWGQGTLVTVSS (SEQ ID NO: 163).

[0176] In particular embodiments, the anti-CA 19-9 binding domain includes a variable light chain including a CDRL1 including the sequence SGSSSNIGSNFVY (SEQ ID NO: 236), a CDRL2 including the sequence RNNQRPS (SEQ ID NO: 237), and CDRL3 including the sequence AAWDDSLGGHYV (SEQ ID NO: 238), and a variable heavy chain including a CDRH1 including the sequence AYAMH (SEQ ID NO: 239), CDRH2 including the sequence SINWNSGRIAYADSVKG (SEQ ID NO: 240), and CDRH3 including the sequence DIRRFSTGGAEFEY (SEQ ID NO: 241), according to Kabat. CDR predictions according to other CDR definitions can also be determined.

[0177] FIG. 14 presents a table of cancer antigens and the primary tumor type by which they are expressed. The table further displays which trafficking signals, retention signals, and inhibitory signals (i.e., down-regulation of trafficking signals) can be used to direct genetically modified cells to a select tissue and avoid a non-selected tissue for the particular cancer types and organs that they commonly affect.

(III-C-2) Transmembrane Domains. As indicated, a transmembrane domain within a recombinant receptor serves to connect the extracellular component and intracellular component through the cell membrane. The transmembrane domain can anchor the expressed molecule in the modified cell's membrane.

[0178] The transmembrane domain can be derived either from a natural and/or a synthetic source. When the source is natural, the transmembrane domain can be derived from any membrane-bound or transmembrane protein. Transmembrane domains can include at least the transmembrane region(s) of the α , β or ζ chain of a T-cell receptor, CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22; CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9. In particular embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40, CD2, CD27, LFA-1 (CD 11a, CD18), ICOS (CD278), 4-1BB

(CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R β , IL2R γ , IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDI Id, ITGAE, CD103, ITGAL, CDI Ia, ITGAM, CDI Ib, ITGAX, CDI Ic, ITGB1, CD29, ITGB2, CD18, ITGB7, TNFR2, DNAM1(CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9(CD229), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, or NKG2C. In particular embodiments, a variety of human hinges can be employed as well including the human Ig (immunoglobulin) hinge (e.g., an IgG4 hinge, an IgD hinge), a GS linker (e.g., a GS linker described herein), a KIR2DS2 hinge or a CD8a hinge. In particular embodiments, the recombinant receptor (e.g., CAR) includes a CD28 transmembrane domain. It has been shown that a CD28 transmembrane domain reduces the antigen-threshold for second-generation 4-1BB CAR T cell activation.

[0179] In particular embodiments, a transmembrane domain has a three-dimensional structure that is thermodynamically stable in a cell membrane, and generally ranges in length from 15 to 30 amino acids. The structure of a transmembrane domain can include an α helix, a β barrel, a β sheet, a β helix, or any combination thereof.

[0180] A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid within the extracellular region of the recombinant receptor (e.g., up to 15 amino acids of the extracellular region) and/or one or more additional amino acids within the intracellular region of the recombinant receptor (e.g., up to 15 amino acids of the intracellular components). In one aspect, the transmembrane domain is from the same protein that the signaling domain, co-stimulatory domain or the hinge domain is derived from. In another aspect, the transmembrane domain is not derived from the same protein that any other domain of the recombinant receptor is derived from. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other unintended members of the receptor complex. In particular embodiments, the transmembrane domain is encoded by the nucleic acid sequence encoding the CD28 transmembrane domain (SEQ ID NOs: 8, 30, 31, 32, or 33). In particular embodiments, the transmembrane domain includes the amino acid sequence of the CD28 transmembrane domain (SEQ ID NOs: 27, 28, or 29).

(III-C-3) Intracellular Components. The intracellular component of a recombinant receptor includes an effector domain that is responsible for activation of the cell in which the recombinant receptor is expressed. The term “effector domain” is thus meant to include any portion of the

intracellular domain sufficient to transduce an activation signal. An effector domain can directly or indirectly promote a biological or physiological response in a cell when receiving the appropriate signal. In certain embodiments, an effector domain is part of a protein or protein complex that receives a signal when bound, or it binds directly to a target molecule, which triggers a signal from the intracellular effector domain. An effector domain may directly promote a cellular response when it contains one or more signaling domains or motifs, such as an immunoreceptor tyrosine-based activation motif (ITAM). In other embodiments, an effector domain will indirectly promote a cellular response by associating with one or more other proteins that directly promote a cellular response, such as co-stimulatory domains.

[0181] Effector domains can provide for activation of at least one function of a modified cell upon binding to the cellular marker expressed by a cancer cell. Activation of the modified cell can include one or more of differentiation, proliferation and/or activation or other effector functions. In particular embodiments, an effector domain can include an intracellular signaling component including a T cell receptor and a co-stimulatory domain which can include the cytoplasmic sequence from co-receptor or co-stimulatory molecule.

[0182] An effector domain can include one, two, three or more intracellular signaling components (e.g., receptor signaling domains, cytoplasmic signaling sequences), co-stimulatory domains, or combinations thereof. Exemplary effector domains include signaling and stimulatory domains selected from: 4-1BB (CD137), CARD11, CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD27, CD28, CD79A, CD79B, DAP10, FcR α , FcR β (Fc ϵ R1b), FcR γ , Fyn, HVEM (LIGHTR), ICOS, LAG3, LAT, Lck, LRP, NKG2D, NOTCH1, pT α , PTCH2, OX40, ROR2, Ryk, SLAMF1, Slp76, TCR α , TCR β , TRIM, Wnt, Zap70, or any combination thereof. In particular embodiments, exemplary intracellular effector domains include signaling and co-stimulatory domains selected from: CD86, Fc γ RIIa, DAP12, CD30, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFFR, SLAMF7, NKp80 (KLRP1), CD127, CD160, CD19, CD4, CD8 α , CD8 β , IL2R β , IL2R γ , IL7R α , ITGA4, VLA1, CD49a, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, GADS, PAG/Cbp, NKp44, NKp30, NKp46, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, or TLR9. In particular embodiments, the effector domain includes a CD3 ζ signaling domain.

[0183] Intracellular signaling domains that act in a stimulatory manner may include iTAMs.

Examples of iTAMs including primary cytoplasmic signaling sequences include those derived from CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD5, CD22, CD66d, CD79a, CD79b, and common FcR γ (FCER1G), Fc γ RIIa, FcR β (Fc ϵ Rib), DAP10, and DAP12. In particular embodiments, variants of CD3 ζ retain at least one, two, three, or all ITAM regions.

[0184] In particular embodiments, an effector domain includes a cytoplasmic portion that associates with a cytoplasmic signaling protein, wherein the cytoplasmic signaling protein is a lymphocyte receptor or signaling domain thereof, a protein including a plurality of ITAMs, a co-stimulatory domain, or any combination thereof.

[0185] Additional examples of intracellular signaling domains include the cytoplasmic sequences of the CD3 ζ chain, and/or co- receptors that act in concert to initiate signal transduction following binding domain engagement.

[0186] A co-stimulatory domain is a domain whose activation can be required for an efficient lymphocyte response to cellular marker binding. Some molecules are interchangeable as intracellular signaling domains or co-stimulatory domains. Examples of costimulatory domains include CD27, CD28, 4-1BB (CD 137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83. For example, CD27 co-stimulation has been demonstrated to enhance expansion, effector function, and survival of human CAR T cells *in vitro* and augments human T cell persistence and anti-cancer activity *in vivo* (Song *et al.* Blood. 2012; 119(3):696-706). Further examples of such co-stimulatory domain molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8 α , CD8 β , IL2R β , IL2R γ , IL7R α , ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), NKG2D, CEACAM1, CRTAM, Ly9 (CD229), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a. In particular embodiments, the co-stimulatory domain includes a CD28 signaling domain.

[0187] In particular embodiments, the nucleic acid sequences encoding the intracellular component include a CD3 ζ encoding sequence (SEQ ID NOs: 47 or 48) and a variant of the CD28 signaling encoding sequence (SEQ ID NOs: 9 or 41). In particular embodiments, the amino acid sequence of the intracellular component includes a variant of CD3 ζ (SEQ ID NOs: 44, 45, or 46) and a portion of the CD28 (SEQ ID NOs: 40) intracellular component.

[0188] In particular embodiments, the intracellular component includes (i) all or a portion of the

signaling domain of CD3 ζ , (ii) all or a portion of the signaling domain of CD28, or (iv) all or a portion of the signaling domain of CD3 ζ and CD28.

[0189] Intracellular components may also include one or more of a protein of a Wnt signaling pathway (e.g., LRP, Ryk, or ROR2), NOTCH signaling pathway (e.g., NOTCH1, NOTCH2, NOTCH3, or NOTCH4), Hedgehog signaling pathway (e.g., PTCH or SMO), receptor tyrosine kinases (RTKs) (e.g., epidermal growth factor (EGF) receptor family, fibroblast growth factor (FGF) receptor family, hepatocyte growth factor (HGF) receptor family, insulin receptor (IR) family, platelet-derived growth factor (PDGF) receptor family, vascular endothelial growth factor (VEGF) receptor family, tropomyosin receptor kinase (Trk) receptor family, ephrin (Eph) receptor family, AXL receptor family, leukocyte tyrosine kinase (LTK) receptor family, tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE) receptor family, receptor tyrosine kinase-like orphan (ROR) receptor family, discoidin domain (DDR) receptor family, rearranged during transfection (RET) receptor family, tyrosine-protein kinase-like (PTK7) receptor family, related to receptor tyrosine kinase (RYK) receptor family, or muscle specific kinase (MuSK) receptor family); G-protein-coupled receptors, GPCRs (Frizzled or Smoothened); serine/threonine kinase receptors (BMPR or TGFR); or cytokine receptors (IL1R, IL2R, IL7R, or IL15R).

[0190] (III-D) Linkers. A linker is a molecule that serves to connect two subcomponents or domains (e.g., of a recombinant receptor). In particular embodiments, linkers can provide flexibility for different components (e.g., different components of a recombinant receptor). Linkers can also include spacer regions and junction amino acids. In certain examples, when a more rigid linker is required, proline-rich linkers can be used. Some linkers serve no purpose other than to link components while many linkers serve an additional purpose, such as a multimerization domain.

[0191] Spacers are used to create appropriate distances and/or flexibility from other sub-components. In particular embodiments, the length of a spacer is customized for binding targeted cells and mediating destruction in a recombinant receptor. In particular embodiments, a spacer length can be selected based upon the location of a cellular marker epitope, affinity of a binding domain for the epitope, and/or the ability of the target-binding agent to mediate cell destruction following target binding.

[0192] Spacers typically include those having 10 to 250 amino acids, 10 to 200 amino acids, 10 to 150 amino acids, 10 to 100 amino acids, 10 to 50 amino acids, or 10 to 25 amino acids.

[0193] In particular embodiments, a spacer is 10 amino acids, 12 amino acids, 14 amino acids, 20 amino acids, 21 amino acids, 26 amino acids, 27 amino acids, 45 amino acids, 50 amino acids, 55 amino acids, 60 amino acids, 65 amino acids, 70 amino acids, or 75 amino acids. These lengths qualify as short spacers.

[0194] In particular embodiments, a spacer is 76 amino acids, 77 amino acids, 80 amino acids, 85 amino acids, 90 amino acids, 95 amino acids, 100 amino acids, 110 amino acids, 120 amino acids, 125 amino acids, 128 amino acids, 131 amino acids, 135 amino acids, 140 amino acids, 150 amino acids, 160 amino acids, or 179 amino acids. These lengths qualify as intermediate spacers.

[0195] In particular embodiments, a spacer is 180 amino acids, 190 amino acids, 200 amino acids, 210 amino acids, 212 amino acids, 214 amino acids, 216 amino acids, 218 amino acids, 220 amino acids, 228 amino acids, 230 amino acids, 240 amino acids, 250 amino acids, 260 amino acids, or 270 amino acids. These lengths qualify as long spacers.

[0196] In particular embodiments, spacers include all or a portion of an immunoglobulin hinge region. An immunoglobulin hinge region may be a wild-type immunoglobulin hinge region or an altered wild-type immunoglobulin hinge region. In certain embodiments, an immunoglobulin hinge region is a human immunoglobulin hinge region. As used herein, a “wild type immunoglobulin hinge region” refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody.

[0197] An immunoglobulin hinge region may be an IgG, IgA, IgD, IgE, or IgM hinge region. An IgG hinge region may be an IgG1, IgG2, IgG3, or IgG4 hinge region. Sequences from IgG1, IgG2, IgG3, IgG4 or IgD can be used alone or in combination with all or a portion of a CH2 region; all or a portion of a CH3 region; or all or a portion of a CH2 region and all or a portion of a CH3 region.

[0198] Other examples of hinge regions that can be used in include the hinge region present in the extracellular regions of type 1 membrane proteins, such as CD8 α , CD4, CD28 and CD7, which may be wild-type or variants thereof.

[0199] In particular embodiments, a spacer includes a hinge region that includes a type II C-lectin interdomain (stalk) region or a cluster of differentiation (CD) molecule stalk region. A “stalk region” of a type II C-lectin or CD molecule refers to the portion of the extracellular domain (ECD) of the type II C-lectin or CD molecule that is located between the C-type lectin-like domain (CTLCD; e.g., similar to CTLCD of natural killer cell receptors) and the hydrophobic portion (transmembrane domain). For example, the ECD of human CD94 (GenBank Accession No. AAC50291.1) corresponds to amino acid residues 34–179, but the CTLCD corresponds to amino acid residues 61–176, so the stalk region of the human CD94 molecule includes amino acid residues 34–60, which are located between the hydrophobic portion (transmembrane domain) and CTLCD (see Boyington *et al.*, Immunity 10:15, 1999; for descriptions of other stalk regions, see also Beavil *et*

al., Proc. Nat'l. Acad. Sci. USA 89:153, 1992; and Figdor *et al.*, Nat. Rev. Immunol. 2:11, 2002). These type II C-lectin or CD molecules may also have junction amino acids (described below) between the stalk region and the transmembrane region or the CTLD. In another example, the 233 amino acid human NKG2A protein (GenBank Accession No. P26715.1) has a hydrophobic portion (transmembrane domain) ranging from amino acids 71–93 and an ECD ranging from amino acids 94–233. The CTLD includes amino acids 119–231 and the stalk region includes amino acids 99–116, which may be flanked by additional junction amino acids. Other type II C-lectin or CD molecules, as well as their extracellular ligand-binding domains, stalk regions, and CTLDs are known in the art (see, e.g., GenBank Accession Nos. NP 001993.2; AAH07037.1; NP 001773.1; AAL65234.1; CAA04925.1; for the sequences of human CD23, CD69, CD72, NKG2A, and NKG2D and their descriptions, respectively).

[0200] Linkers can, for example, link VL and VH of antibody derived binding domains of scFvs and serve as junction amino acids between subcomponent portions of a recombinant receptor or other protein. In particular embodiments a linker includes the Whitlow linker (GSTSGSGKPGSGEGSTKG; SEQ ID NO: 248).

[0201] Linkers can be flexible, rigid, or semi-rigid, depending on the desired function of the linker. Linkers can include junction amino acids. For example, in particular embodiments, linkers provide flexibility and room for conformational movement between different components. Commonly used flexible linkers include Gly-Ser linkers. In particular embodiments, the linker sequence includes sets of glycine and serine repeats such as from one to ten repeats of $(\text{Gly}_x\text{Ser}_y)_n$, wherein x and y are independently an integer from 0 to 10 provided that x and y are not both 0 and wherein n is an integer of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10). Particular examples include $(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 164), $(\text{Gly}_3\text{Ser})_n(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 165), $(\text{Gly}_3\text{Ser})_n(\text{Gly}_2\text{Ser})_n$ (SEQ ID NO: 166), or $(\text{Gly}_3\text{Ser})_n(\text{Gly}_4\text{Ser})_1$ (SEQ ID NO: 167). In particular embodiments, the linker is $(\text{Gly}_4\text{Ser})_4$ (SEQ ID NO: 168), $(\text{Gly}_4\text{Ser})_3$ (SEQ ID NO: 169), $(\text{Gly}_4\text{Ser})_2$ (SEQ ID NO: 170), $(\text{Gly}_4\text{Ser})_1$ (SEQ ID NO: 171), $(\text{Gly}_3\text{Ser})_2$ (SEQ ID NO: 172), $(\text{Gly}_3\text{Ser})_1$ (SEQ ID NO: 173), $(\text{Gly}_2\text{Ser})_2$ (SEQ ID NO: 174) or $(\text{Gly}_2\text{Ser})_1$, GSGGGSGSG (SEQ ID NO: 175), GSGGGSGSG (SEQ ID NO: 176), or GSGGGSG (SEQ ID NO: 177).

[0202] In particular embodiments, a linker region is $(\text{GGGS})_n$ (SEQ ID NO: 164) wherein n is an integer including, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more. In particular embodiments, the linker is $(\text{EAAAK})_n$ (SEQ ID NO: 178) wherein n is an integer including 1, 2, 3, 4, 5, 6, 7, 8, 9, or more.

[0203] In some situations, flexible linkers may be incapable of maintaining a distance or position (e.g., of a recombinant receptor) needed for a particular use. In these instances, rigid or semi-rigid linkers may be useful. Examples of rigid or semi-rigid linkers include proline-rich linkers. In

particular embodiments, a proline-rich linker is a peptide sequence having more proline residues than would be expected based on chance alone. In particular embodiments, a proline-rich linker is one having at least 30%, at least 35%, at least 36%, at least 39%, at least 40%, at least 48%, at least 50%, or at least 51% proline residues. Particular examples of proline-rich linkers include fragments of proline-rich salivary proteins (PRPs).

[0204] Linkers can be susceptible to cleavage (cleavable linker), such as, acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage. Alternatively, linkers can be substantially resistant to cleavage (e.g., stable linker or noncleavable linker). In some aspects, the linker is a procharged linker, a hydrophilic linker, or a dicarboxylic acid-based linker.

[0205] Junction amino acids can be a linker which can be used to connect sequences when the distance provided by a spacer is not needed and/or wanted. For example, junction amino acids can be short amino acid sequences that can be used to connect co-stimulatory intracellular signaling components. In particular embodiments, junction amino acids are 9 amino acids or less (e.g., 2, 3, 4, 5, 6, 7, 8, or 9 amino acids). In particular embodiments, a glycine-serine doublet can be used as a suitable junction amino acid linker. In particular embodiments, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable junction amino acid.

[0206] In particular embodiments, the recombinant receptor can optionally include a multimerization domain. Protein biological activities depend upon their tertiary and quaternary structure. The quaternary structure requires the physical and chemical interaction of different protein subunits or polypeptides. A "multimerization domain" is a domain that causes two or more proteins (monomers) to interact with each other through covalent and/or non-covalent association(s). Multimerization domains present in proteins can result in protein interactions that form dimers, trimers, tetramers, pentamers, hexamers, heptamers, etc., depending on the number of units/monomers incorporated into the multimer.

[0207] (III-E) Control Features Including Tag Cassettes, Transduction Markers, and/or Suicide Switches. In particular embodiments, genetic constructs can include one or more tag cassettes and/or transduction markers. Tag cassettes and transduction markers can be used to activate, promote proliferation of, detect, enrich for, isolate, track, deplete and/or eliminate genetically modified cells *in vitro*, *in vivo* and/or *ex vivo*. "Tag cassette" refers to a unique synthetic peptide sequence affixed to, fused to, or that is part of a genetic construct, to which a cognate binding molecule (e.g., ligand, antibody, or other binding partner) is capable of specifically binding where the binding property can be used to activate, promote proliferation of, detect, enrich for, isolate, track, deplete and/or eliminate the tagged protein and/or cells expressing the tagged protein.

Transduction markers can serve the same purposes but are derived from naturally occurring molecules and are often expressed using a skipping element that separates the transduction marker from the rest of the expressed molecule (e.g., CAR, trafficking signal, retention signal).

[0208] Tag cassettes that bind cognate binding molecules include, for example, His tag (HHHHHH; SEQ ID NO: 179), Flag tag (DYKDDDDK; SEQ ID NO: 180), Xpress tag (DLYDDDDK; SEQ ID NO: 181), Avi tag (GLNDIFEAQKIEWHE; SEQ ID NO: 182), Calmodulin tag (KRRWKKNFIAVSAANRFKKISSSGAL; SEQ ID NO: 183), Polyglutamate tag, HA tag (YPYDVPDYA; SEQ ID NO: 184), Myc tag (EQKLISEEDL; SEQ ID NO: 185), Strep tag (which refers the original STREP® tag (WRHPQFGG; SEQ ID NO: 186), STREP® tag II (WSHPQFEK SEQ ID NO: 187 (IBA Institut für Bioanalytik, Germany); see, e.g., US 7,981,632), Softag 1 (SLAELLNAGLGGS; SEQ ID NO: 188), Softag 3 (TQDPSRVG; SEQ ID NO: 189), and V5 tag (GKPIPNPLLGLDST; SEQ ID NO: 190).

[0209] Conjugate binding molecules that specifically bind tag cassette sequences disclosed herein are commercially available. For example, His tag antibodies are commercially available from suppliers including Life Technologies, Pierce Antibodies, and GenScript. Flag tag antibodies are commercially available from suppliers including Pierce Antibodies, GenScript, and Sigma-Aldrich. Xpress tag antibodies are commercially available from suppliers including Pierce Antibodies, Life Technologies and GenScript. Avi tag antibodies are commercially available from suppliers including Pierce Antibodies, IsBio, and Genecopoeia. Calmodulin tag antibodies are commercially available from suppliers including Santa Cruz Biotechnology, Abcam, and Pierce Antibodies. HA tag antibodies are commercially available from suppliers including Pierce Antibodies, Cell Signal and Abcam. Myc tag antibodies are commercially available from suppliers including Santa Cruz Biotechnology, Abcam, and Cell Signal. Strep tag antibodies are commercially available from suppliers including Abcam, Iba, and Qiagen.

[0210] Transduction markers may be selected from at least one of Cluster of Differentiation 90 (CD90 or CD90.1); a truncated HER2 extracellular protein (Her2tG); a truncated CD19 (tCD19; see Budde *et al.*, Blood 122: 1660, 2013); a truncated human EGFR (tEGFR or EGFRt; see Wang *et al.*, Blood 118: 1255, 2011); an ECD of human CD34; RQR8 which combines target epitopes from CD34 (see Fehse *et al.*, Mol. Therapy 1(5 Pt 1); 448–456, 2000) and/or CD20 antigens (see Philip *et al.*, Blood 124: 1277–1278). In particular embodiments, cells are genetically modified to express CD90.1, Her2tG, EGFRt, or tCD19.

[0211] In particular embodiments, genetic constructs can include a polynucleotide that encodes a self-cleaving polypeptide, wherein the polynucleotide encoding the self-cleaving polypeptide is located between the polynucleotide encoding an expressed molecule within the genetic construct

and a polynucleotide encoding a transduction marker (e.g., CD90.1 or EGFRt).

[0212] Exemplary self-cleaving polypeptides include 2A peptide from porcine teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or variants thereof. Further exemplary nucleic acid and amino acid sequences of 2A peptides are set forth in, for example, Kim *et al.* (*PLOS One* 6:e18556 (2011)). In particular embodiments, cells are genetically modified to include a self-cleaving polypeptide. In particular embodiments, the self-cleaving polypeptide includes T2A.

[0213] Control features may be present in multiple copies in a genetic construct or can be expressed as distinct molecules with the use of a skipping element. For example, a genetic construct can have one, two, three, four or five tag cassettes and/or one, two, three, four, or five transduction markers could also be expressed. For example, embodiments can include a genetic construct having two Myc tag cassettes, or a His tag and an HA tag cassette, or a HA tag and a Softag 1 tag cassette, or a Myc tag and a SBP tag cassette. Exemplary transduction markers and cognate pairs are described in US 13/463,247.

[0214] In certain embodiments, modified cells expressing a genetic construct may be detected or tracked *in vivo* by using antibodies that bind with specificity to a control feature (e.g., anti-Tag antibodies), or by other cognate binding molecules that specifically bind the control feature, which binding partners for the control feature are conjugated to a fluorescent dye, radio-tracer, iron-oxide nanoparticle or other imaging agent known in the art for detection by X-ray, CT-scan, MRI-scan, PET-scan, ultrasound, flow-cytometry, near infrared imaging systems, or other imaging modalities (see, e.g., Yu, *et al.*, *Theranostics* 2:3, 2012).

[0215] One advantage of including at least one control feature in a genetic construct is that cells expressing a genetic construct administered to a subject can be increased or depleted using the cognate binding molecule to a tag cassette. In certain embodiments, the present disclosure provides a method for depleting a modified cell expressing a genetic construct by using an antibody specific for the tag cassette, using a cognate binding molecule specific for the control feature, or by using a second modified cell expressing a genetic construct and having specificity for the control feature. Elimination of modified cells may be accomplished using depletion agents specific for a control feature. For example, if EGFRt is used, then an anti-EGFRt binding domain (e.g., antibody, scFv) fused to or conjugated to a cell-toxic reagent (such as a toxin, radiometal) may be used, or an anti-EGFRt /anti-CD3 bispecific scFv, or an anti-EGFRt CAR T cell may be used.

[0216] A control feature can also include a suicide gene or suicide switch. The term "suicide gene" as used herein refers to a suicide or apoptosis-inducing gene operably linked to a promoter, which

may be constitutive or inducible. Examples of a suicide gene include, but are not limited to, a herpes simplex virus thymidine kinase (HSV-TK), the cytoplasmic domain of Fas, a caspase such as caspase-8 or caspase-9, cytosine deaminase, E1A, FHIT, and other known suicide or apoptosis-inducing genes.

[0217] The term "suicide switch" as used herein refers to the expression product of the suicide gene. In particular embodiments, a polynucleotide encoding an iCaspase9 construct (iCasp9) may be inserted into a genetic construct as a suicide gene.

[0218] Modified cells expressing at least one control feature within a genetic construct can be, e.g., more readily identified, isolated, sorted, induced to proliferate, tracked, and/or eliminated as compared to a modified cell without a tag cassette.

[0219] (IV) Characterization of Genetically Modified Cells. In particular embodiments, the modified (i.e., engineered) cells can be assessed for expression of the genetic constructs. In particular embodiments, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% of the genetically modified cells express a detectable level of the genetic constructs.

[0220] Surface protein expression can be determined by flow cytometry or other appropriate methods well known to those of ordinary skill in the art. By labeling a population of cells with an element that targets the desired cell surface marker (e.g., an antibody) and is tagged with a fluorescent molecule, flow cytometry can be used to quantify the portion of the population that is positive for the surface marker, as well as the level of surface marker expression.

[0221] Genomic incorporation of a genetic construct within genetically modified cells can be determined by digital droplet PCR (ddPCR). Digital PCR enables quantification of DNA concentration in a sample. Digital PCR is performed by fractionating a mixture of a PCR reaction (e.g., containing a sample of nucleic acid molecules and copies of a PCR probe) such that some fractions contain no PCR probe copy, while other fractions contain one or more PCR probe copies. A PCR amplification of the fractions is performed and the fractions are analyzed for a PCR reaction. A fraction containing one or more probes and one or more target DNA molecules yields a positive end-point, while a fraction containing no PCR probe yields a negative end-point. The fraction of positive reactions is then fitted to a Poisson distribution to determine the absolute copy number of target DNA molecules per given volume of the unfractionated sample (i.e., copies per microliter of sample) (see Hindson, B. *et al.*, (2011) *Anal Chem.* 83:8604-8610). Digital droplet PCR is a variation of digital PCR wherein a sample of nucleic acids is fractionated into droplets using a water-oil emulsion. PCR amplification is performed on the droplets collectively, whereupon a fluidics system is used to separate the droplets and provide analysis of each

individual droplet. For one skilled in the art, ddPCR is used to provide an absolute quantification of DNA in a sample, to perform a copy number variation analysis, or to assess efficiency of genomic edits.

[0222] Genetically modified cells can also be assessed for cytokine-independent growth. Genetically modified cells are expected to only grow in the presence of stimulatory cytokines (e.g., IL-2, IL-7). Growth in the absence of cytokines is an indicator of tumorigenic potential. In particular embodiments, genetically modified cells are grown for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, or 20 days in either the presence or in the absence of one or more stimulatory cytokines (e.g., IL-2, IL-7). In particular embodiments, proliferation is assessed by cell count and viability using conventional methods (e.g., flow cytometry, microscopy, optical density, metabolic activity). In particular embodiments, proliferation is assessed starting on day 1, day 2, day 3, day 4, day 5, day 6. In particular embodiments, proliferation is assessed every 1 day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, or every 8 days. In particular embodiments, growth in the absence of cytokines is assessed at the end of a growth period. In some particular embodiments, genetically modified cells with no growth in the absence of cytokines is defined as lacking tumorigenic potential. In particular embodiments, no growth is defined as an expansion of the population that is less than 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, or 1.5 fold between the end of the growth period relative to the beginning of the growth period. In particular embodiments, the genetically modified cells do not proliferate in the absence of cytokine stimulation, growth factor stimulation, or antigen stimulation.

[0223] (V) Cell Activating Culture Conditions. Cell populations can be incubated in a culture-initiating composition to expand cell populations. The incubation can be carried out in a culture vessel, such as a bag, cell culture plate, flask, chamber, chromatography column, cross-linked gel, cross-linked polymer, column, culture dish, hollow fiber, microtiter plate, silica-coated glass plate, tube, tubing set, well, vial, or other container for culture or cultivating cells.

[0224] In particular embodiments, the cell population can be incubated in the culture-initiating composition before or after genetic engineering the cell populations. In particular embodiments, the incubation can be carried out for 1 day to 6 days, 1 day to 5 days, 1 day to 4 days, 1 day to 3 days, 1 day to 2 days, or 1 day before genetically engineering the cell populations. In particular embodiments, the incubation can be carried out for 1 day to 6 days, 1 day to 5 days, 1 day to 4 days, 1 day to 3 days, 1 day to 2 days, or 1 day after genetically engineering the cell populations. In particular embodiments, the incubation can be carried out at the same time as genetically engineering the cell populations.

[0225] Culture conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, e.g., nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0226] In some aspects, incubation is carried out in accordance with techniques such as those described in US 6,040,177, Klebanoff *et al.* (2012) J Immunother. 35(9): 651-660, Terakura *et al.* (2012) Blood. 1:72-82, and/or Wang *et al.* (2012) J Immunother. 35(9):689-701.

[0227] Exemplary culture media for culturing T cells include (i) RPMI supplemented with non-essential amino acids, sodium pyruvate, and penicillin/streptomycin; (ii) RPMI with HEPES, 5-15% human serum, 1-3% L-Glutamine, 0.5-1.5% penicillin/streptomycin, and 0.25×10^{-4} - 0.75×10^{-4} M β -MercaptoEthanol; (iii) RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 10mM HEPES, 100 U/ml penicillin and 100 m/mL streptomycin; (iv) DMEM medium supplemented with 10% FBS, 2mM L-glutamine, 10mM HEPES, 100 U/ml penicillin and 100 m/mL streptomycin; and (v) X-Vivo 15 medium (Lonza, Walkersville, MD) supplemented with 5% human AB serum (Gemcell, West Sacramento, CA), 1% HEPES (Gibco, Grand Island, NY), 1% Pen-Strep (Gibco), 1% GlutaMax (Gibco), and 2% N-acetyl cysteine (Sigma-Aldrich, St. Louis, MO). T cell culture media are also commercially available from Hyclone (Logan, UT). Additional T cell activating components that can be added to such culture media are described in more detail below.

[0228] In some embodiments, the T cells are expanded by adding to the culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g., for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can include gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells.

[0229] Optionally, the incubation may further include adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least 10: 1.

[0230] In some embodiments, the stimulating conditions include temperature suitable for the growth of human T lymphocytes, for example, at least 25°C, at least 30°C, or 37°C.

[0231] The activating culture conditions for T cells include conditions whereby T cells of the culture-initiating composition proliferate or expand. T cell activating conditions can include one or more cytokines, for example, interleukin (IL)-2, IL-7, IL-15 and/or IL-21. IL-2 can be included at a range of 10 – 100 ng/ml (e.g., 40, 50, or 60 ng/ml). IL-7, IL-15, and/or IL-21 can be individually included at a range of 0.1 – 50 ng/ml (e.g., 5, 10, or 15 ng/ml). Particular embodiments utilize IL-2 at 50 ng/ml. Particular embodiments utilize, IL-7, IL-15 and IL-21 individually included at 10 ng/ml.

[0232] In particular embodiments, T cell activating culture conditions can include T cell stimulating epitopes. T cell stimulating epitopes include CD3, CD27, CD2, CD4, CD5, CD7, CD8, CD28, CD30, CD40, CD56, CD83, CD90, CD95, 4-1BB (CD 137), B7-H3, CTLA-4, Frizzled-1 (FZD1), FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, HVEM, ICOS, IL-1R, LAT, LFA-1, LIGHT, MHCI, MHCII, NKG2D, OX40, ROR2, and RTK.

[0233] In particular embodiments, a T-cell activating culture media includes a fluorescence-activated cell sorting (FACS)-sorted T cell population cultured within RPMI with HEPES, 5-15% human serum, 1-3% L-Glutamine, 0.5-1.5% Pen/strep, 0.25×10^{-4} - 0.75×10^{-4} M β -MercaptoEthanol, with IL-7, IL-15 and IL-21 individually included at 5-15 (e.g., 10) ng/ml. The culture is carried out on a flat-bottom well plate with 0.1 - 0.5×10^6 plated cells/well. On Day 3 post activation cells are transferred to a tissue culture (TC)-treated plate.

[0234] In particular embodiments, a T-cell activating culture media includes a FACS-sorted CD8+ T population cultured within RPMI with HEPES, 10% human serum, 2% L-Glutamine, 1% Pen/strep, 0.5×10^{-4} M β -MercaptoEthanol, with IL-7, IL-15 and IL-21 individually included at 5-15 (e.g., 10) ng/ml. The culture is carried out on a flat-bottom non-tissue culture-treated 96/48-well plate with 0.1 - 0.5×10^6 plated cells/well. On Day 3 post activation cells are transferred to TC-treated plate.

[0235] Culture conditions for HSC/HSP can include expansion with a Notch agonist (see, e.g., US 7,399,633; US 5,780,300; US 5,648,464; US 5,849,869; and US 5,856,441 and growth factors present in the culture condition as follows: 25-300 ng/ml SCF, 25-300 ng/ml Flt-3L, 25-100 ng/ml TPO, 25-100 ng/ml IL-6 and 10 ng/ml IL-3. In more specific embodiments, 50, 100, or 200 ng/ml SCF; 50, 100, or 200 ng/ml of Flt-3L; 50 or 100 ng/ml TPO; 50 or 100 ng/ml IL-6; and 10 ng/ml IL-3 can be used.

[0236] In particular embodiments, culturing immune cells and/or stem cells includes placing immune cells and/or stem cells in a medium including potassium ion at a concentration higher than 40 mM. In particular embodiments, the potassium ion concentration is at least 40 mM, at least 45 mM, 50 mM, at least 55 mM, at least 60 mM, at least 65 mM, at least 70 mM, at least 75

mM, at least 80 mM, at least 85 mM, at least 90 mM, or at least 100mM.

[0237] In particular embodiments, the medium further includes one or more cytokines selected from IL-2, IL-21, IL-15, IL-7, or any combination thereof. In particular embodiments, the medium includes potassium ion at a concentration higher than 40 mM and IL-2. In particular embodiments, the medium includes potassium ion at a concentration higher than 40 mM, IL-2, IL-7, and IL-15. In particular embodiments, the medium includes potassium ion at a concentration higher than 40 mM, IL-7, and IL-21. In particular embodiments, the medium includes potassium ion at a concentration higher than 40 mM, IL-15, and IL-21. In particular embodiments, the IL-2 is at a concentration of 0.1 ng/mL to 20 ng/mL. In particular embodiments, the IL-21 is at a concentration of 0.1 ng/mL to 20 ng/mL. In particular embodiments, the IL-7 is at a concentration of 0.1 ng/mL to 20 ng/mL. In particular embodiments, the IL-15 is at a concentration of 0.1 ng/mL to 20 ng/mL.

[0238] In particular embodiments, the medium is hypotonic. In particular embodiments, the medium is isotonic. In particular embodiments, the medium includes a cell expansion agent. In particular embodiments, the cell expansion agent includes a GSK3B inhibitor, an ACLY inhibitor, a PI3K inhibitor (e.g., LY294002, pictilisib, CAL101, IC87114), an AKT inhibitor (e.g., MK2206, A443654, AKTi-VIII), or any combination thereof.

[0239] In particular embodiments, the medium includes a sodium ion. In particular embodiments, the sodium ion is at a concentration of 25 mM to 100mM. In particular embodiments, the medium includes glucose. In particular embodiments, the concentration of glucose is more than 10 mM. In particular embodiments, the medium includes calcium ion. In particular embodiments, the calcium ion concentration is more than 0.4 mM. Additional detailed methods for formulating the cell medium can be found in US Publication No. 2021/0332326.

[0240] (VI) *Ex Vivo* Manufactured Cell Formulations. In particular embodiments, genetically modified cells can be harvested from a culture medium and washed and concentrated into a carrier in a therapeutically-effective amount. Herein, cell formulations refers to the formulations including cells genetically modified to include a genetic construct (e.g., a genetic construct encoding a recombinant receptor, trafficking signal, and/or retention signal or a genetic construct resulting in down-regulated expression of a trafficking signal and/or retention signal) and prepared for administration. Exemplary carriers include saline, buffered saline, physiological saline, water, Hanks' solution, Ringer's solution, Normosol-R (Abbott Labs), PLASMA-LYTE A® (Baxter Laboratories, Inc., Morton Grove, IL), and combinations thereof.

[0241] In particular embodiments, carriers can be supplemented with human serum albumin (HSA) or other human serum components or fetal bovine serum. In particular embodiments, a carrier for infusion includes buffered saline with 5% HSA or dextrose. Additional isotonic agents

include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0242] Carriers can include buffering agents, such as citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0243] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which helps to prevent cell adherence to container walls. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as HSA, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran.

[0244] Where necessary or beneficial, cell formulations can include a local anesthetic such as lidocaine to ease pain at a site of injection.

[0245] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0246] In cell formulations disclosed herein, cells are generally in a volume of a liter or less, 500 ml or less, 250 ml or less or 100 ml or less. Hence the density of administered cells is typically greater than 10^4 cells/ml, 10^7 cells/ml or 10^8 cells/ml.

[0247] In particular embodiments, cell formulations can include one or more genetically modified cell types (e.g., modified T cells, NK cells, or stem cells). Cell formulations can include different types of genetically-modified cells (e.g., T cells, NK cells, and/or stem cells in combination).

[0248] Different types of genetically-modified cells or cell subsets (e.g., modified T cells, NK cells, and/or stem cells) can be provided in different ratios e.g., a 1:1:1 ratio, 2:1:1 ratio, 1:2:1 ratio, 1:1:2 ratio, 5:1:1 ratio, 1:5:1 ratio, 1:1:5 ratio, 10:1:1 ratio, 1:10:1 ratio, 1:1:10 ratio, 2:2:1 ratio, 1:2:2 ratio, 2:1:2 ratio, 5:5:1 ratio, 1:5:5 ratio, 5:1:5 ratio, 10:10:1 ratio, 1:10:10 ratio, 10:1:10 ratio, etc. These ratios can also apply to numbers of cells expressing the same or different genetic

construct components.

[0249] The cell formulations disclosed herein can be prepared for administration by, e.g., injection, infusion, perfusion, instillation, or lavage. The cell formulations can further be formulated for bone marrow, intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, intrathecal, intratumoral, intramuscular, intravesical, and/or subcutaneous injection. The cell formulation can further be formulated for physical locoregional delivery such as hepatic arterial infusion pump, isolated limb perfusion, instillation of cells into the abdominal cavity, or instillation of cells into the thoracic cavity.

[0250] (VII) Targeted Viral Vectors & Nanoparticles for *In Vivo* Cell Modification. Targeted viral vectors and/or nanoparticles can also be used to genetically-modify immune cells *in vivo* or *ex vivo*. Modifying formulations refer to targeted viral vectors and/or nanoparticles used to genetically modify immune cells to include a genetic construct disclosed herein within a pharmaceutically acceptable carrier. Viral vectors that can be used to deliver genes to cells are described elsewhere herein, and numerous targeted (e.g., pseudotyped) viral vectors and nanoparticles are known in the art.

[0251] Exemplary cell-targeted nanoparticles include a cell targeting ligand (e.g., that binds CD3, CD4, CD8, CD34) on the surface of the nanoparticle wherein the cell targeting ligand results in selective uptake of the nanoparticle by a selected cell type (e.g., by an immune cell). The nanoparticle then delivers gene modifying components that result in expression of the genetic construct or down-regulated expression of a targeted gene.

[0252] Exemplary nanoparticles include liposomes (microscopic vesicles including at least one concentric lipid bilayer surrounding an aqueous core), liposomal nanoparticles (a liposome structure used to encapsulate another smaller nanoparticle within its core); and lipid nanoparticles (liposome-like structures that lack the continuous lipid bilayer characteristic of liposomes). Other polymer-based nanoparticles can also be used as well as porous nanoparticles constructed from any material capable of forming a porous network. Exemplary materials include metals, transition metals and metalloids (e.g., lithium, magnesium, zinc, aluminum and silica).

[0253] Therapeutically effective amounts of vectors and/or nanoparticles within modifying formulations can range from 0.1 to 5 µg/kg or from 0.5 to 1 µg /kg. In other examples, a dose can include 1 µg /kg, 30 µg /kg, 90 µg/kg, 150 µg/kg, 500 µg/kg, 750 µg/kg, 0.1 to 5 mg/kg or from 0.5 to 1 mg/kg. In other examples, a dose can include 1 mg/kg, 10 mg/kg, 30 mg/kg, 50 mg/kg, 70 mg/kg, 100 mg/kg, 300 mg/kg, 500 mg/kg, 700 mg/kg, 1000 mg/kg or more.

[0254] (VIII) Methods of Use. Methods disclosed herein include treating subjects (humans, non-human primates, veterinary animals (dogs, cats, reptiles, birds, etc.) livestock (horses, cattle,

goats, pigs, chickens, etc.) and research animals (monkeys, rats, mice, fish, etc.)) with formulations disclosed herein (cell formulations and/or modifying formulations). Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic treatments.

[0255] An "effective amount" is the amount of a formulation necessary to result in a desired physiological change in the subject. For example, an effective amount can provide an immunogenic anti-cancer effect or anti-infection effect. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically significant effect in an animal model or *in vitro* assay relevant to the assessment of a cancer's or infection's development or progression. An immunogenic formulation can be provided in an effective amount, wherein the effective amount stimulates an immune response.

[0256] A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a cancer or infection or displays only early signs or symptoms of a cancer or infection such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the cancer or infection further. Thus, a prophylactic treatment functions as a preventative treatment against cancer or infection. In particular embodiments, prophylactic treatments reduce, delay, or prevent metastasis from a primary a cancer tumor site from occurring.

[0257] A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a cancer or infection and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the cancer or infection. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the cancer or infection and/or reduce control or eliminate side effects of the cancer or infection.

[0258] Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

[0259] In particular embodiments, therapeutically effective amounts provide anti-cancer effects. Anti-cancer effects include a decrease in the number of cancer cells, decrease in the number of metastases, a decrease in tumor volume, an increase in life expectancy, induced chemo- or radiosensitivity in cancer cells, inhibited angiogenesis near cancer cells, inhibited cancer cell proliferation, inhibited tumor growth, reduced metastases, prolonged subject life, reduced cancer-associated pain, and/or reduced relapse or re-occurrence of cancer following treatment.

[0260] A "tumor" is a swelling or lesion formed by an abnormal growth of cells (called neoplastic cells or tumor cells). A "tumor cell" is an abnormal cell that grows by a rapid, uncontrolled cellular

proliferation and continues to grow after the stimuli that initiated the new growth cease. Tumors show partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue, which may be benign, pre-malignant or malignant.

[0261] In particular embodiments, therapeutically effective amounts induce an immune response. The immune response can be against a target cell (e.g., cancer cell or infected cell (e.g., infected with bacteria, virus, fungi, parasite, or arthropod)).

[0262] "Target cell" refers to a cell that expresses a target antigen on its surface. In some embodiments, expression of an antigen on the cell surface is determined, for example, using antibodies to the target in a method such as immunohistochemistry, FACS, etc. Alternatively, target mRNA expression is considered to correlate to target expression on the cell surface and can be determined by, for example, *in situ* hybridization and/or RT-PCR (including quantitative RT-PCR).

[0263] In particular embodiments, therapeutically effective amounts can be used to direct cells expressing a genetic construct to a select tissue and not to non-selected tissues. Example uses of genetic constructs and/or genetically modified cells disclosed herein include cancer treatment, tissue engineering, and genetic engineering.

[0264] Examples of cancers that can be treated with genetic constructs disclosed herein include colorectal cancer, pancreatic cancer, breast cancer, colon cancer, gastric cancer, prostate cancer, hepatocellular carcinoma, ovarian cancer, endometrial cancer, uterine cancer, esophageal cancer, lung cancer, head and neck squamous cell cancer, cholangiocarcinoma, and bladder cancer. In particular embodiments, breast cancer includes triple-negative breast cancer (TNBC) and basal breast cancer. In particular embodiments, bladder cancer includes urothelial carcinoma, squamous cell carcinoma, and adenocarcinoma. In particular embodiments, genetic constructs disclosed herein are used to treat metastases of the cancers described above.

[0265] Particular embodiments include providing localized treatment to the liver to a subject having a primary gastric cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary colon cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary colorectal cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary breast cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary pancreatic cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary ovarian cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary endometrial cancer. Particular

embodiments include providing localized treatment to the liver to a subject having a primary uterine cancer. Particular embodiments include providing localized treatment to the liver to a subject having a primary hepatocellular carcinoma.

[0266] Particular embodiments include providing localized treatment to the bone to a subject having a primary breast cancer. Particular embodiments include providing localized treatment to the bone to a subject having a primary prostate cancer.

[0267] Particular embodiments include providing localized treatment to the pancreas to a subject having a primary pancreatic cancer.

[0268] In particular embodiments, a treatment of a cancer in a subject includes i) assessing the type of cancer and choosing a recombinant receptor binding domain that will bind a target antigen expressed on the cancer cell; ii) determining desired site of activity (e.g., target tissue) and choosing a trafficking signal corresponding to the desired site of activity; iii) determine if there is a site of on-target/off-tumor toxicity and choose a trafficking signal to knockout in order to avoid trafficking to that site; iv) determine if a retention signal is desired; v) prepare a genetic construct; and vi) deliver a therapeutically effective amount of the treatment to the subject. In particular embodiments, the genetic construct is used to genetically engineer cells *ex vivo* and then administer the cells to the subject. In particular embodiments, the genetic construct is associated with a nanoparticle and administered to the subject in order to genetically engineer cells *in vivo*.

[0269] In tissue engineering, genetically modified cells can be directed to a select tissue to increase the population of cells within a tissue. For example, fibroblasts can be directed to skin for wound healing, hepatocytes can be directed to the liver, or chondrocytes can be directed to cartilage. In particular embodiments, a stem cell can be directed to a particular tissue. In particular embodiments, the stem cell can include a genetic construct to direct the stem cell's differentiation or activity.

[0270] In genetic engineering, genetically modified cells can include a genetic construct encoding a therapeutic protein. In particular embodiments, genetically modified cells are directed to a select tissue where it can express the therapeutic protein. In particular embodiments, the therapeutic protein includes an enzyme, an antibody, a peptide, a growth factor, a transcription factor, or any other useful protein or molecule. For example, beta cells can be genetically engineered to go to and reside in the pancreas where they can produce insulin.

[0271] For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from *in vitro* assays and/or animal model studies. Such information can be used to more accurately determine useful doses in subjects of interest. The actual dose amount administered to a particular subject can be determined by a physician,

veterinarian or researcher taking into account parameters such as physical and physiological factors including target, body weight, severity of condition, type of cancer, stage of cancer, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

[0272] Therapeutically effective amounts of cell formulations can include 10^4 to 10^9 cells/kg body weight, or 10^3 to 10^{11} cells/kg body weight. Therapeutically effective amounts to administer can include greater than 10^2 cells, greater than 10^3 cells, greater than 10^4 cells, greater than 10^5 cells, greater than 10^6 cells, greater than 10^7 cells, greater than 10^8 cells, greater than 10^9 cells, greater than 10^{10} cells, or greater than 10^{11} .

[0273] Therapeutically effective amounts of modifying formulations can range from 0.1 to 5 $\mu\text{g/kg}$ or from 0.5 to 1 $\mu\text{g/kg}$. In other examples, a dose can include 1 $\mu\text{g/kg}$, 30 $\mu\text{g/kg}$, 90 $\mu\text{g/kg}$, 150 $\mu\text{g/kg}$, 500 $\mu\text{g/kg}$, 750 $\mu\text{g/kg}$, 0.1 to 5 mg/kg or from 0.5 to 1 mg/kg . In other examples, a dose can include 1 mg/kg , 10 mg/kg , 30 mg/kg , 50 mg/kg , 70 mg/kg , 100 mg/kg , 300 mg/kg , 500 mg/kg , 700 mg/kg , 1000 mg/kg or more.

[0274] Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (e.g., daily, every other day, every 3 days, every 4 days, every 5 days, every 6 days, weekly, every 2 weeks, every 3 weeks, monthly, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every 8 months, every 9 months, every 10 months, every 11 months or yearly). In particular embodiments, the treatment protocol may be dictated by a clinical trial protocol or an FDA-approved treatment protocol.

[0275] Therapeutically effective amounts can be administered by, e.g., injection, infusion, perfusion, instillation, or lavage. Routes of administration can include bolus intravenous, intradermal, intraarterial, intraparenteral, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesical, limb perfusion, intrathoracic, intraabdominal, and/or subcutaneous administration.

[0276] In certain embodiments, cell formulations and/or modifying formulations are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities. In particular embodiments, cells may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycoplienolic acid, steroids, FR901228, cytokines, and irradiation.

[0277] In certain embodiments, cell formulations and/or modifying formulations (or formulations)

may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents; alkyl sulfonates; aziridines; ethylenimines and methylamelamines; nitrogen mustards; nitrosureas; antibiotics; anti-metabolites; folic acid analogues; purine analogs; pyrimidine analogs; androgens; anti-adrenals; folic acid replenisher; platinum analogs; retinoic acid; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and anti-androgens and pharmaceutically acceptable salts, acids or derivatives of any of the above. Combinations of chemotherapeutic agents are also administered where appropriate, including, CHOP, i.e., Cyclophosphamide (Cytoxan®), Doxorubicin (hydroxydoxorubicin), Vincristine (Oncovin®), and Prednisone.

[0278] In some embodiments, the chemotherapeutic agent is administered at the same time or within one week after the administration of the cell formulation and/or modifying formulation. In other embodiments, the chemotherapeutic agent is administered from 1 to 4 weeks or from 1 week to 1 month, 1 week to 2 months, 1 week to 3 months, 1 week to 6 months, 1 week to 9 months, or 1 week to 12 months after the administration of the cell formulation and/or modifying formulation. In other embodiments, the chemotherapeutic agent is administered at least 1 month before administering the cell formulation and/or modifying formulation. In some embodiments, the methods further include administering two or more chemotherapeutic agents.

[0279] In additional embodiments, formulations can be administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDS) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

[0280] In certain embodiments, formulations described herein are administered in conjunction with a cytokine. "Cytokine" as used herein is meant to refer to proteins released by one cell population that act on another cell as intercellular mediators. Examples of cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor (HGF); fibroblast growth factor (FGF); prolactin; placental lactogen; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve

growth factors (NGFs) such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and - gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte- macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL- 1 alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

[0281] (IX) Kits. The current disclosure also includes kits. Kits can include various components to practice methods disclosed herein. For example, depending on the aspect of the methods practiced, kits could include one or more of nucleic acids encoding a recombinant receptor disclosed herein; nucleic acids encoding a CAR disclosed herein; nucleic acids encoding a trafficking signal (e.g., CXCR6, integrin $\beta 2$ (LFA-1), CCR1, CCR2, CXCR4, $\beta 7$ integrin, CCR4, CXCR3, CCR3) disclosed herein; nucleic acids encoding a retention signal (e.g., CD69, Runx3, Hobit, CD103) disclosed herein; nucleic acids resulting in down-regulated expression of a trafficking signal disclosed herein; nucleic acids resulting in down-regulated expression of a retention signal disclosed herein; a vector (e.g., lentiviral vector or retroviral vector) carrying a genetic construct; a nucleic acid encoding an scFv; a nucleic acid encoding a VL; a nucleic acid encoding a VH; a nucleic acid encoding a transmembrane domain; a nucleic acid encoding CD90.1; cells (e.g., immune cells, T-cells, CD4 T cells, CD8 T cells, B cells, natural killer (NK) cells, NK-T cells, monocytes/macrophages, lymphocytes, stem or progenitor cells, hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPC), adult cells, untransduced T cells, CAR T cells); cell lines (e.g., Kasumi2, LS174T, OT-I, KPC); tissue samples (e.g., colorectal, pancreatic, breast, colon, gastric, prostate, endometrial, uterine, ovaries, esophagus, bladder, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, lymph node, liver, lung, stomach, intestine, kidney, bone, cervix, testes, tonsil, or other organ, and/or cells derived therefrom); genetic expression components (e.g., genes for expression provided by vectors (e.g., lentiviral vector, retroviral vector), CRISPR components, ZFNs, TALENs, MegaTALs, targeted viral vectors and/or nanoparticles); cell formulation or activation components (e.g., saline, buffered saline, phosphate buffered saline (PBS); biocompatible buffers such as, $\text{Ca}^{++}/\text{Mg}^{++}$ free PBS; physiological saline, water, Hanks' solution, Ringer's solution, T cell stimulating epitopes (e.g., anti-CD3/anti-CD28 conjugated beads; OKT3, TGN1412), culture-initiating compositions, RPMI, non-essential amino acids, sodium pyruvate,

penicillin/streptomycin, non-dividing EBV-transformed lymphoblastoid cells (LCL), human serum albumin (HSA) or other human serum components or fetal bovine serum, dextrose, stabilizers, preservatives); combination therapy components (e.g., local anesthetics, chemotherapeutic agents, immunosuppressive agents, anti-inflammatory agents); an antibody tagged with a fluorescent molecule; animal models (C57B6 mice or transgenic mice); PCR amplification sequences; cytokines (e.g., IL-2, IL-7, IL-15, IL-21); culture vessels; reference levels; GAPDH; enzyme-linked immunosorbent assay (ELISA); culture plates; administration components (e.g., syringes, catheters, tubing, needles); etc.

[0282] The Exemplary Embodiments and Example below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

[0283] (X) Exemplary Embodiments.

1. A method of treating a subject in need thereof including
identifying a subject with a cancer; and
administering to the subject a therapeutically effective amount of a cell genetically modified to express
 - i) a chimeric antigen receptor including a binding domain that binds a target antigen of the cancer; and
 - ii) a (a) trafficking signal that directs the cell to a treatment location,
(b) a retention signal that maintains the cell within the treatment location, and/or
(c) an inhibitory signal that reduces migration of the cell to an off-target location;
thereby treating the subject in need thereof.
2. The method of embodiment 1, wherein the cancer is a primary cancer that has metastasized to a second location; and
the cell is genetically modified to express
 - i) a chimeric antigen receptor including a binding domain that binds a target antigen of the primary cancer; and
 - ii) a (a) trafficking signal that directs the cell to the second location,
(b) a retention signal that maintains the cell within the second location, and/or
(c) an inhibitory signal that reduces migration of the cell to an off-target location;
thereby treating the subject in need thereof.
3. The method of embodiment 2, wherein
the primary cancer is colorectal cancer, the second location is liver, the target antigen

- is CEA, the trafficking signal includes CXC motif chemokine receptor 6 (CXCR6), and the retention signal includes CD69.
4. The method of embodiments 2 or 3, wherein
- the primary cancer is colorectal cancer, the second location is liver, the target antigen is CEA, and the trafficking signal includes CXCR6;
 - the primary cancer is breast, colon, or gastric cancer; the second location is liver, the target antigen is HER2, and the trafficking signal CXCR6;
 - the primary cancer is breast cancer, the second location is bone, the target antigen is HER2, and the trafficking signal includes CXCR4;
 - the primary cancer is prostate cancer, the second location is bone, the target antigen is PSA, and the trafficking signal includes CXCR4;
 - the primary cancer is pancreatic cancer, the second location is liver, the target antigen is mesothelin or CA19-9, and the trafficking signal includes CXCR6;
 - the primary cancer is ovarian or endometrial cancer, the second location is liver, the target antigen is FOLR1, and the trafficking signal includes CXCR6; or
 - the primary cancer is breast, uterine, ovarian or endometrial cancer; the second location is liver, the target antigen is MUC1, and the trafficking signal includes CXCR6.
5. The method of any of embodiments 2-4, wherein the retention signal includes CD69.
6. The method of any of embodiments 2-5, wherein the off-target location is small intestine or colon.
7. The method of embodiment 6, wherein the inhibitory signal includes knockout of $\beta 7$ integrin.
8. The method of any of embodiments 2-5, wherein the off-target location is prostate gland.
9. The method of embodiment 8, wherein the inhibitory signal includes knockout of CXCR3.
10. The method of any of embodiments 2-5, wherein the off-target location is lung.
11. The method of embodiment 10, wherein the inhibitory signal includes knockout of CCR4, CXCR3, and CCR3.
12. The method of any of embodiments 2-5, wherein the off-target location is lung, kidney, or stomach.
13. The method of embodiment 12, wherein the inhibitory signal includes knockout of $\beta 7$ integrin, CCR4, CXCR3, and CCR3.
14. The method of any of embodiments 2-13, further including surgically removing the primary cancer.
15. The method of any of embodiments 1-14, wherein the cell is genetically modified to express
- i) a chimeric antigen receptor including a binding domain that binds a target

- antigen of the cancer; and
- ii) a (a) trafficking signal that directs the cell to an organ in which the cancer is located,
(b) a retention signal that maintains the cell within organ in which the cancer is located, and/or
(c) an inhibitory signal that reduces migration of the cell to an off-target location;
thereby treating the subject in need thereof.
16. The method of any of embodiments 1-15, wherein the cancer is a primary cancer that has metastasized to a second location; and
the cell is genetically modified to express
- i) a chimeric antigen receptor including a binding domain that binds a target antigen of the primary cancer; and
- ii) a (a) trafficking signal that directs the cell to the organ of the primary cancer location,
(b) a retention signal that maintains the cell within the primary cancer location, and/or
(c) an inhibitory signal that reduces migration of the cell to the second location and/or an off-target location;
thereby treating the subject in need thereof.
17. The method of embodiments 15 or 16, wherein the off-target location is an organ, gland, tissue, or bone.
18. A cell genetically modified to achieve at least two of:
expression of a recombinant receptor,
modulation of a trafficking signal, or
modulation of a retention signal.
19. The cell of embodiment 18, wherein the trafficking signal includes CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
20. The cell of embodiments 18 or 19, wherein modulation of the trafficking signal includes up- or down-regulating expression of CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
21. The cell of any of embodiments 18-20, genetically modified to express CXCR6, integrin β 2, CCR1, CCR2, or CXCR4.
22. The cell of any of embodiments 18-21, genetically modified to express CXCR6.
23. The cell of any of embodiments 18-21, genetically modified to express integrin β 2.

24. The cell of any of embodiments 18-21, genetically modified to express CCR1.
25. The cell of any of embodiments 18-21, genetically modified to express CCR2.
26. The cell of any of embodiments 18-21, genetically modified to express CXCR4.
27. The cell of any of embodiments 18-20, genetically modified to down-regulate expression of $\beta 7$ integrin, CCR4, CXCR3, or CCR3.
28. The cell of any of embodiments 18-27, genetically modified to down-regulate expression of $\beta 7$ integrin.
29. The cell of any of embodiments 18-27, genetically modified to down-regulate expression of CXCR3.
30. The cell of any of embodiments 18-27, genetically modified to down-regulate expression of CCR4.
31. The cell of any of embodiments 18-27, genetically modified to down-regulate expression of CXCR3.
32. The cell of any of embodiments 27-31, wherein down-regulated expression is by administration of a guide RNA (gRNA) and a nuclease to the cell.
33. The cell of embodiment 32, wherein the gRNA includes a sequence as set forth in one of SEQ ID NOs: 77-103.
34. The cell of embodiment 33, wherein the gRNA includes single guide RNA (sgRNA).
35. The cell of embodiment 34, wherein the sgRNA targeting $\beta 7$ integrin includes a sequence as set forth in SEQ ID Nos: 245-247.
36. The cell of embodiments 34 or 35, wherein the sgRNA targeting CXCR3 includes a sequence as set forth in SEQ ID Nos: 242-244.
37. The cell of any of embodiments 32-36, wherein the nuclease includes Cas9 or Cpf1.
38. The cell of any of embodiments 18-37, wherein the retention signal includes CD69, Runx3, Hobit, or CD103.
39. The cell of any of embodiments 18-38, wherein modulation of the retention signal includes up- or down regulating expression of CD69, Runx3, Hobit, or CD103.
40. The cell of any of embodiments 18-39, genetically modified to express CD69, Runx3, Hobit, or CD103.
41. The cell of any of embodiments 18-40, genetically modified to express CD69.
42. The cell of any of embodiments 18-41, genetically modified to down-regulate expression of CD69, Runx3, Hobit, or CD103.
43. The cell of embodiment 42, wherein the down-regulated expression is by administration of a gRNA and a nuclease to the cell.

44. The cell of embodiment 43, wherein the gRNA includes a sequence as set forth in one of SEQ ID NOs: 107-121 or 192-194.
45. The cell of embodiments 43 or 44, wherein the nuclease includes Cas9 or Cpf1.
46. The cell of any of embodiments 18-45, genetically modified to express the recombinant receptor.
47. The cell of any of embodiments 18-46, wherein the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a CAR/TCR hybrid.
48. The cell of any of embodiments 18-47, wherein the recombinant receptor includes i) an extracellular component including a binding domain that binds an antigen expressed on a surface of targeted cells; and ii) an intracellular component.
49. The cell of embodiment 48, wherein the targeted cells include cancer cells or infected cells.
50. The cell of embodiments 48 or 49, wherein the targeted cells include cancer cells.
51. The cell of any of embodiments 48-50, wherein the antigen includes a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9).
52. The cell of any of embodiments 48-51, wherein the antigen includes a CEA.
53. The cell of any of embodiments 48-52, wherein the binding domain includes a binding domain of an anti-CEA antibody.
54. The cell of embodiment 53, wherein the anti-CEA antibody includes AHB7.
55. The cell of embodiments 53 or 54, wherein the anti-CEA antibody includes a variable light chain including a complementarity determining region (CDR)L1 as set forth in SEQ ID NO: 207, a CDRL2 as set forth in SEQ ID NO: 208, and a CDRL3 as set forth in SEQ ID NO: 209, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 210, a CDRH2 as set forth in SEQ ID NO: 211, and a CDRH3 as set forth in SEQ ID NO: 212.
56. The cell of any of embodiments 53-55, wherein the anti-CEA antibody includes a variable light chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 205.
57. The cell of any of embodiments 53-56, wherein the anti-CEA antibody includes a variable light chain having the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 205.
58. The cell of any of embodiments 53-57, wherein the anti-CEA antibody includes a variable light

chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 63 and a variable heavy chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 64.

59. The cell of any of embodiments 53-58, wherein the anti-CEA antibody includes a variable light chain encoded by the sequence as set forth in SEQ ID NO: 63 and a variable heavy chain encoded by the sequence as set forth in SEQ ID NO: 64.
60. The cell of any of embodiments 55-59, wherein the anti-CEA antibody includes a linker between the variable light chain and variable heavy chain.
61. The cell of embodiment 60, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 65.
62. The cell of embodiment 48, wherein the antigen includes EpCAM.
63. The cell of embodiments 48 or 62, wherein the binding domain includes a binding domain of an anti-EpCAM antibody.
64. The cell of embodiment 63, wherein the anti-EpCAM antibody includes G8.8, ab71916, AUA-1, Ber-EP4, HEA125, EpMab-16, MOC31, or VU-1D9.
65. The cell of any of embodiments 63 or 64, wherein the anti-EpCAM antibody includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 127, a CDRL2 as set forth in SEQ ID NO: 128, and a CDRL3 as set forth in SEQ ID NO: 129, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 124, a CDRH2 as set forth in SEQ ID NO: 125, and a CDRH3 as set forth in SEQ ID NO: 126.
66. The cell of any of embodiments 63-65, wherein the anti-EpCAM antibody includes a variable light chain including a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain including a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 196.
67. The cell of any of embodiments 63-66, wherein the anti-EpCAM antibody includes a variable light chain having the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 196.
68. The cell of any of embodiments 63-67, wherein the anti-EpCAM antibody includes a linker between the variable light chain and variable heavy chain.
69. The cell of embodiment 68, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 197.
70. The cell of any of embodiments 48-69, wherein the intracellular component includes a CD3ζ signaling domain, a 4-1BB signaling domain, and/or a CD28 signaling domain.
71. The cell of any of embodiments 48-70, wherein the intracellular component includes a CD3ζ

signaling and a CD28 signaling domain.

72. The cell of any of embodiments 48-71, wherein the intracellular component is linked to the extracellular component through a transmembrane domain.
73. The cell of embodiment 72, wherein the transmembrane domain includes a CD28 transmembrane domain.
74. The cell of any of embodiments 18-73, wherein the cell is an autologous cell or an allogeneic cell in reference to a subject.
75. The cell of any of embodiments 18-74, wherein the cell is *in vivo* or *ex vivo*.
76. The cell of any of embodiments 18-75, wherein the cell is an immune cell, a stem or progenitor cell, or an adult cell.
77. The cell of embodiment 76, wherein the immune cell is a lymphocyte.
78. The cell of embodiment 77, wherein the lymphocyte includes a T cell, B cell, natural killer (NK) cell, or NK-T cell.
79. The cell of any of embodiments 76-78, wherein the immune cell is a T cell selected from a CD3⁺ T cell, a CD4⁺ T cell, a CD8⁺ T cell, a central memory T cell, an effector memory T cell, and/or a naïve T cell.
80. The cell of any of embodiments 76-79, wherein the immune cell is a CD8⁺ T cell.
81. The cell of any of embodiments 76-79, wherein the immune cell is a CD4⁺ T cell.
82. The cell of embodiment 76, wherein the stem or progenitor cell includes a hematopoietic stem cell (HSC), a hematopoietic progenitor cell (HPC), a pluripotent stem cell, a mesenchymal stem cell, a neural stem cell, or an endothelial stem cell.
83. The cell of embodiment 76, wherein the adult cell includes a fibroblast, a hepatocyte, a myoblast, a neuron, an osteoblast, an osteoclast, a kidney cell, a chondrocyte, or an adipocyte.
84. The cell of any of embodiments 18-83, wherein the cell expresses an anti-CEA CAR, CXCR6, and CD69.
85. The cell of embodiment 84, wherein the cell expresses the anti-CEA CAR, CXCR6, and CD69 by administration of a sequence as set forth in SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, or SEQ ID NO: 62.
86. The cell of any of embodiments 18-83, wherein the cell expresses an anti-EpCAM CAR, CXCR6, and CD69.
87. The cell of any of embodiments 86, wherein the cell expresses the anti-EpCAM CAR, CXCR6, and CD69 by administration of a sequence as set forth in SEQ ID NO: 203.
88. The cell of any of embodiments 18-83, wherein the cell expresses anti-CEA CAR and down-

- regulates expression of CXCR3.
89. The cell of embodiment 88, wherein the cell expresses the anti-CEA CAR and down-regulates expression of CXCR3 by administration of an sgRNA sequence as set forth in SEQ ID NOs: 242-244 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 201.
90. The cell of any of embodiments 18-83, wherein the cell expresses anti-CEA CAR and down-regulates expression of $\beta 7$ integrin.
91. The cell of embodiment 90, wherein the cell expresses the anti-CEA CAR and down-regulates expression of $\beta 7$ integrin by administration of an sgRNA sequence as set forth in SEQ ID NOs: 245-247 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 201.
92. The cell of any of embodiments 18-83, wherein the cell expresses anti-EpCAM CAR and down-regulates expression of CXCR3.
93. The cell of embodiment 92, wherein the cell expresses the anti-EpCAM CAR and down-regulates expression of CXCR3 by administration of an sgRNA sequence as set forth in SEQ ID NOs: 242-244 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 202.
94. The cell of any of embodiments 18-83, wherein the cell expresses anti-EpCAM CAR and down-regulates expression of $\beta 7$ integrin.
95. The cell of embodiment 94, wherein the cell expresses the anti-EpCAM CAR and down-regulates expression of $\beta 7$ integrin by administration of an sgRNA sequence as set forth in SEQ ID NOs: 245-247 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 202.
96. A genetic construct including at least two of:
a sequence encoding a recombinant receptor;
a sequence encoding a trafficking signal or a sequence down-regulating a trafficking signal;
or
a sequence encoding a retention signal or a sequence down-regulating a retention signal.
97. The genetic construct of embodiment 96, wherein the trafficking signal includes CXCR6, integrin $\beta 2$, CCR1, CCR2, CXCR4, $\beta 7$ integrin, CCR4, CXCR3, or CCR3.
98. The genetic construct of embodiments 96 or 97, wherein the genetic construct includes a sequence encoding CXCR6, integrin $\beta 2$, CCR1, CCR2, or CXCR4.
99. The genetic construct of any of embodiments 96-98, wherein the genetic construct includes a sequence down-regulating expression of $\beta 7$ integrin, CCR4, CXCR3, or CCR3.
100. The genetic construct of any of embodiments 96-99, wherein the genetic construct

includes or encodes a trafficking signal gRNA and nuclease.

101. The genetic construct of embodiment 100, wherein the trafficking signal gRNA includes a sequence as set forth in one of SEQ ID NOs: 77-103.
102. The genetic construct of embodiments 100 or 101, wherein the trafficking signal gRNA includes a trafficking signal single guide RNA (sgRNA).
103. The genetic construct of embodiment 102, wherein the trafficking signal sgRNA includes an sgRNA targeting $\beta 7$ integrin.
104. The genetic construct of embodiment 103, wherein the sgRNA targeting $\beta 7$ integrin includes a sequence as set forth in SEQ ID Nos: 245-247.
105. The genetic construct of embodiment 102, wherein the trafficking signal sgRNA includes an sgRNA targeting CXCR3.
106. The genetic construct of embodiment 105, wherein the sgRNA targeting CXCR3 includes a sequence as set forth in SEQ ID Nos: 242-244.
107. The genetic construct of any of embodiments 100-106, wherein the nuclease includes Cas9 or Cpf1.
108. The genetic construct of any of embodiments 96-107, wherein the retention signal includes CD69, Runx3, Hobit, or CD103.
109. The genetic construct of any of embodiments 96-108, wherein the genetic construct includes or encodes a retention signal gRNA and nuclease.
110. The genetic construct of embodiment 109, wherein the retention signal gRNA includes a sequence as set forth in one of SEQ ID NOs: 107-121 or 192-194.
111. The genetic construct of embodiments 109 or 110, wherein the nuclease includes Cas9 or Cpf1.
112. The genetic construct of any of embodiments 96-111, wherein the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a CAR/TCR hybrid.
113. The genetic construct of any of embodiments 96-112, wherein the recombinant receptor includes i) an extracellular component including a binding domain that binds a target antigen, wherein the target antigen is expressed on a surface of targeted cells; and ii) an intracellular component.
114. The genetic construct of embodiment 113, wherein the targeted cells include cancer cells or infected cells.
115. The genetic construct of embodiments 113 or 114, wherein the targeted cells include cancer cells.

116. The genetic construct of any of embodiments 113-115, wherein the target antigen includes a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9).
117. The genetic construct of any of embodiments 113-116, wherein the target antigen includes CEA.
118. The genetic construct of any of embodiments 113-117, wherein the binding domain includes a binding domain of an anti-CEA antibody.
119. The genetic construct of embodiment 118, wherein the anti-CEA antibody includes AHB7.
120. The genetic construct of embodiments 118 or 119, wherein the anti-CEA antibody includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 207, a CDRL2 as set forth in SEQ ID NO: 208, and a CDRL3 as set forth in SEQ ID NO: 209, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 210, a CDRH2 as set forth in SEQ ID NO: 211, and a CDRH3 as set forth in SEQ ID NO: 212.
121. The genetic construct of any of embodiments 118-120, wherein the anti-CEA antibody includes a variable light chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 205.
122. The genetic construct of any of embodiments 118-121, wherein the anti-CEA antibody includes a variable light chain having the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 205.
123. The genetic construct of any of embodiments 118-122, wherein the anti-CEA antibody includes a variable light chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 63 and a variable heavy chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 64.
124. The genetic construct of any of embodiments 118-123, wherein the anti-CEA antibody includes a variable light chain encoded by the sequence as set forth in SEQ ID NO: 63 and variable heavy chain encoded by the sequence as set forth in SEQ ID NO: 64.
125. The genetic construct of any of embodiments 121-124, wherein the anti-CEA antibody includes a linker between the variable light chain and variable heavy chain.
126. The genetic construct of embodiment 125, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 65.

127. The genetic construct of any of embodiments 113-117, wherein the target antigen includes EpCAM.
128. The genetic construct of any of embodiments 113-117 or 127, wherein the binding domain includes a binding domain of an anti-EpCAM antibody.
129. The cell of embodiment 128, wherein the anti-EpCAM antibody includes G8.8, ab71916, AUA-1, Ber-EP4, HEA125, EpMab-16, MOC31, or VU-1D9.
130. The genetic construct of embodiments 128 or 129, wherein the anti-EpCAM antibody includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 127, a CDRL2 as set forth in SEQ ID NO: 128, and a CDRL3 as set forth in SEQ ID NO: 129, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 124, a CDRH2 as set forth in SEQ ID NO: 125, and a CDRH3 as set forth in SEQ ID NO: 126.
131. The genetic construct of any of embodiments 128-130, wherein the anti-EpCAM antibody includes a variable light chain including a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain including a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 196.
132. The genetic construct of any of embodiments 128-131, wherein the anti-EpCAM antibody includes a variable light chain having the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 196.
133. The genetic construct of any of embodiments 128-132, wherein the anti-EpCAM antibody includes a linker between the variable light chain and variable heavy chain.
134. The genetic construct of embodiment 133, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 197.
135. The genetic construct of any of embodiments 113-134, wherein the intracellular component includes a CD3 ζ signaling domain, a 4-1BB signaling domain and/or a CD28 signaling domain.
136. The genetic construct of any of embodiments 113-135, wherein the intracellular component includes a CD3 ζ signaling domain and a CD28 signaling domain.
137. The genetic construct of any of embodiments 113-136, wherein the intracellular component is linked to the extracellular component through a transmembrane domain.
138. The genetic construct of embodiment 137, wherein the transmembrane domain includes a CD28 transmembrane domain.
139. The genetic construct of embodiment 96-138, further including a control feature.
140. The genetic construct of embodiment 139, wherein the control feature includes a transduction marker.

141. The genetic construct of embodiment 140, wherein the transduction marker includes CD90.1, Her2tG, epidermal growth factor receptor (EGFRt), or tCD19.
142. The genetic construct of any of embodiments 139-141, wherein the control feature includes a selection cassette.
143. The genetic construct of any of embodiments 139-142, wherein the control feature includes a tag cassette.
144. The genetic construct of embodiment 143, wherein the tag cassette includes a sequence as set forth in SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, or SEQ ID NO: 190.
145. The genetic construct of any of embodiments 96-144, further including a skip sequence.
146. The genetic construct of embodiment 145, wherein the skip sequence includes a 2A skip sequence.
147. The genetic construct of embodiment 146, wherein the 2A skip sequence encodes a T2A, P2A, E2A, or F2A self-cleaving polypeptide.
148. The genetic construct of any of embodiments 96-147, further including a suicide gene.
149. The genetic construct of any of embodiments 96-148, including a sequence as set forth in SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 1, and/or SEQ ID NO: 4 or a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 1, and/or SEQ ID NO: 4.
150. The genetic construct of embodiment 149, wherein the genetic construct includes from 5' to 3': a sequence encoding a trafficking signal; a first skip sequence; a sequence encoding a retention signal; a second skip sequence; and a sequence encoding a recombinant receptor.
151. The genetic construct of any of embodiments 96-150, wherein the genetic construct includes from 5' to 3': a sequence encoding a trafficking signal; a first skip sequence; a sequence encoding a retention signal; a second skip sequence; a sequence encoding a recombinant receptor; a third skip sequence; and a transduction marker.
152. The genetic construct of any of embodiments 96-150, wherein the genetic construct includes from 5' to 3': a sequence downregulating a trafficking signal; a first skip sequence; and a sequence encoding a recombinant receptor.
153. The genetic construct of any of embodiments 96-150, wherein the genetic construct includes from 5' to 3': a sequence downregulating a trafficking signal; a first skip sequence; a

sequence encoding a recombinant receptor; a second skip sequence; and a transduction marker.

154. The genetic construct of any of embodiments 96-153, wherein the genetic construct includes the sequence as set forth in SEQ ID NOs: 59, 60, 61, or 62 or a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NOs: 59, 60, 61, or 62.
155. The genetic construct of any of embodiments 96-154, wherein the genetic construct includes the sequence as set forth in SEQ ID NO: 203 or a sequence having at least 90% sequence identity to a sequence as set forth in SEQ ID NOs: 203.
156. A vector including the genetic construct of any of embodiments 96-155.
157. The vector of embodiment 156, wherein the vector is a viral vector.
158. The vector of embodiment 157, wherein the viral vector includes a retroviral vector or a lentiviral vector.
159. A nanoparticle encapsulating the genetic construct of any of embodiments 96-155.
160. A population of cells genetically modified to include the genetic construct of any of embodiments 96-155 or the vector any of embodiments 156-158.
161. A formulation including (i) the population of cells of embodiment 160 or a cell of any of embodiments 18-95 and (ii) a pharmaceutically acceptable carrier.
162. A method of treating a subject in need thereof including administering a therapeutically effective amount of the formulation of embodiment 161 or nanoparticle of embodiment 159 to the subject, thereby treating the subject in need thereof.
163. The method of embodiment 162, wherein the administering a therapeutically effective amount includes physical locoregional delivery.
164. The method of embodiment 162, wherein the administering a therapeutically effective amount includes systemic delivery.
165. The method of any of embodiments 162-164, wherein the administering a therapeutically effective amount includes administering intravenously, intravesically, intradermally, intraarterially, intraparenterally, intranodally, intralymphatically, intraperitoneally, intralesionally, intraprostatically, intravaginally, intrarectally, topically, intrathecally, intratumorally, intramuscularly, or subcutaneously.
166. The method of any of embodiments 162-165, wherein the administering a therapeutically effective amount includes hepatic arterial infusion, isolated limb perfusion, instillation within an abdominal cavity, or instillation within a thoracic cavity.
167. A method of treating a cancer within a subject in need thereof including administering a therapeutically effective amount of a formulation to the subject, wherein the

formulation includes cells genetically modified to

- i) express a recombinant receptor including a binding domain that binds a target antigen, and
- ii) direct location of the cell to a target tissue and/or away from a non-targeted tissue;

thereby treating the subject in need thereof.

168. The method of embodiment 167, wherein the subject has colorectal cancer and the target antigen is CEA.

169. The method of embodiments 167 or 168, wherein the target tissue is a liver and the cell expresses CXCR6.

170. The method of any of embodiments 167-169, wherein the non-targeted tissue is a colon and the cell has down-regulated expression of $\beta 7$ integrin.

171. The method of embodiment 167, wherein the subject has pancreatic cancer and the target antigen is CEA, mesothelin, or CA19-9.

172. The method of embodiments 167 or 171, wherein the target tissue is a liver and the cell expresses CXCR6.

173. The method of embodiments 167 or 171, wherein the target tissue is a pancreas and the cell expresses integrin $\beta 2$, CCR1, or CCR2.

174. The method of any of embodiments 167 -173, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.

175. The method of any of embodiments 167 -173, wherein the non-targeted tissue is a small intestine or colon and the cell has down-regulated expression of $\beta 7$ integrin.

176. The method of embodiment 167, wherein the subject has breast cancer and the target antigen is HER2 or MUC1.

177. The method of embodiments 167 or 176, wherein the target tissue is a liver and the cell expresses CXCR6.

178. The method of embodiments 167 or 176, wherein the target tissue is a bone and the cell expresses CXCR4.

179. The method of any of embodiments 167-178, wherein the non-targeted tissue is a stomach, lung, or kidney and the cell has down-regulated expression of CCR4, CXCR3, CCR3, or $\beta 7$ integrin.

180. The method of any of embodiments 167 -178, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.

181. The method of embodiment 167, wherein the subject has colon cancer or gastric cancer

and the target antigen is HER2.

182. The method of embodiments 167 or 181, wherein the target tissue is a liver and the cell expresses CXCR6.
183. The method of any of embodiments 167-182, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.
184. The method of embodiment 167, wherein the subject has prostate cancer and the target antigen is PEA.
185. The method of embodiments 167 or 184, wherein the target tissue is a bone and the cell expresses CXCR4.
186. The method of any of embodiments 167-185, wherein the non-targeted tissue is a prostate and the cell has down-regulated expression of CXCR3.
187. The method of embodiment 167, wherein the subject has hepatocellular carcinoma and the target antigen is GPC3.
188. The method of embodiments 167 or 187, wherein the target tissue is a liver and the cell expresses CXCR6.
189. The method of any of embodiments 167-188, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.
190. The method of embodiment 167, wherein the subject has ovarian cancer or endometrial cancer and the target antigen is FOLR1 or MUC1.
191. The method of embodiments 167 or 190, wherein the target tissue is a liver and the cell expresses CXCR6.
192. The method of any of embodiments 167-191, wherein the non-targeted tissue is a stomach, lung or kidney and the cell has down-regulated expression of CCR4, CXCR3, CCR3, or $\beta 7$ integrin.
193. The method of embodiment 167, wherein the subject has uterine cancer and the target antigen is MUC1.
194. The method of embodiments 167 or 193, wherein the target tissue is a liver and the cell expresses CXCR6.
195. The method of any of embodiments 167-194, wherein the non-targeted tissue is a stomach, lung, or kidney and the cell has down-regulated expression of CCR4, CXCR3, CCR3, or $\beta 7$ integrin.
196. The method of any of embodiments 167-195, wherein the cell expresses CD69, Runx3, Hobit, or CD103.
197. The method of any of embodiments 167-196, wherein the cell expresses CD69.

198. The method of any of embodiments 167-195, wherein the cell has down-regulated expression of CD69, Runx3, Hobit, or CD103.
199. A kit including a genetic construct of any of embodiments 97-155, wherein the genetic construct results in:
- expression of a binding domain that binds a target antigen,
 - modulated expression of a trafficking signal, and/or
 - modulated expression of a retention signal.
200. The kit of embodiment 199, wherein the modulated expression includes up- or down-regulated expression.
201. The kit of embodiments 199 or 200, wherein the trafficking signal includes CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
202. The kit of any of embodiments 199-201, wherein the genetic construct includes sequences encoding CXCR6, integrin β 2, CCR1, CCR2, or CXCR4.
203. The kit of any of embodiments 199-201, wherein the genetic construct includes a sequence down-regulating the expression of β 7 integrin, CCR4, CXCR3, or CCR3.
204. The kit of any of embodiments 199-203, wherein the genetic construct includes a trafficking signal gRNA.
205. The kit of embodiment 204, wherein the trafficking signal gRNA includes a sequence as set forth in one of SEQ ID NOs: 77-103.
206. The kit of any of embodiments 199-205, wherein the retention signal includes CD69, Runx3, Hobit, or CD103.
207. The kit of any of embodiments 199-206, wherein the genetic construct includes a sequence encoding CD69, Runx3, Hobit, or CD103.
208. The kit of any of embodiments 199-207, wherein the genetic construct includes a sequence encoding CD69.
209. The kit of any of embodiments 199-206, wherein the genetic construct includes a sequence down-regulating the expression of CD69, Runx3, Hobit, or CD103.
210. The kit of any of embodiments 199-209, wherein the genetic construct includes a retention signal gRNA.
211. The kit of embodiment 210, wherein the retention signal gRNA includes a sequence as set forth in one of SEQ ID NOs: 107-121 or 192-194.
212. The kit of any of embodiments 209-211, further including a nuclease or sequence encoding a nuclease.
213. The kit of embodiment 212, wherein the nuclease includes Cas9 or Cpf1.

214. The kit of any of embodiments 199-213, wherein the binding domain is part of a recombinant receptor.
215. The kit of embodiment 214, wherein the recombinant receptor includes a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a CAR/TCR hybrid.
216. The kit of embodiment 215, wherein the CAR includes an extracellular component including the binding domain; an intracellular component; and a transmembrane domain linking the extracellular component to the intracellular component.
217. The kit of embodiment 216, wherein the intracellular component includes a CD3 ζ signaling domain, a 4-1BB signaling domain, and/or a CD28 signaling domain.
218. The kit of embodiments 216 or 217, wherein the intracellular component includes a CD3 ζ signaling domain and a CD28 signaling domain.
219. The kit of any of embodiments 216-218, wherein the transmembrane domain includes a CD28 transmembrane domain.
220. The kit of any of embodiments 199-219, wherein the genetic construct further encodes a control feature.
221. The kit of embodiment 220, wherein the control feature includes a transduction marker.
222. The kit of embodiment 221, wherein the transduction marker includes CD90.1, Her2tG, epidermal growth factor receptor (EGFRt), or tCD19.
223. The kit of any of embodiments 220-222, wherein the control feature includes a selection cassette.
224. The kit of any of embodiments 220-223, wherein the control feature includes a tag cassette.
225. The kit of embodiment 224, wherein the tag cassette includes a sequence as set forth in SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, or SEQ ID NO: 190.
226. The kit of any of embodiments 199-225, wherein the genetic construct further includes a skip sequence.
227. The kit of embodiment 226, wherein the skip sequence includes a 2A skip sequence.
228. The kit of embodiment 227, wherein the 2A skip sequence encodes a T2A, P2A, E2A, or F2A self-cleaving polypeptide.
229. The kit of any of embodiments 199-228, wherein the target antigen includes a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3

(GPC3), a mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9).

230. The kit of any of embodiments 199-229, wherein the target antigen includes CEA and the binding domain binds CEA.
231. The kit of embodiment 230, wherein the binding domain that binds CEA includes an anti-CEA antibody.
232. The kit of embodiment 231, wherein the anti-CEA antibody includes AHB7.
233. The kit of embodiment 232, wherein the anti-CEA antibody includes a variable light chain including a complementarity determining region (CDRL)1 as set forth in SEQ ID NO: 207, a CDRL2 as set forth in SEQ ID NO: 208, and a CDRL3 as set forth in SEQ ID NO: 209, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 210, a CDRH2 as set forth in SEQ ID NO: 211, and a CDRH3 as set forth in SEQ ID NO: 212.
234. The kit of any of embodiments 199-229, wherein the target antigen includes EpCAM and the binding domain binds EpCAM.
235. The kit of embodiment 234, wherein the binding domain that binds EpCAM includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 127, a CDRL2 as set forth in SEQ ID NO: 128, and a CDRL3 as set forth in SEQ ID NO: 129, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 124, a CDRH2 as set forth in SEQ ID NO: 125, and a CDRH3 as set forth in SEQ ID NO: 126.
236. The kit of any of embodiments 199-229, wherein the target antigen includes HER2 and the binding domain binds HER2.
237. The kit of embodiment 236, wherein the binding domain that binds HER2 includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 218, a CDRL2 as set forth in SEQ ID NO: 219, and a CDRL3 as set forth in SEQ ID NO: 220, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 221, a CDRH2 as set forth in SEQ ID NO: 222, and a CDRH3 as set forth in SEQ ID NO: 223.
238. The kit of any of embodiments 199-229, wherein the target antigen includes PSA and the binding domain binds PSA.
239. The kit of embodiment 238, wherein the binding domain that binds PSA includes a variable light chain including a CDRL1 including the sequence as set forth in SEQ ID NO: 134, a CDRL2 including the sequence as set forth in SEQ ID NO: 135, and a CDRL3 including the sequence as set forth in SEQ ID NO: 136; and a variable heavy chain including a CDRH1 including the sequence as set forth in SEQ ID NO: 137, a CDRH2 including the sequence as set forth in SEQ ID NO: 138, and a CDRH3 including the sequence as set forth in SEQ ID

NO: 139.

240. The kit of any of embodiments 199-229, wherein the target antigen includes GPC3 and the binding domain binds GPC3.
241. The kit of embodiment 240, wherein the binding domain that binds GPC3 includes a variable light chain including a complementarity determining region (CDRL)1 including the sequence as set forth in SEQ ID NO: 140, a CDRL2 including the sequence as set forth in SEQ ID NO: 141, and a CDRL3 including the sequence as set forth in SEQ ID NO: 142; and a variable heavy chain including a CDRH1 including the sequence as set forth in SEQ ID NO: 143, a CDRH2 including the sequence as set forth in SEQ ID NO: 144, and a CDRH3 including the sequence as set forth in SEQ ID NO: 145.
242. The kit of any of embodiments 199-229, wherein the target antigen includes mesothelin and the binding domain binds mesothelin.
243. The kit of embodiment 240, wherein the binding domain that binds mesothelin includes a variable light chain including a CDRL1 including the sequence as set forth in SEQ ID NO: 146, a CDRL2 including the sequence as set forth in SEQ ID NO: 147, and a CDRL3 including the sequence as set forth in SEQ ID NO: 148; and a variable heavy chain including a CDRH1 including the sequence as set forth in SEQ ID NO: 149, a CDRH2 including the sequence as set forth in SEQ ID NO: 150, and a CDRH3 including the sequence as set forth in SEQ ID NO: 151.
244. The kit of any of embodiments 199-229, wherein the target antigen includes FOLR1 and the binding domain binds FOLR1.
245. The kit of any of embodiments 244, wherein the binding domain that binds FOLR1 includes a variable light chain including a CDRL1 including the sequence as set forth in SEQ ID NO: 154, a CDRL2 including the sequence as set forth in SEQ ID NO: 155, and a CDRL3 including the sequence as set forth in SEQ ID NO: 156; and a variable heavy chain including a CDRH1 including the sequence as set forth in SEQ ID NO: 157, a CDRH2 including the sequence as set forth in SEQ ID NO: 158, and a CDRH3 including the sequence as set forth in SEQ ID NO: 159.
246. The kit of any of embodiments 199-229, wherein the target antigen includes MUC1 and the binding domain binds MUC1.
247. The kit of embodiment 246, wherein the binding domain that binds MUC1 includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 230, a CDRL2 as set forth in SEQ ID NO: 231, and a CDRL3 as set forth in SEQ ID NO: 232, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 233, a CDRH2 as set forth in SEQ ID NO: 234,

and a CDRH3 as set forth in SEQ ID NO: 235.

248. The kit of any of embodiments 199-229, wherein target antigen includes CA19-9 and the binding domain binds CA19-9.
249. The kit of embodiment 248, wherein the binding domain that binds CA19-9 includes a variable light chain including a CDRL1 as set forth in SEQ ID NO: 236, a CDRL2 as set forth in SEQ ID NO: 241, and a CDRL3 as set forth in SEQ ID NO: 238, and a variable heavy chain including a CDRH1 as set forth in SEQ ID NO: 239, a CDRH2 as set forth in SEQ ID NO: 240, and a CDRH3 as set forth in SEQ ID NO: 241.
250. The kit of any of embodiments 199-249, wherein the genetic construct is within a nanoparticle.
251. The kit of any of embodiments 199-250, wherein the genetic construct is within a cell.
252. The kit of embodiment 251, wherein the cell is within a formulation.
253. The kit of embodiments 251 or 252, wherein the cell is cultured in a hypotonic or isotonic medium.
254. The kit of any of embodiments 251-253, wherein the cell is cultured in a culture medium.
255. The kit of embodiment 254, wherein the culture medium includes at least 40 mM potassium ion.
256. The kit of embodiments 254 or 255, wherein the culture medium includes IL-2, IL-21, IL-15, or IL-7.
257. The kit of any of embodiments 254-256, wherein the culture medium includes IL-2 at a concentration of 0.1 ng/mL to 20 ng/mL.
258. The kit of any of embodiments 254-257, wherein the culture medium includes IL-21 at a concentration of 0.1 ng/mL to 20 ng/mL.
259. The kit of any of embodiments 254-258, wherein the culture medium includes IL-15 at a concentration of 0.1 ng/mL to 20 ng/mL.
260. The kit of any of embodiments 254-259, wherein the culture medium includes IL-7 at a concentration of 0.1 ng/mL to 20 ng/mL.
261. The kit of any of embodiments 254-260, wherein the culture medium includes a cell expansion agent.
262. The kit of any of embodiments 261, wherein the cell expansion agent includes a GSK3B inhibitor, an ACLY inhibitor, a PI3K inhibitor, or an AKT inhibitor.
263. The kit of any of embodiments 254-262, wherein the culture medium includes a sodium ion, glucose, or a calcium ion.
264. The kit of any of embodiments 254-263, wherein the culture medium includes a sodium

ion at a concentration of 25 mM to 100 mM.

265. The kit of any of embodiments 254-264, wherein the culture medium includes glucose at a concentration of at least 10 mM.

266. The kit of any of embodiments 254-265, wherein the culture medium includes a calcium ion at a concentration of at least 0.4 mM.

[0284] (XI) Experimental Example. Example 1. Materials and Methods. Mice and cell lines. C57BL/6 mice (obtained from Jackson Laboratories, Bar Harbor, ME) of 6–8 weeks of age were used as recipient hosts for adoptive transfer unless otherwise indicated. OT-1 Ly5.1 transgenic mice or C57BL/6 mice were used at cell source for adoptive cell transfer experiments, as indicated. To obtain OT-1 Ly5.1 mice OT-1 (C57BL/6-Tg (Tcr α Tcr β)1100Mjb/J) were crossed with Ly5.1 mice (B6.SJL-PtprcaPepcb/BoyJ). All mice were maintained under specific pathogen-free conditions. Kasumi-2 cells were maintained in RPMI 16540 (Gibco) with 10% fetal bovine serum (FBS), 1% glutamine, and 1% penicillin-streptomycin. LS174T cell line was obtained from American Type Culture Collection (ATCC). KPC cells were transduced to express full length human carcinoembryonic antigen (CEA), mouse epithelial cell adhesion molecule (EpCAM), or firefly luciferase (pLX313-Firefly luciferase (Addgene plasmid # 118017) to generate KPC-hCEA, KPC-EpCAM, or KPC luciferase overexpression (OE) cell lines, respectively. KPC EpCAM knockout (KO) cells were generated via CRISPR-Cas9 mediated deletion of *Epcam*. Briefly, *Epcam* targeted single guide RNAs (sgRNAs) were purchased from Synthego. Guides were complexed with Cas9 and mixed with KPC cells; mixtures were electroporated with the Lonza 4D Nucleofector. Knockout was confirmed by flow cytometry staining from mouse EpCAM. Cell lines were used for *in vitro* co-culture assays and *in vivo* experiments, as indicated. Cell lines were maintained in complete media DMEM (Gibco) with 10% FBS, 1% glutamine and 1% penicillin–streptomycin.

[0285] *In vitro* activation and culture of T cells. CD8⁺ T cells from OT-1 or C57BL/6 mice were isolated using a negative bead selection (StemCell) and stimulated *in vitro* with plate-bound anti-CD3 and anti-CD28 (5 μ g/ml; BioXcell) and expanded in RPMI 1640 containing 10% FBS and 100 IU/mL of recombinant human IL-2 (Peprotech). Human blood samples were enriched for T cells using negative bead selection (StemCell) and stimulated *in vitro* in media containing a 1:100 titer of TransAct (Miltenyi Biotec), 5% FBS and 100 IU/mL of rhIL-2.

[0286] Intracellular cytokine staining and flow cytometry. For all flow cytometry experiments, T cells were stained with a fixable live/dead stain (Invitrogen) in phosphate buffered saline (PBS) followed by surface antibody staining in fluorescence-activated cell sorting (FACS) buffer (PBS with 0.5% bovine serum albumin (BSA) and 0.1% sodium azide). For intracellular cytokine

staining, cells were first stained for surface markers and later stained for intracellular molecules following fixation and permeabilization following the manufacturer's protocols (BD Cytotfix/Cytoperm). Antibodies for surface staining and intracellular cytokine staining were purchased from BD Biosciences, BioLegend, eBiosciences and Invitrogen. All experiments were conducted on a BD (Becton Dickinson) Fortessa or CyTek Biosciences Aurora cytometer and analyzed with FlowJo software (Tree Star, Inc).

[0287] *In vitro* co-culture. Transduced T cells were mixed 1:1 with tumor cell lines as indicated. Cells were incubated overnight (16 hours) in the presence of protein transport inhibitor (BD Golgi Stop and BD GolgiPlug). After 16 hours, cells were washed and stained for flow cytometry as described.

[0288] Retroviral transduction. Platinum-E ecotropic or Platinum-A amphotropic packaging cells (Cell Biolabs) were plated one day before transfections on poly-D-lysine-coated 10-cm plates (Corning) at a concentration of 6×10^6 cells per plate. Packaging cells were transfected with retroviral plasmid DNA, pCL-Eco or pCL-Ampho plasmid DNA, and jetOPTIMUS reagent for 8 h, according to manufacturer instructions (Polyplus). Lenti-X packaging cells (ATCC) were plated one day before transfections on poly-D-lysine-coated 10-cm plates (Corning) at a concentration of 6×10^6 cells per plate. Packaging cells were transfected with lentivirus plasmid DNA (Addgene plasmid #118017), pMLDg/ pRRE plasmid DNA, pRSV/ REV plasmid DNA, pMD2.G plasmid DNA, and jetOPTIMUS reagent for 8 h, according to manufacturer instructions (Polyplus). Medium was replaced 8 h after transfection and cells were incubated for a further 48 h. Viral supernatants were then collected and spun at 2,000g for 2 h at 32 °C onto 24-well non-tissue-culture-treated plates coated overnight in Retronectin (Takara Bio) or spininfected with T cells for 90 minutes at 32°C in 1 $\mu\text{g mL}^{-1}$ polybrene, which was then removed after an additional 4 hour incubation.

[0289] Genetic ablation in CD8⁺ T cells. Non-targeting control sgRNA, *Cxcr3*, *Itgb7* targeted modified single guide RNAs (sgRNAs) were purchased from and synthesized by Synthego. *Cxcr3* loci were targeted with sgRNAs mixed in equal parts (murine *Cxcr3* sgRNAs AGGCUGAAAUCCUGUGGGCA (SEQ ID NO: 242), UCCCCAUAAUCGUAGGGAG (SEQ ID NO: 243), ACGUCAAGUGCUAGAUGCCU (SEQ ID NO: 244)). *Itgb7* loci were targeted with sgRNAs mixed in equal parts (murine *Itgb7* sgRNAs AGAAUACACUUCUGGCAGGA (SEQ ID NO: 245), GUCAGGAUCCUCCCAUUCUG (SEQ ID NO: 246), UUUCUGCUGGUUCUGGGCGG (SEQ ID NO: 247)). Mixtures of complexed sgRNA and Cas9 (0.3 nmol synthetic sgRNA + 62 μmol Cas9 nuclease) and $2-10 \times 10^6$ enriched murine CD8⁺ T cells were suspended in 25ul of P3 electroporation buffer (Lonza) and electroporated using the Lonza 4D Nucleofector (pulse code DN100), activated via 5 mcg/ mL anti-CD3 and anti-CD28 for 24 hours, and maintained in 100 IU

mL⁻¹ until day 4 of culture for analysis or adoptive transfer. Knockout efficiency was assessed by flow cytometry staining for Cxcr3 or the complex $\alpha 4\beta 7$ integrin.

[0290] Organ distribution of CD8⁺ T cells in response to acute viral infection. For experiments assessing the organ distribution of CD8⁺ T cells in response to acute viral infection, 2e6 total OT-1 (1e6 of control retrovirus/liver tissue residency programmed (TRP) transduced) cells were transferred into Ly5.2⁺ recipient C57BL/6 mice. Following transfer, mice were vaccinated with 1×10^6 plaque-forming units (PFU) of recombinant vaccinia virus expressing ovalbumin (OVA).

[0291] Adoptive cell transfer (ACT). C57BL/6 were sub-lethally irradiated (500cGy), randomized, and injected intravenously with transduced T cells, as described elsewhere herein. Mice received intraperitoneal injection of interleukin-2 (IL-2) in PBS (5×10^4 IU in 0.5 mL) once daily for 2 days starting on the day of cell transfer. Mice were monitored for changes in body weight, body composition, or behavior. Jaundice was evaluated by eye with criteria being yellowing of skin. For endpoint analysis of transferred cells, organs were collected and processed to single cell suspensions prior to staining for flow cytometry.

[0292] Murine tumor models. For subcutaneous tumor experiments, C57BL/6 mice were implanted with KPC (5×10^5), KPC EpCAM KO (5×10^5), KPC EpCAM OE (1×10^6), or KPC hCEA OE (5×10^5) cells. 11 days after tumor implantation, mice were enrolled in the adoptive cell transfer experiment and treated as described above. Tumors were measured using digital calipers. Tumor size was measured in a blinded fashion every two-three days after transfer and tumor area was calculated as length $\times$ width of the tumor. Mice with tumors greater than 400 mm² were euthanized. The products of the perpendicular tumor diameters are presented as mean $\pm$ standard error of mean (SEM) at the indicated times after ACT. For intrahepatic tumor experiments, C57BL/6 mice were implanted with KPC-Luciferase (7.5×10^5) or KPC hCEA (5×10^5) cells via intrahepatic injection. For mice with Luciferase-expressing tumors, four days after implantation, mice were imaged using the IVIS Spectrum *In Vivo* Imaging System (Perkin Elmer) and D-Luciferin (GoldBio), according to manufacturer instructions. Images were analyzed using the automated ROI tool in Living Image software (Perkin Elmer). Mice without detectable tumors were excluded from the example. Mice with IVIS-confirmed tumors were enrolled and treated as described with ACT. IVIS imaging was repeated two-three times a week for the duration of the experiment. For mice with KPC-hCEA intrahepatic tumors, autopsy time-course studies showed 100% of mice developed tumors. At the example's endpoint, mice were euthanized and tumors excised from the liver and weighed.

[0293] Histology. Tissues were formalin-fixed and paraffin embedded (FFPE). FFPE tissues were stained with hematoxylin and eosin.

[0294] Statistical analysis. Data were analyzed using unpaired two-tailed Student's t tests, two-way ANOVA. For adoptive transfer experiments, recipient mice were randomized before cell transfer. Tumor measurements were captured in a blinded fashion and plotted as the mean $\pm$ SEM for each data point, and tumor treatment graphs were compared by using the Wilcoxon rank sum test and analysis of animal survival was assessed using a log-rank test. In all cases, two tailed test with P values of less than 0.05 were considered significant. Statistics were calculated using GraphPad Prism 7 software (GraphPad Software Inc). Experimental sample sizes were chosen using power calculations, preliminary experiments, or were based on previous experience of variability in similar experiments. Samples which had undergone technical failure during processing were excluded from subsequent analysis.

[0295] (XII) Closing Paragraphs. The nucleic acid and amino acid sequences provided herein are shown using letter abbreviations for nucleotide bases and amino acid residues, as defined in 37 C.F.R. §1.831-1.835 and set forth in WIPO Standard ST.26 (implemented on July 1, 2022). Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included in embodiments where it would be appropriate.

[0296] Variants of the sequences disclosed and referenced herein are also included. Functional variants include one or more residue additions or substitutions that do not substantially impact the physiological effects of the protein. Functional fragments include one or more deletions or truncations that do not substantially impact the physiological effects of the protein. A lack of substantial impact can be confirmed by observing experimentally comparable results in an activation study or a binding study. Functional variants and functional fragments of intracellular domains (e.g., intracellular signaling components) transmit activation or inhibition signals comparable to a wild-type reference when in the activated state of the current disclosure. Functional variants and functional fragments of binding domains bind their cognate antigen or ligand at a level comparable to a wild-type reference.

[0297] Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity can be found using computer programs well known in the art, such as DNASTAR™ (Madison, Wisconsin) software. Preferably, amino acid changes in the protein variants disclosed herein are conservative amino acid changes, i.e., substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains.

[0298] In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and generally can be made without altering a biological activity of a resulting molecule. Those of skill in this art recognize that, in general, single amino acid

substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p. 224). Naturally occurring amino acids are generally divided into conservative substitution families as follows: Group 1: Alanine (Ala), Glycine (Gly), Serine (Ser), and Threonine (Thr); Group 2: (acidic): Aspartic acid (Asp), and Glutamic acid (Glu); Group 3: (acidic; also classified as polar, negatively charged residues and their amides): Asparagine (Asn), Glutamine (Gln), Asp, and Glu; Group 4: Gln and Asn; Group 5: (basic; also classified as polar, positively charged residues): Arginine (Arg), Lysine (Lys), and Histidine (His); Group 6 (large aliphatic, nonpolar residues): Isoleucine (Ile), Leucine (Leu), Methionine (Met), Valine (Val) and Cysteine (Cys); Group 7 (uncharged polar): Tyrosine (Tyr), Gly, Asn, Gln, Cys, Ser, and Thr; Group 8 (large aromatic residues): Phenylalanine (Phe), Tryptophan (Trp), and Tyr; Group 9 (non-polar): Proline (Pro), Ala, Val, Leu, Ile, Phe, Met, and Trp; Group 11 (aliphatic): Gly, Ala, Val, Leu, and Ile; Group 10 (small aliphatic, nonpolar or slightly polar residues): Ala, Ser, Thr, Pro, and Gly; and Group 12 (sulfur-containing): Met and Cys. Additional information can be found in Creighton (1984) Proteins, W.H. Freeman and Company.

[0299] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, J. Mol. Biol. 157(1), 105-32). Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (−0.4); Thr (−0.7); Ser (−0.8); Trp (−0.9); Tyr (−1.3); Pro (−1.6); His (−3.2); Glutamate (−3.5); Gln (−3.5); aspartate (−3.5); Asn (−3.5); Lys (−3.9); and Arg (−4.5).

[0300] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e., still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity.

[0301] As detailed in US 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Thr (−0.4); Pro (−0.5 $\pm$ 1); Ala (−0.5); His (−0.5); Cys (−1.0); Met (−1.3); Val (−1.5); Leu (−1.8); Ile (−1.8); Tyr (−2.3); Phe (−2.5); Trp (−3.4). It is understood that

an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

[0302] As outlined above, amino acid substitutions may be based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. As indicated elsewhere, variants of gene sequences can include codon optimized variants, sequence polymorphisms, splice variants, and/or mutations that do not affect the function of an encoded product to a statistically-significant degree.

[0303] Variants of the protein, nucleic acid, and gene sequences disclosed herein also include sequences with at least 70% sequence identity, 80% sequence identity, 85% sequence identity, 90% sequence identity, 95% sequence identity, 96% sequence identity, 97% sequence identity, 98% sequence identity, or 99% sequence identity to the protein, nucleic acid, or gene sequences disclosed herein.

[0304] "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between protein, nucleic acid, or gene sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily calculated by known methods, including those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp CABIOS, 5, 151-153 (1989) with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul, *et al.*, J. Mol. Biol. 215:403-410 (1990); DNASTAR (DNASTAR, Inc.,

Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.). Within the context of this disclosure it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program referenced. As used herein "default values" will mean any set of values or parameters, which originally load with the software when first initialized.

[0305] Variants also include nucleic acid molecules that hybridize under stringent hybridization conditions to a sequence disclosed herein and provide the same function as the reference sequence. Exemplary stringent hybridization conditions include an overnight incubation at 42 °C in a solution including 50% formamide, 5XSSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5XDenhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1XSSC at 50 °C. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37°C in a solution including 6XSSPE (20XSSPE=3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 °C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5XSSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

[0306] "Specifically binds" refers to an association of a binding domain (of, for example, a CAR binding domain) to its cognate binding molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1} , while not significantly associating with any other molecules or components in a relevant environment sample. Binding domains may be classified as "high affinity" or "low affinity". In particular embodiments, "high affinity" binding domains refer to those binding domains with a K_a of at least 10^7 M^{-1} , at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , at least 10^{11} M^{-1} , at least 10^{12} M^{-1} , or at least 10^{13} M^{-1} . In particular embodiments, "low affinity" binding domains refer to those

binding domains with a K_a of up to 10^7 M^{-1} , up to 10^6 M^{-1} , up to 10^5 M^{-1} . Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., 10^{-5} M to 10^{-13} M). In certain embodiments, a binding domain may have "enhanced affinity," which refers to a selected or engineered binding domains with stronger binding to a cognate binding molecule than a wild type (or parent) binding domain. For example, enhanced affinity may be due to a K_a (equilibrium association constant) for the cognate binding molecule that is higher than the reference binding domain or due to a K_d (dissociation constant) for the cognate binding molecule that is less than that of the reference binding domain, or due to an off-rate (K_{off}) for the cognate binding molecule that is less than that of the reference binding domain. A variety of assays are known for detecting binding domains that specifically bind a particular cognate binding molecule as well as determining binding affinities, such as Western blot, ELISA, and BIACORE® analysis (see also, e.g., Scatchard, *et al.*, 1949, *Ann. N.Y. Acad. Sci.* 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0307] Unless otherwise indicated, the practice of the present disclosure can employ conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA. These methods are described in the following publications. See, e.g., Sambrook, *et al.* Molecular Cloning: A Laboratory Manual, 2nd Edition (1989); F. M. Ausubel, *et al.* eds., Current Protocols in Molecular Biology, (1987); the series Methods IN Enzymology (Academic Press, Inc.); M. MacPherson, *et al.*, PCR: A Practical Approach, IRL Press at Oxford University Press (1991); MacPherson *et al.*, eds. PCR 2: Practical Approach, (1995); Harlow and Lane, eds. Antibodies, A Laboratory Manual, (1988); and R. I. Freshney, ed. Animal Cell Culture (1987).

[0308] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms "include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." The transition term "comprise" or "comprises" means has, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase "consisting of" excludes any element, step, ingredient or component not specified. The transition phrase "consisting essentially of" limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would reduce the ability of a localized therapy to treat metastases from a primary tumor site.

[0309] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless

indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term “about” has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of $\pm 20\%$ of the stated value; $\pm 19\%$ of the stated value; $\pm 18\%$ of the stated value; $\pm 17\%$ of the stated value; $\pm 16\%$ of the stated value; $\pm 15\%$ of the stated value; $\pm 14\%$ of the stated value; $\pm 13\%$ of the stated value; $\pm 12\%$ of the stated value; $\pm 11\%$ of the stated value; $\pm 10\%$ of the stated value; $\pm 9\%$ of the stated value; $\pm 8\%$ of the stated value; $\pm 7\%$ of the stated value; $\pm 6\%$ of the stated value; $\pm 5\%$ of the stated value; $\pm 4\%$ of the stated value; $\pm 3\%$ of the stated value; $\pm 2\%$ of the stated value; or $\pm 1\%$ of the stated value.

[0310] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0311] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0312] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is

anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0313] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0314] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0315] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0316] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0317] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or

essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Eds. Attwood T *et al.*, Oxford University Press, Oxford, 2006).

CLAIMS

What is claimed is:

1. A method of treating a subject in need thereof comprising identifying a subject with a cancer; and administering to the subject a therapeutically effective amount of a cell genetically modified to express
 - i) a chimeric antigen receptor comprising a binding domain that binds a target antigen of the cancer; and
 - ii) a (a) trafficking signal that directs the cell to a treatment location, (b) a retention signal that maintains the cell within the treatment location, and/or (c) an inhibitory signal that reduces migration of the cell to an off-target location; thereby treating the subject in need thereof.
2. The method of claim 1, wherein the cancer is a primary cancer that has metastasized to a second location; and the cell is genetically modified to express
 - i) a chimeric antigen receptor comprising a binding domain that binds a target antigen of the primary cancer; and
 - ii) a (a) trafficking signal that directs the cell to the second location, (b) a retention signal that maintains the cell within the second location, and/or (c) an inhibitory signal that reduces migration of the cell to an off-target location; thereby treating the subject in need thereof.
3. The method of claim 2, wherein
the primary cancer is colorectal cancer, the second location is liver, the target antigen is CEA, the trafficking signal comprises CXC motif chemokine receptor 6 (CXCR6), and the retention signal comprises CD69.
4. The method of claim 2, wherein
the primary cancer is colorectal cancer, the second location is liver, the target antigen is CEA, and the trafficking signal comprises CXCR6;
the primary cancer is breast, colon, or gastric cancer; the second location is liver, the target antigen is HER2, and the trafficking signal CXCR6;
the primary cancer is breast cancer, the second location is bone, the target antigen is HER2, and the trafficking signal comprises CXCR4;
the primary cancer is prostate cancer, the second location is bone, the target antigen is PSA, and the trafficking signal comprises CXCR4;

the primary cancer is pancreatic cancer, the second location is liver, the target antigen is mesothelin or CA19-9, and the trafficking signal comprises CXCR6;

the primary cancer is ovarian or endometrial cancer, the second location is liver, the target antigen is FOLR1, and the trafficking signal comprises CXCR6; or

the primary cancer is breast, uterine, ovarian or endometrial cancer; the second location is liver, the target antigen is MUC1, and the trafficking signal comprises CXCR6.

5. The method of claim 2, wherein the retention signal comprises CD69.
6. The method of claim 2, wherein the off-target location is small intestine or colon.
7. The method of claim 6, wherein the inhibitory signal comprises knockout of $\beta 7$ integrin.
8. The method of claim 2, wherein the off-target location is prostate gland.
9. The method of claim 8, wherein the inhibitory signal comprises knockout of CXCR3.
10. The method of claim 2, wherein the off-target location is lung.
11. The method of claim 10, wherein the inhibitory signal comprises knockout of CCR4, CXCR3, and CCR3.
12. The method of claim 2, wherein the off-target location is lung, kidney, or stomach.
13. The method of claim 12, wherein the inhibitory signal comprises knockout of $\beta 7$ integrin, CCR4, CXCR3, and CCR3.
14. The method of claim 2, further comprising surgically removing the primary cancer.
15. The method of claim 1, wherein the cell is genetically modified to express
 - i) a chimeric antigen receptor comprising a binding domain that binds a target antigen of the cancer; and
 - ii) a (a) trafficking signal that directs the cell to an organ in which the cancer is located,
 - (b) a retention signal that maintains the cell within organ in which the cancer is located, and/or
 - (c) an inhibitory signal that reduces migration of the cell to an off-target location; thereby treating the subject in need thereof.
16. The method of claim 1, wherein the cancer is a primary cancer that has metastasized to a second location; and the cell is genetically modified to express
 - i) a chimeric antigen receptor comprising a binding domain that binds a target antigen of the primary cancer; and
 - ii) a (a) trafficking signal that directs the cell to the organ of the primary cancer location,

(b) a retention signal that maintains the cell within the primary cancer location, and/or

(c) an inhibitory signal that reduces migration of the cell to the second location and/or an off-target location;

thereby treating the subject in need thereof.

17. The method of claim 15, wherein the off-target location is an organ, gland, or bone.
18. A cell genetically modified to express i) a chimeric antigen receptor comprising a CEA binding domain; ii) a CXCR6; and iii) CD69.
19. A cell genetically modified to achieve at least two of:
expression of a recombinant receptor,
modulation of a trafficking signal, or
modulation of a retention signal.
20. The cell of claim 19, wherein the trafficking signal comprises CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
21. The cell of claim 19, wherein modulation of the trafficking signal comprises up- or down-regulating expression of CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
22. The cell of claim 19, genetically modified to express CXCR6, integrin β 2, CCR1, CCR2, or CXCR4.
23. The cell of claim 19, genetically modified to express CXCR6.
24. The cell of claim 19, genetically modified to express integrin β 2.
25. The cell of claim 19, genetically modified to express CCR1.
26. The cell of claim 19, genetically modified to express CCR2.
27. The cell of claim 19, genetically modified to express CXCR4.
28. The cell of claim 19, genetically modified to down-regulate expression of β 7 integrin, CCR4, CXCR3, or CCR3.
29. The cell of claim 19, genetically modified to down-regulate expression of β 7 integrin.
30. The cell of claim 19, genetically modified to down-regulate expression of CXCR3.
31. The cell of claim 19, genetically modified to down-regulate expression of CCR4.
32. The cell of claim 19, genetically modified to down-regulate expression of CXCR3.
33. The cell of claim 28, wherein down-regulated expression is by administration of a guide RNA (gRNA) and a nuclease to the cell.
34. The cell of claim 33, wherein the gRNA comprises a sequence as set forth in one of SEQ ID NOs: 77-103.

35. The cell of claim 34, wherein the gRNA comprises single guide RNA (sgRNA).
36. The cell of claim 35, wherein the sgRNA targeting $\beta 7$ integrin comprises a sequence as set forth in SEQ ID Nos: 245-247.
37. The cell of claim 35, wherein the sgRNA targeting CXCR3 comprises a sequence as set forth in SEQ ID Nos: 242-244.
38. The cell of claim 33, wherein the nuclease comprises Cas9 or Cpf1.
39. The cell of claim 19, wherein the retention signal comprises CD69, Runx3, Hobit, or CD103.
40. The cell of claim 19, wherein modulation of the retention signal comprises up- or down regulating expression of CD69, Runx3, Hobit, or CD103.
41. The cell of claim 19, genetically modified to express CD69, Runx3, Hobit, or CD103.
42. The cell of claim 19, genetically modified to express CD69.
43. The cell of claim 19, genetically modified to down-regulate expression of CD69, Runx3, Hobit, or CD103.
44. The cell of claim 43, wherein the down-regulated expression is by administration of a gRNA and a nuclease to the cell.
45. The cell of claim 44, wherein the gRNA comprises a sequence as set forth in one of SEQ ID NOs: 107-121 or 192-194.
46. The cell of claim 44, wherein the nuclease comprises Cas9 or Cpf1.
47. The cell of claim 19, genetically modified to express the recombinant receptor.
48. The cell of claim 19, wherein the recombinant receptor comprises a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a CAR/TCR hybrid.
49. The cell of claim 19, wherein the recombinant receptor comprises i) an extracellular component comprising a binding domain that binds an antigen expressed on a surface of targeted cells; and ii) an intracellular component.
50. The cell of claim 49, wherein the targeted cells comprise cancer cells or infected cells.
51. The cell of claim 49, wherein the targeted cells comprise cancer cells.
52. The cell of claim 49, wherein the antigen comprises a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9).
53. The cell of claim 49, wherein the antigen comprises a CEA.
54. The cell of claim 49, wherein the binding domain comprises a binding domain of an anti-CEA antibody.
55. The cell of claim 54, wherein the anti-CEA antibody comprises AHB7.

56. The cell of claim 54, wherein the anti-CEA antibody comprises a variable light chain comprising a complementarity determining region (CDR)L1 as set forth in SEQ ID NO: 207, a CDRL2 as set forth in SEQ ID NO: 208, and a CDRL3 as set forth in SEQ ID NO: 209, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 210, a CDRH2 as set forth in SEQ ID NO: 211, and a CDRH3 as set forth in SEQ ID NO: 212.
57. The cell of claim 54, wherein the anti-CEA antibody comprises a variable light chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 205.
58. The cell of claim 54, wherein the anti-CEA antibody comprises a variable light chain having the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 205.
59. The cell of claim 54, wherein the anti-CEA antibody comprises a variable light chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 63 and a variable heavy chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 64.
60. The cell of claim 54, wherein the anti-CEA antibody comprises a variable light chain encoded by the sequence as set forth in SEQ ID NO: 63 and a variable heavy chain encoded by the sequence as set forth in SEQ ID NO: 64.
61. The cell of claim 56, wherein the anti-CEA antibody comprises a linker between the variable light chain and variable heavy chain.
62. The cell of claim 61, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 65.
63. The cell of claim 49, wherein the antigen comprises EpCAM.
64. The cell of claim 49, wherein the binding domain comprises a binding domain of an anti-EpCAM antibody.
65. The cell of claim 64, wherein the anti-EpCAM antibody comprises G8.8, ab71916, AUA-1, Ber-EP4, HEA125, EpMab-16, MOC31, or VU-1D9.
66. The cell of claim 64, wherein the anti-EpCAM antibody comprises a variable light chain comprising a CDRL1 as set forth in SEQ ID NO: 127, a CDRL2 as set forth in SEQ ID NO: 128, and a CDRL3 as set forth in SEQ ID NO: 129, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 124, a CDRH2 as set forth in SEQ ID NO: 125, and a CDRH3 as set forth in SEQ ID NO: 126.
67. The cell of claim 64, wherein the anti-EpCAM antibody comprises a variable light chain

comprising a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain comprising a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 196.

68. The cell of claim 64, wherein the anti-EpCAM antibody comprises a variable light chain having the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 196.
69. The cell of claim 66, wherein the anti-EpCAM antibody comprises a linker between the variable light chain and variable heavy chain.
70. The cell of claim 69, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 197.
71. The cell of claim 49, wherein the intracellular component comprises a CD3 ζ signaling domain, a 4-1BB signaling domain, and/or a CD28 signaling domain.
72. The cell of claim 49, wherein the intracellular component comprises a CD3 ζ signaling and a CD28 signaling domain.
73. The cell of claim 49, wherein the intracellular component is linked to the extracellular component through a transmembrane domain.
74. The cell of claim 73, wherein the transmembrane domain comprises a CD28 transmembrane domain.
75. The cell of claim 19, wherein the cell is an autologous cell or an allogeneic cell in reference to a subject.
76. The cell of claim 19, wherein the cell is *in vivo* or *ex vivo*.
77. The cell of claim 19, wherein the cell is an immune cell, a stem or progenitor cell, or an adult cell.
78. The cell of claim 77, wherein the immune cell is a lymphocyte.
79. The cell of claim 78, wherein the lymphocyte comprises a T cell, B cell, natural killer (NK) cell, or NK-T cell.
80. The cell of claim 77, wherein the immune cell is a T cell selected from a CD3⁺ T cell, a CD4⁺ T cell, a CD8⁺ T cell, a central memory T cell, an effector memory T cell, and/or a naïve T cell.
81. The cell of claim 77, wherein the immune cell is a CD8⁺ T cell.
82. The cell of claim 77, wherein the immune cell is a CD4⁺ T cell.
83. The cell of claim 77, wherein the stem or progenitor cell comprises a hematopoietic stem cell (HSC), a hematopoietic progenitor cell (HPC), a pluripotent stem cell, a mesenchymal stem cell, a neural stem cell, or an endothelial stem cell.

84. The cell of claim 77, wherein the adult cell comprises a fibroblast, a hepatocyte, a myoblast, a neuron, an osteoblast, an osteoclast, a kidney cell, a chondrocyte, or an adipocyte.
85. The cell of claim 19, wherein the cell expresses an anti-CEA CAR, CXCR6, and CD69.
86. The cell of claim 85, wherein the cell expresses the anti-CEA CAR, CXCR6, and CD69 by administration of a sequence as set forth in SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, or SEQ ID NO: 62.
87. The cell of claim 19, wherein the cell expresses an anti-EpCAM CAR, CXCR6, and CD69.
88. The cell of claim 87, wherein the cell expresses the anti-EpCAM CAR, CXCR6, and CD69 by administration of a sequence as set forth in SEQ ID NO: 203.
89. The cell of claim 19, wherein the cell expresses anti-CEA CAR and down-regulates expression of CXCR3.
90. The cell of claim 89, wherein the cell expresses the anti-CEA CAR and down-regulates expression of CXCR3 by administration of an sgRNA sequence as set forth in SEQ ID NOs: 242-244 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 201.
91. The cell of claim 19, wherein the cell expresses anti-CEA CAR and down-regulates expression of $\beta 7$ integrin.
92. The cell of claim 91, wherein the cell expresses the anti-CEA CAR and down-regulates expression of $\beta 7$ integrin by administration of an sgRNA sequence as set forth in SEQ ID NOs: 245-247 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 201.
93. The cell of claim 19, wherein the cell expresses anti-EpCAM CAR and down-regulates expression of CXCR3.
94. The cell of claim 93, wherein the cell expresses the anti-EpCAM CAR and down-regulates expression of CXCR3 by administration of an sgRNA sequence as set forth in SEQ ID NOs: 242-244 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 202.
95. The cell of claim 19, wherein the cell expresses anti-EpCAM CAR and down-regulates expression of $\beta 7$ integrin.
96. The cell of claim 95, wherein the cell expresses the anti-EpCAM CAR and down-regulates expression of $\beta 7$ integrin by administration of an sgRNA sequence as set forth in SEQ ID NOs: 245-247 and a nuclease and administration of a sequence as set forth in SEQ ID NO: 202.
97. A genetic construct comprising at least two of:
 - a sequence encoding a recombinant receptor;
 - a sequence encoding a trafficking signal or a sequence down-regulating a trafficking signal;

or

a sequence encoding a retention signal or a sequence down-regulating a retention signal.

98. The genetic construct of claim 97, wherein the trafficking signal comprises CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
99. The genetic construct of claim 97, wherein the genetic construct comprises a sequence encoding CXCR6, integrin β 2, CCR1, CCR2, or CXCR4.
100. The genetic construct of claim 97, wherein the genetic construct comprises a sequence down-regulating expression of β 7 integrin, CCR4, CXCR3, or CCR3.
101. The genetic construct of claim 97, wherein the genetic construct comprises or encodes a trafficking signal gRNA and nuclease.
102. The genetic construct of claim 101, wherein the trafficking signal gRNA comprises a sequence as set forth in one of SEQ ID NOs: 77-103.
103. The genetic construct of claim 101, wherein the trafficking signal gRNA comprises a trafficking signal single guide RNA (sgRNA).
104. The genetic construct of claim 103, wherein the trafficking signal sgRNA comprises an sgRNA targeting β 7 integrin.
105. The genetic construct of claim 104, wherein the sgRNA targeting β 7 integrin comprises a sequence as set forth in SEQ ID Nos: 245-247.
106. The genetic construct of claim 103, wherein the trafficking signal sgRNA comprises an sgRNA targeting CXCR3.
107. The genetic construct of claim 106, wherein the sgRNA targeting CXCR3 comprises a sequence as set forth in SEQ ID Nos: 242-244.
108. The genetic construct of claim 101, wherein the nuclease comprises Cas9 or Cpf1.
109. The genetic construct of claim 97, wherein the retention signal comprises CD69, Runx3, Hobit, or CD103.
110. The genetic construct of claim 97, wherein the genetic construct comprises or encodes a retention signal gRNA and nuclease.
111. The genetic construct of claim 110, wherein the retention signal gRNA comprises a sequence as set forth in one of SEQ ID NOs: 107-121 or 192-194.
112. The genetic construct of claim 110, wherein the nuclease comprises Cas9 or Cpf1.
113. The genetic construct of claim 97, wherein the recombinant receptor comprises a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a CAR/TCR hybrid.
114. The genetic construct of claim 97, wherein the recombinant receptor comprises i) an extracellular component comprising a binding domain that binds a target antigen, wherein the

- target antigen is expressed on a surface of targeted cells; and ii) an intracellular component.
115. The genetic construct of claim 114, wherein the targeted cells comprise cancer cells or infected cells.
116. The genetic construct of claim 114, wherein the targeted cells comprise cancer cells.
117. The genetic construct of claim 114, wherein the target antigen comprises a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9).
118. The genetic construct of claim 114, wherein the target antigen comprises CEA.
119. The genetic construct of claim 114, wherein the binding domain comprises a binding domain of an anti-CEA antibody.
120. The genetic construct of claim 119, wherein the anti-CEA antibody comprises AHB7.
121. The genetic construct of claim 119, wherein the anti-CEA antibody comprises a variable light chain comprising a CDRL1 as set forth in SEQ ID NO: 207, a CDRL2 as set forth in SEQ ID NO: 208, and a CDRL3 as set forth in SEQ ID NO: 209, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 210, a CDRH2 as set forth in SEQ ID NO: 211, and a CDRH3 as set forth in SEQ ID NO: 212.
122. The genetic construct of claim 119, wherein the anti-CEA antibody comprises a variable light chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 205.
123. The genetic construct of claim 119, wherein the anti-CEA antibody comprises a variable light chain having the sequence as set forth in SEQ ID NO: 204 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 205.
124. The genetic construct of claim 119, wherein the anti-CEA antibody comprises a variable light chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 63 and a variable heavy chain encoded by a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 64.
125. The genetic construct of claim 119, wherein the anti-CEA antibody comprises a variable light chain encoded by the sequence as set forth in SEQ ID NO: 63 and variable heavy chain encoded by the sequence as set forth in SEQ ID NO: 64.
126. The genetic construct of claim 122, wherein the anti-CEA antibody comprises a linker between the variable light chain and variable heavy chain.

127. The genetic construct of claim 126, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 65.
128. The genetic construct of claim 114, wherein the target antigen comprises EpCAM.
129. The genetic construct of claim 114, wherein the binding domain comprises a binding domain of an anti-EpCAM antibody.
130. The cell of claim 129, wherein the anti-EpCAM antibody comprises G8.8, ab71916, AUA-1, Ber-EP4, HEA125, EpMab-16, MOC31, or VU-1D9.
131. The genetic construct of claim 129, wherein the anti-EpCAM antibody comprises a variable light chain comprising a CDRL1 as set forth in SEQ ID NO: 127, a CDRL2 as set forth in SEQ ID NO: 128, and a CDRL3 as set forth in SEQ ID NO: 129, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 124, a CDRH2 as set forth in SEQ ID NO: 125, and a CDRH3 as set forth in SEQ ID NO: 126.
132. The genetic construct of claim 129, wherein the anti-EpCAM antibody comprises a variable light chain comprising a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain comprising a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 196.
133. The genetic construct of claim 129, wherein the anti-EpCAM antibody comprises a variable light chain having the sequence as set forth in SEQ ID NO: 195 and a variable heavy chain having the sequence as set forth in SEQ ID NO: 196.
134. The genetic construct of claim 131, wherein the anti-EpCAM antibody comprises a linker between the variable light chain and variable heavy chain.
135. The genetic construct of claim 134, wherein the linker is encoded by the sequence as set forth in SEQ ID NO: 197.
136. The genetic construct of claim 114, wherein the intracellular component comprises a CD3 ζ signaling domain, a 4-1BB signaling domain and/or a CD28 signaling domain.
137. The genetic construct of claim 114, wherein the intracellular component comprises a CD3 ζ signaling domain and a CD28 signaling domain.
138. The genetic construct of claim 114, wherein the intracellular component is linked to the extracellular component through a transmembrane domain.
139. The genetic construct of claim 138, wherein the transmembrane domain comprises a CD28 transmembrane domain.
140. The genetic construct of claim 97, further comprising a control feature.
141. The genetic construct of claim 140, wherein the control feature comprises a transduction marker.

142. The genetic construct of claim 141, wherein the transduction marker comprises CD90.1, Her2tG, epidermal growth factor receptor (EGFRt), or tCD19.
143. The genetic construct of claim 140, wherein the control feature comprises a selection cassette.
144. The genetic construct of claim 140, wherein the control feature comprises a tag cassette.
145. The genetic construct of claim 144, wherein the tag cassette comprises a sequence as set forth in SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, or SEQ ID NO: 190.
146. The genetic construct of claim 97, further comprising a skip sequence.
147. The genetic construct of claim 146, wherein the skip sequence comprises a 2A skip sequence.
148. The genetic construct of claim 147, wherein the 2A skip sequence encodes a T2A, P2A, E2A, or F2A self-cleaving polypeptide.
149. The genetic construct of claim 97, further comprising a suicide gene.
150. The genetic construct of claim 97, comprising a sequence as set forth in SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 1, and/or SEQ ID NO: 4 or a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 1, and/or SEQ ID NO: 4.
151. The genetic construct of claim 150, wherein the genetic construct comprises from 5' to 3': a sequence encoding a trafficking signal; a first skip sequence; a sequence encoding a retention signal; a second skip sequence; and a sequence encoding a recombinant receptor.
152. The genetic construct of claim 97, wherein the genetic construct comprises from 5' to 3': a sequence encoding a trafficking signal; a first skip sequence; a sequence encoding a retention signal; a second skip sequence; a sequence encoding a recombinant receptor; a third skip sequence; and a transduction marker.
153. The genetic construct of claim 97, wherein the genetic construct comprises from 5' to 3': a sequence downregulating a trafficking signal; a first skip sequence; and a sequence encoding a recombinant receptor.
154. The genetic construct of claim 97, wherein the genetic construct comprises from 5' to 3': a sequence downregulating a trafficking signal; a first skip sequence; a sequence encoding a recombinant receptor; a second skip sequence; and a transduction marker.

155. The genetic construct of claim 97, wherein the genetic construct comprises the sequence as set forth in SEQ ID NOs: 59, 60, 61, or 62 or a sequence having at least 90% sequence identity to the sequence as set forth in SEQ ID NOs: 59, 60, 61, or 62.
156. The genetic construct of claim 97, wherein the genetic construct comprises the sequence as set forth in SEQ ID NO: 203 or a sequence having at least 90% sequence identity to a sequence as set forth in SEQ ID NOs: 203.
157. A vector comprising the genetic construct of claim 97.
158. The vector of claim 157, wherein the vector is a viral vector.
159. The vector of claim 158, wherein the viral vector comprises a retroviral vector or a lentiviral vector.
160. A nanoparticle encapsulating the genetic construct of claim 97.
161. A population of cells genetically modified to comprise the genetic construct of claim 97 or the vector claim 157.
162. A formulation comprising (i) the population of cells of claim 161 or a cell of claim 19 and (ii) a pharmaceutically acceptable carrier.
163. A method of treating a subject in need thereof comprising administering a therapeutically effective amount of the formulation of claim 162 or nanoparticle of claim 160 to the subject, thereby treating the subject in need thereof.
164. The method of claim 163, wherein the administering a therapeutically effective amount comprises physical locoregional delivery.
165. The method of claim 163, wherein the administering a therapeutically effective amount comprises systemic delivery.
166. The method of claim 163, wherein the administering a therapeutically effective amount comprises administering intravenously, intravesically, intradermally, intraarterially, intraparenterally, intranodally, intralymphatically, intraperitoneally, intralesionally, intraprostatically, intravaginally, intrarectally, topically, intrathecally, intratumorally, intramuscularly, or subcutaneously.
167. The method of claim 163, wherein the administering a therapeutically effective amount comprises hepatic arterial infusion, isolated limb perfusion, instillation within an abdominal cavity, or instillation within a thoracic cavity.
168. A method of treating a cancer within a subject in need thereof comprising administering a therapeutically effective amount of a formulation to the subject, wherein the formulation comprises cells genetically modified to
- i) express a recombinant receptor comprising a binding domain that binds a

target antigen, and

- ii) direct location of the cell to a target tissue and/or away from a non-targeted tissue;

thereby treating the subject in need thereof.

169. The method of claim 168, wherein the subject has colorectal cancer and the target antigen is CEA.
170. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.
171. The method of claim 168, wherein the non-targeted tissue is a colon and the cell has down-regulated expression of $\beta 7$ integrin.
172. The method of claim 168, wherein the subject has pancreatic cancer and the target antigen is CEA, mesothelin, or CA19-9.
173. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.
174. The method of claim 168, wherein the target tissue is a pancreas and the cell expresses integrin $\beta 2$, CCR1, or CCR2.
175. The method of claim 168, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.
176. The method of claim 168, wherein the non-targeted tissue is a small intestine or colon and the cell has down-regulated expression of $\beta 7$ integrin.
177. The method of claim 168, wherein the subject has breast cancer and the target antigen is HER2 or MUC1.
178. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.
179. The method of claim 168, wherein the target tissue is a bone and the cell expresses CXCR4.
180. The method of claim 168, wherein the non-targeted tissue is a stomach, lung, or kidney and the cell has down-regulated expression of CCR4, CXCR3, CCR3, or $\beta 7$ integrin.
181. The method of claim 168, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.
182. The method of claim 168, wherein the subject has colon cancer or gastric cancer and the target antigen is HER2.
183. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.

184. The method of claim 168, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.
185. The method of claim 168, wherein the subject has prostate cancer and the target antigen is PEA.
186. The method of claim 168, wherein the target tissue is a bone and the cell expresses CXCR4.
187. The method of claim 168, wherein the non-targeted tissue is a prostate and the cell has down-regulated expression of CXCR3.
188. The method of claim 168, wherein the subject has hepatocellular carcinoma and the target antigen is GPC3.
189. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.
190. The method of claim 168, wherein the non-targeted tissue is a lung and the cell has down-regulated expression of CCR4, CXCR3, or CCR3.
191. The method of claim 168, wherein the subject has ovarian cancer or endometrial cancer and the target antigen is FOLR1 or MUC1.
192. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.
193. The method of claim 168, wherein the non-targeted tissue is a stomach, lung or kidney and the cell has down-regulated expression of CCR4, CXCR3, CCR3, or β 7 integrin.
194. The method of claim 168, wherein the subject has uterine cancer and the target antigen is MUC1.
195. The method of claim 168, wherein the target tissue is a liver and the cell expresses CXCR6.
196. The method of claim 168, wherein the non-targeted tissue is a stomach, lung, or kidney and the cell has down-regulated expression of CCR4, CXCR3, CCR3, or β 7 integrin.
197. The method of claim 168, wherein the cell expresses CD69, Runx3, Hobit, or CD103.
198. The method of claim 168, wherein the cell expresses CD69.
199. The method of claim 168, wherein the cell has down-regulated expression of CD69, Runx3, Hobit, or CD103.
200. A kit comprising a genetic construct of claim 97,
wherein the genetic construct results in:
expression of a binding domain that binds a target antigen,
modulated expression of a trafficking signal, and/or

modulated expression of a retention signal.

- 201. The kit of claim 200, wherein the modulated expression comprises up- or down-regulated expression.
- 202. The kit of claim 200, wherein the trafficking signal comprises CXCR6, integrin β 2, CCR1, CCR2, CXCR4, β 7 integrin, CCR4, CXCR3, or CCR3.
- 203. The kit of claim 200, wherein the genetic construct comprises sequences encoding CXCR6, integrin β 2, CCR1, CCR2, or CXCR4.
- 204. The kit of claim 200, wherein the genetic construct comprises a sequence down-regulating the expression of β 7 integrin, CCR4, CXCR3, or CCR3.
- 205. The kit of claim 200, wherein the genetic construct comprises a trafficking signal gRNA.
- 206. The kit of claim 205, wherein the trafficking signal gRNA comprises a sequence as set forth in one of SEQ ID NOs: 77-103.
- 207. The kit of claim 200, wherein the retention signal comprises CD69, Runx3, Hobit, or CD103.
- 208. The kit of claim 200, wherein the genetic construct comprises a sequence encoding CD69, Runx3, Hobit, or CD103.
- 209. The kit of claim 200, wherein the genetic construct comprises a sequence encoding CD69.
- 210. The kit of claim 200, wherein the genetic construct comprises a sequence down-regulating the expression of CD69, Runx3, Hobit, or CD103.
- 211. The kit of claim 200, wherein the genetic construct comprises a retention signal gRNA.
- 212. The kit of claim 211, wherein the retention signal gRNA comprises a sequence as set forth in one of SEQ ID NOs: 107-121 or 192-194.
- 213. The kit of claim 200, further comprising a nuclease or sequence encoding a nuclease.
- 214. The kit of claim 213, wherein the nuclease comprises Cas9 or Cpf1.
- 215. The kit of claim 200, wherein the binding domain is part of a recombinant receptor.
- 216. The kit of claim 215, wherein the recombinant receptor comprises a chimeric antigen receptor (CAR), an engineered T cell receptor (eTCR), or a CAR/TCR hybrid.
- 217. The kit of claim 216, wherein the CAR comprises an extracellular component comprising the binding domain; an intracellular component; and a transmembrane domain linking the extracellular component to the intracellular component.
- 218. The kit of claim 217, wherein the intracellular component comprises a CD3 ζ signaling domain, a 4-1BB signaling domain, and/or a CD28 signaling domain.
- 219. The kit of claim 217, wherein the intracellular component comprises a CD3 ζ signaling domain and a CD28 signaling domain.

220. The kit of claim 217, wherein the transmembrane domain comprises a CD28 transmembrane domain.
221. The kit of claim 200, wherein the genetic construct further encodes a control feature.
222. The kit of claim 221, wherein the control feature comprises a transduction marker.
223. The kit of claim 222, wherein the transduction marker comprises CD90.1, Her2tG, epidermal growth factor receptor (EGFRt), or tCD19.
224. The kit of claim 221, wherein the control feature comprises a selection cassette.
225. The kit of claim 221, wherein the control feature comprises a tag cassette.
226. The kit of claim 225, wherein the tag cassette comprises a sequence as set forth in SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, or SEQ ID NO: 190.
227. The kit of claim 200, wherein the genetic construct further comprises a skip sequence.
228. The kit of claim 227, wherein the skip sequence comprises a 2A skip sequence.
229. The kit of claim 228, wherein the 2A skip sequence encodes a T2A, P2A, E2A, or F2A self-cleaving polypeptide.
230. The kit of claim 200, wherein the target antigen comprises a carcinoembryonic antigen (CEA), an epithelial cell adhesion molecule (EpCAM), a human epidermal growth factor receptor 2 (HER2), a prostate-specific antigen (PSA), a glypican-3 (GPC3), a mesothelin, a folate receptor alpha (FOLR1), a mucin short variant S1 (MUC1), or a carbohydrate antigen 19-9 (CA19-9).
231. The kit of claim 200, wherein the target antigen comprises CEA and the binding domain binds CEA.
232. The kit of claim 231, wherein the binding domain that binds CEA comprises an anti-CEA antibody.
233. The kit of claim 232, wherein the anti-CEA antibody comprises AHB7.
234. The kit of claim 233, wherein the anti-CEA antibody comprises a variable light chain comprising a complementarity determining region (CDRL)1 as set forth in SEQ ID NO: 207, a CDRL2 as set forth in SEQ ID NO: 208, and a CDRL3 as set forth in SEQ ID NO: 209, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 210, a CDRH2 as set forth in SEQ ID NO: 211, and a CDRH3 as set forth in SEQ ID NO: 212.
235. The kit of claim 200, wherein the target antigen comprises EpCAM and the binding domain binds EpCAM.
236. The kit of claim 235, wherein the binding domain that binds EpCAM comprises a variable

light chain comprising a CDRL1 as set forth in SEQ ID NO: 127, a CDRL2 as set forth in SEQ ID NO: 128, and a CDRL3 as set forth in SEQ ID NO: 129, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 124, a CDRH2 as set forth in SEQ ID NO: 125, and a CDRH3 as set forth in SEQ ID NO: 126.

237. The kit of claim 200, wherein the target antigen comprises HER2 and the binding domain binds HER2.
238. The kit of claim 237, wherein the binding domain that binds HER2 comprises a variable light chain comprising a CDRL1 as set forth in SEQ ID NO: 218, a CDRL2 as set forth in SEQ ID NO: 219, and a CDRL3 as set forth in SEQ ID NO: 220, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 221, a CDRH2 as set forth in SEQ ID NO: 222, and a CDRH3 as set forth in SEQ ID NO: 223.
239. The kit of claim 200, wherein the target antigen comprises PSA and the binding domain binds PSA.
240. The kit of claim 239, wherein the binding domain that binds PSA comprises a variable light chain comprising a CDRL1 comprising the sequence as set forth in SEQ ID NO: 134, a CDRL2 comprising the sequence as set forth in SEQ ID NO: 135, and a CDRL3 comprising the sequence as set forth in SEQ ID NO: 136; and a variable heavy chain comprising a CDRH1 comprising the sequence as set forth in SEQ ID NO: 137, a CDRH2 comprising the sequence as set forth in SEQ ID NO: 138, and a CDRH3 comprising the sequence as set forth in SEQ ID NO: 139.
241. The kit of claim 200 wherein the target antigen comprises GPC3 and the binding domain binds GPC3.
242. The kit of claim 241, wherein the binding domain that binds GPC3 comprises a variable light chain comprising a complementarity determining region (CDRL)1 comprising the sequence as set forth in SEQ ID NO: 140, a CDRL2 comprising the sequence as set forth in SEQ ID NO: 141, and a CDRL3 comprising the sequence as set forth in SEQ ID NO: 142; and a variable heavy chain comprising a CDRH1 comprising the sequence as set forth in SEQ ID NO: 143, a CDRH2 comprising the sequence as set forth in SEQ ID NO: 144, and a CDRH3 comprising the sequence as set forth in SEQ ID NO: 145.
243. The kit of claim 200, wherein the target antigen comprises mesothelin and the binding domain binds mesothelin.
244. The kit of claim 241, wherein the binding domain that binds mesothelin comprises a variable light chain comprising a CDRL1 comprising the sequence as set forth in SEQ ID NO: 146, a CDRL2 comprising the sequence as set forth in SEQ ID NO: 147, and a CDRL3

comprising the sequence as set forth in SEQ ID NO: 148; and a variable heavy chain comprising a CDRH1 comprising the sequence as set forth in SEQ ID NO: 149, a CDRH2 comprising the sequence as set forth in SEQ ID NO: 150, and a CDRH3 comprising the sequence as set forth in SEQ ID NO: 151.

245. The kit of claim 200, wherein the target antigen comprises FOLR1 and the binding domain binds FOLR1.
246. The kit of claim 245, wherein the binding domain that binds FOLR1 comprises a variable light chain comprising a CDRL1 comprising the sequence as set forth in SEQ ID NO: 154, a CDRL2 comprising the sequence as set forth in SEQ ID NO: 155, and a CDRL3 comprising the sequence as set forth in SEQ ID NO: 156; and a variable heavy chain comprising a CDRH1 comprising the sequence as set forth in SEQ ID NO: 157, a CDRH2 comprising the sequence as set forth in SEQ ID NO: 158, and a CDRH3 comprising the sequence as set forth in SEQ ID NO: 159.
247. The kit of claim 200, wherein the target antigen comprises MUC1 and the binding domain binds MUC1.
248. The kit of claim 247, wherein the binding domain that binds MUC1 comprises a variable light chain comprising a CDRL1 as set forth in SEQ ID NO: 230, a CDRL2 as set forth in SEQ ID NO: 231, and a CDRL3 as set forth in SEQ ID NO: 232, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 233, a CDRH2 as set forth in SEQ ID NO: 234, and a CDRH3 as set forth in SEQ ID NO: 235.
249. The kit of claim 200, wherein target antigen comprises CA19-9 and the binding domain binds CA19-9.
250. The kit of claim 249, wherein the binding domain that binds CA19-9 comprises a variable light chain comprising a CDRL1 as set forth in SEQ ID NO: 236, a CDRL2 as set forth in SEQ ID NO: 241, and a CDRL3 as set forth in SEQ ID NO: 238, and a variable heavy chain comprising a CDRH1 as set forth in SEQ ID NO: 239, a CDRH2 as set forth in SEQ ID NO: 240, and a CDRH3 as set forth in SEQ ID NO: 241.
251. The kit of claim 200, wherein the genetic construct is within a nanoparticle.
252. The kit of claim 200, wherein the genetic construct is within a cell.
253. The kit of claim 252, wherein the cell is within a formulation.
254. The kit of claim 252, wherein the cell is cultured in a hypotonic or isotonic medium.
255. The kit of claim 252, wherein the cell is cultured in a culture medium.
256. The kit of claim 255, wherein the culture medium comprises at least 40 mM potassium ion.
257. The kit of claim 255, wherein the culture medium comprises IL-2, IL-21, IL-15, or IL-7.

- 258. The kit of claim 255, wherein the culture medium comprises IL-2 at a concentration of 0.1 ng/mL to 20 ng/mL.
- 259. The kit of claim 255, wherein the culture medium comprises IL-21 at a concentration of 0.1 ng/mL to 20 ng/mL.
- 260. The kit of claim 255, wherein the culture medium comprises IL-15 at a concentration of 0.1 ng/mL to 20 ng/mL.
- 261. The kit of claim 255, wherein the culture medium comprises IL-7 at a concentration of 0.1 ng/mL to 20 ng/mL.
- 262. The kit of claim 255, wherein the culture medium comprises a cell expansion agent.
- 263. The kit of claim 262, wherein the cell expansion agent comprises a GSK3B inhibitor, an ACLY inhibitor, a PI3K inhibitor, or an AKT inhibitor.
- 264. The kit of claim 255, wherein the culture medium comprises a sodium ion, glucose, or a calcium ion.
- 265. The kit of claim 255, wherein the culture medium comprises a sodium ion at a concentration of 25 mM to 100 mM.
- 266. The kit of claim 255, wherein the culture medium comprises glucose at a concentration of at least 10 mM.
- 267. The kit of claim 255, wherein the culture medium comprises a calcium ion at a concentration of at least 0.4 mM.

FIG. 1A

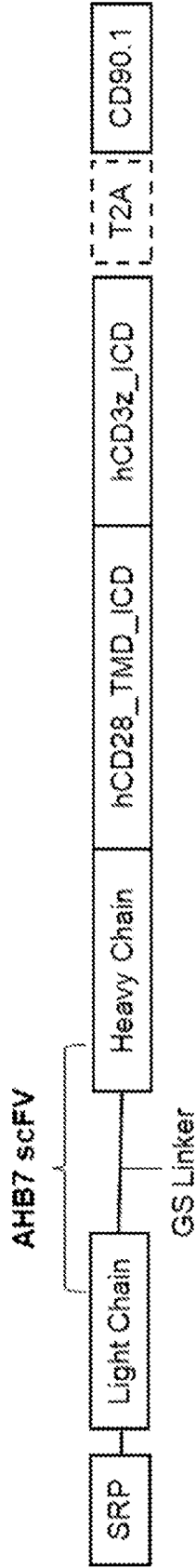


FIG. 1B

LS174T (hCEA⁺) Co-culture

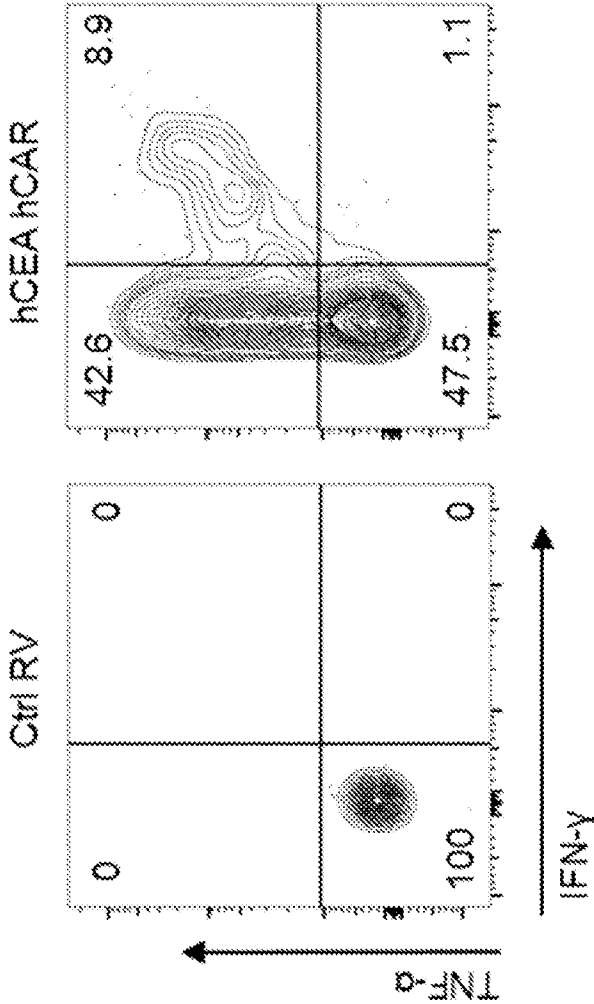


FIG. 1C

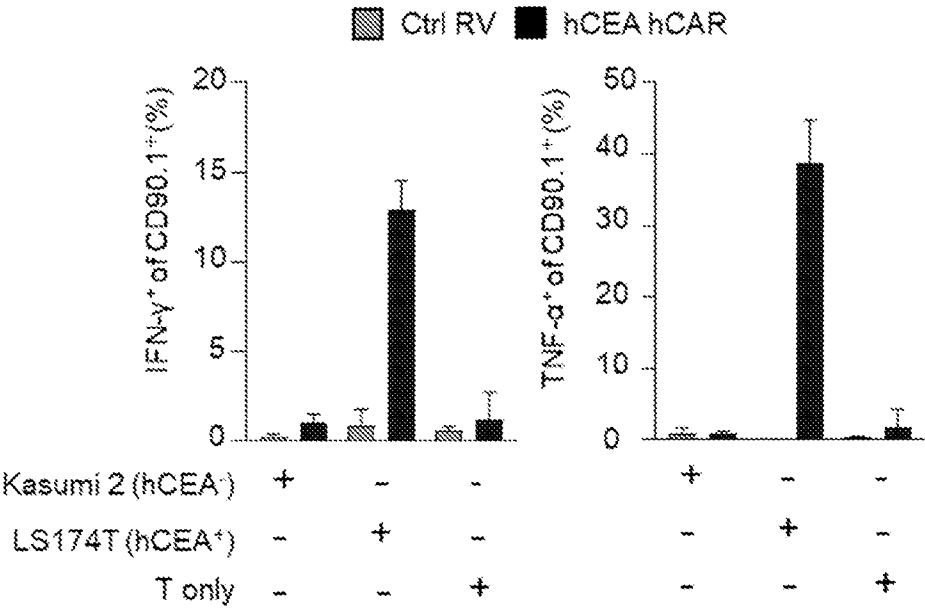
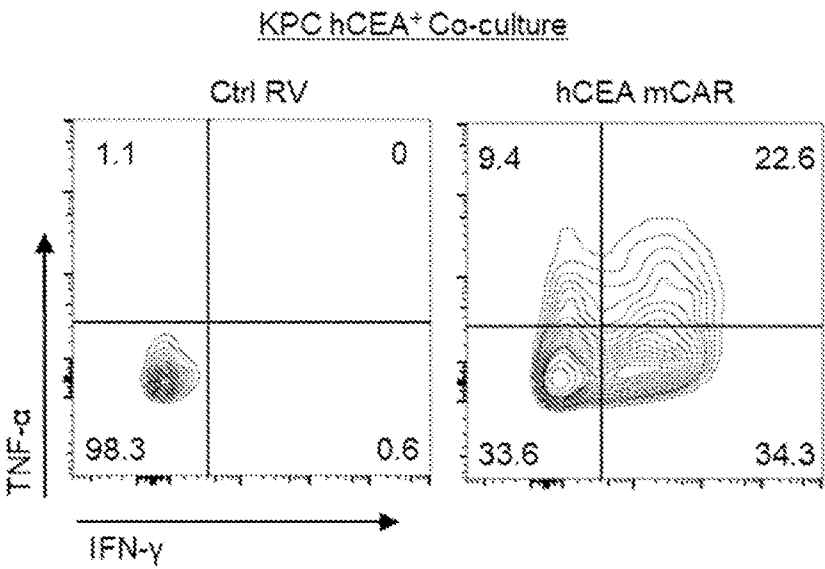


FIG. 1D



FIG. 1E



3/53

FIG. 1F

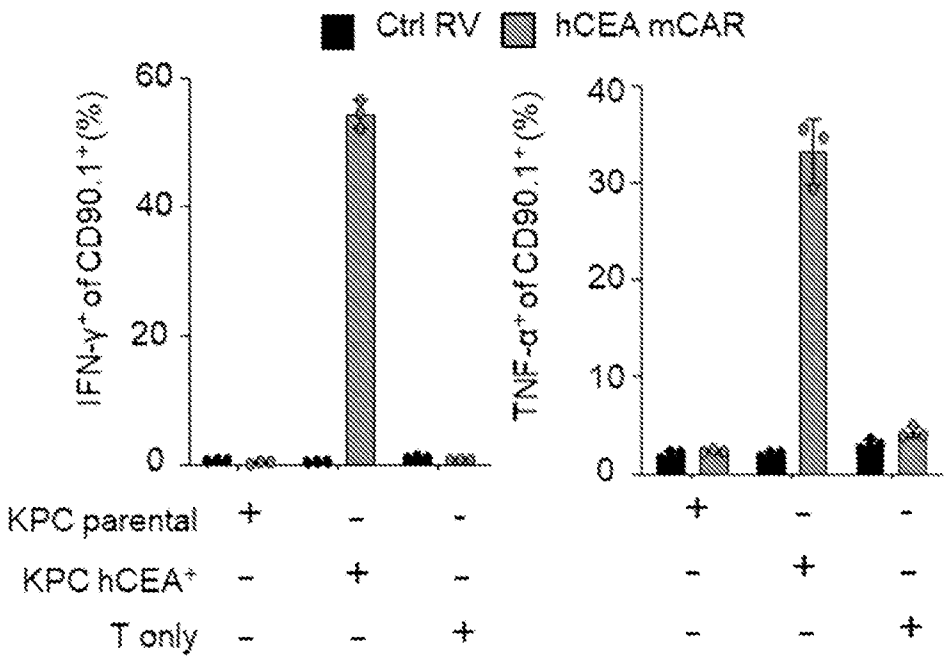


FIG. 2A

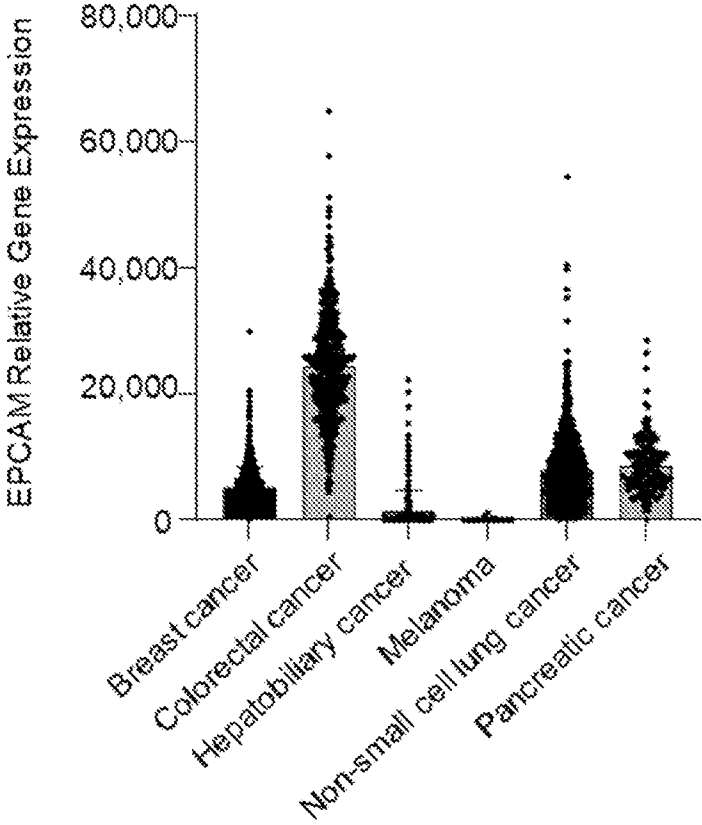


FIG. 2B

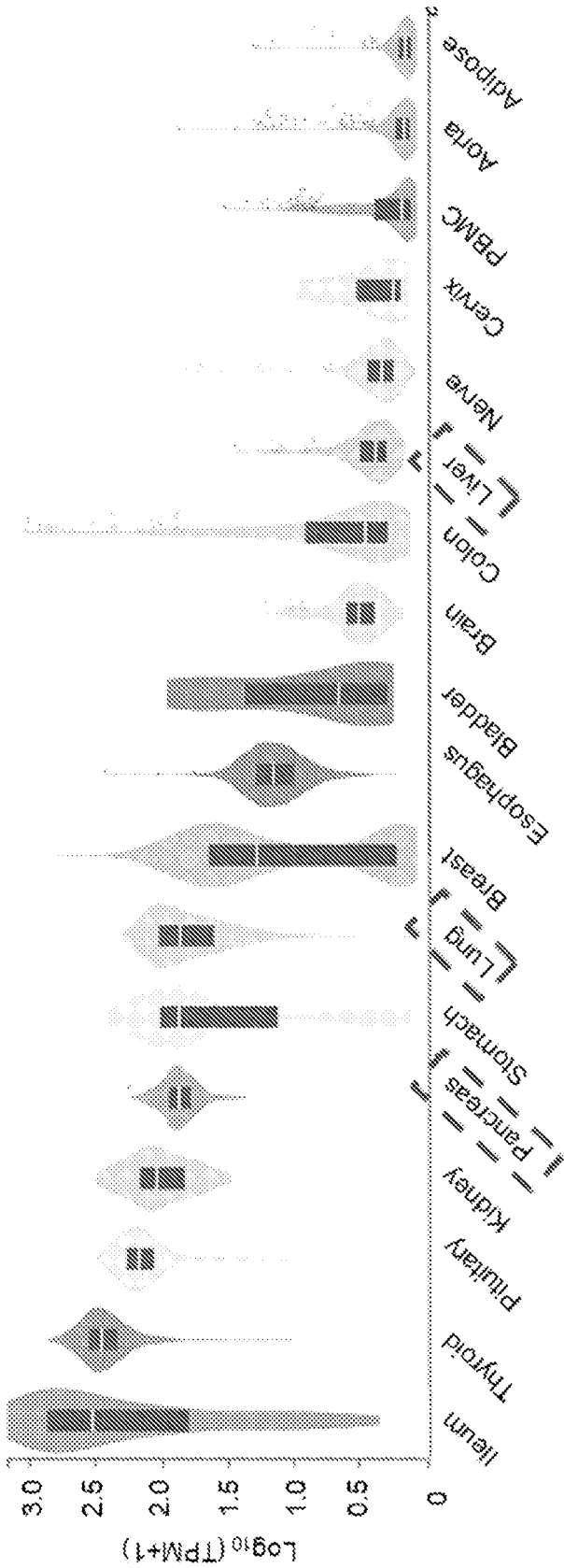
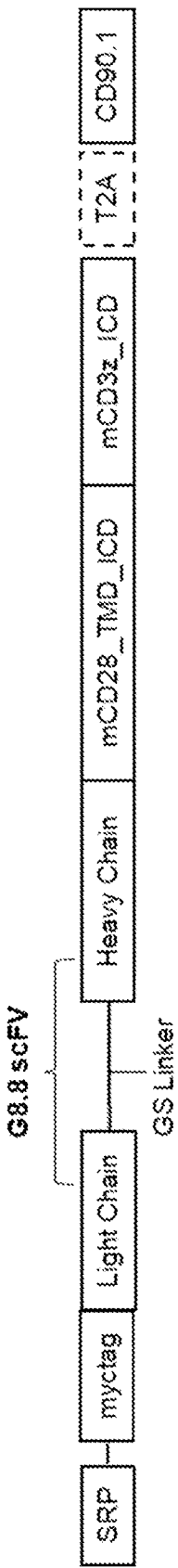


FIG. 2C



5/53

FIG. 2D

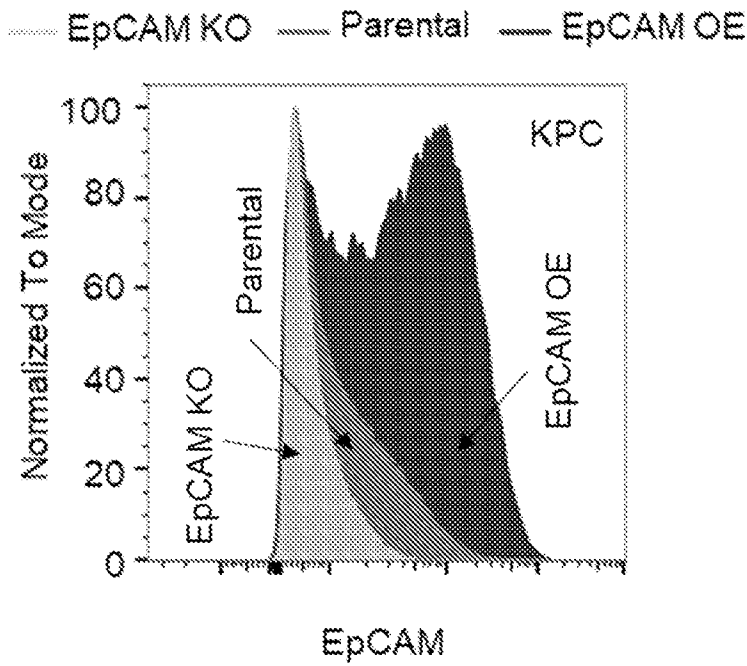
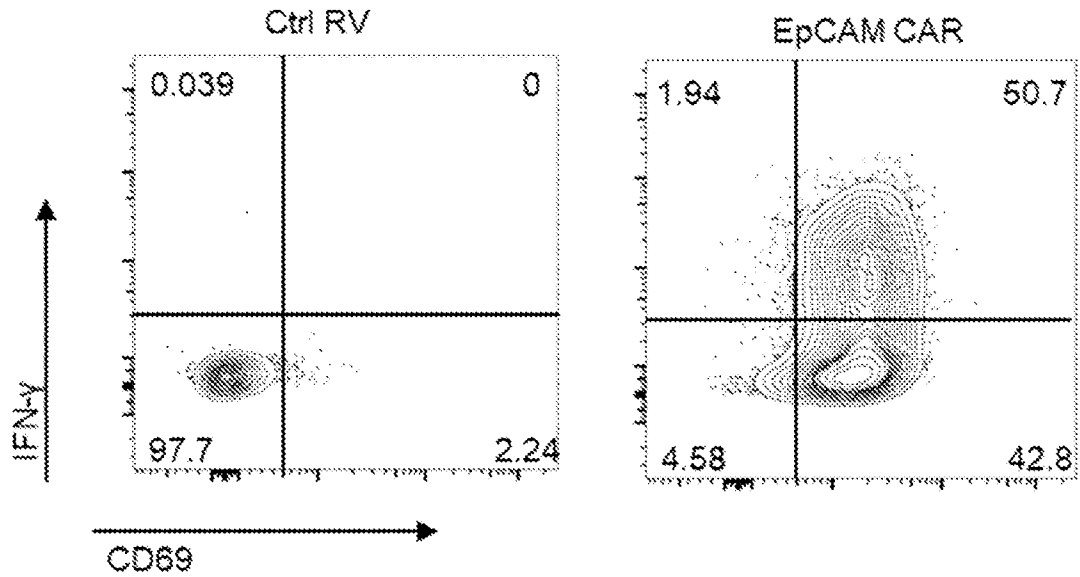


FIG. 2E



6/53

FIG. 2F

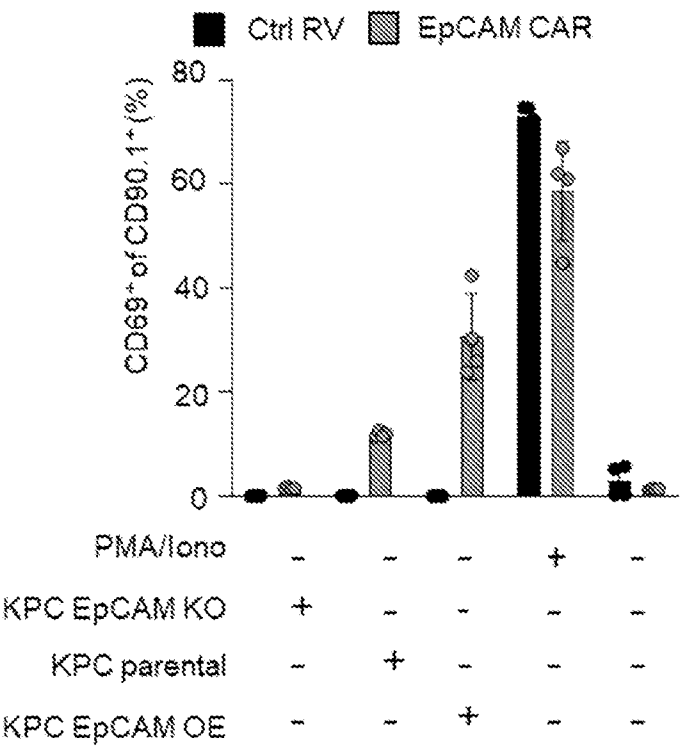
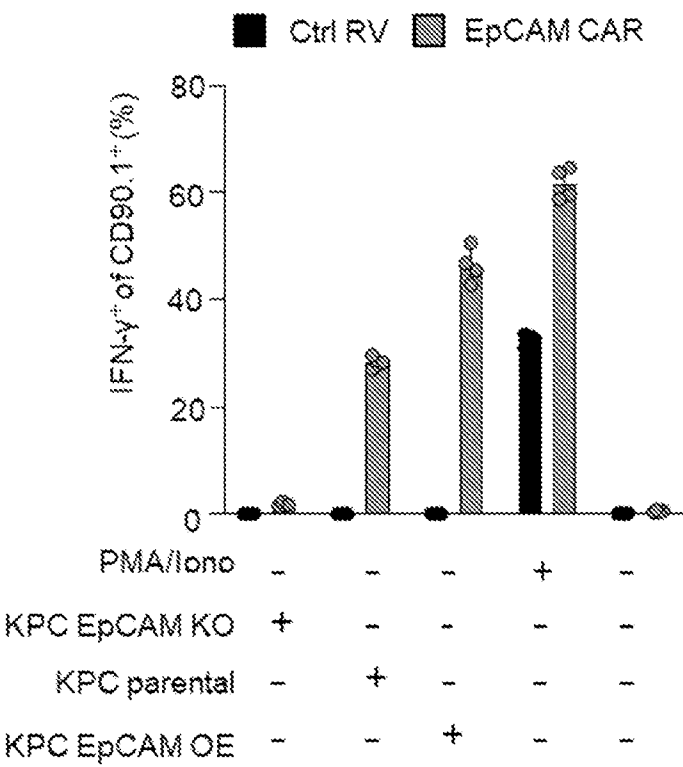


FIG. 2G



7/53

FIG. 2H

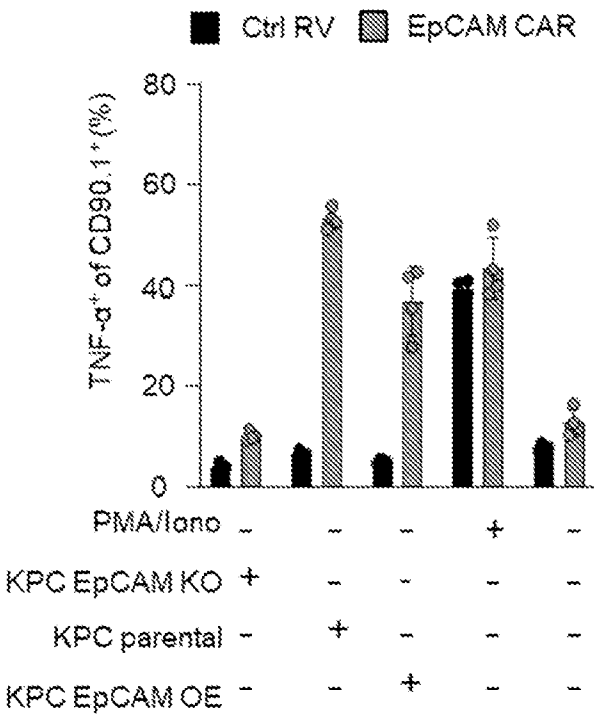
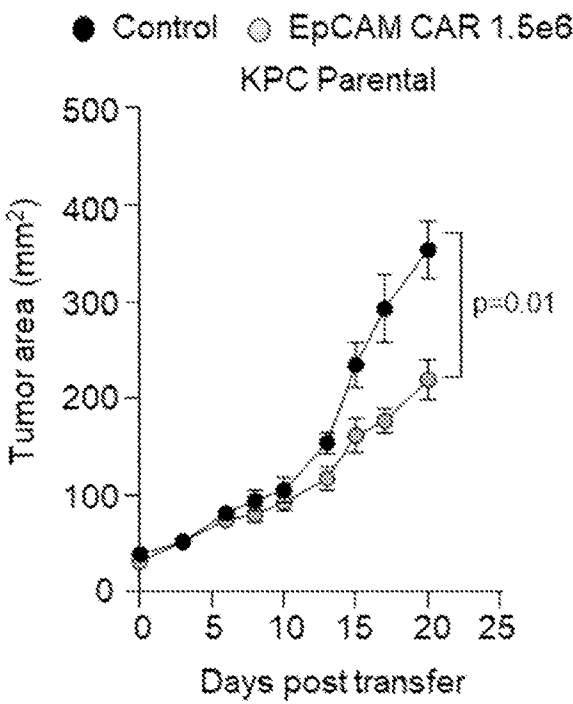


FIG. 3A



8/53

FIG. 3B

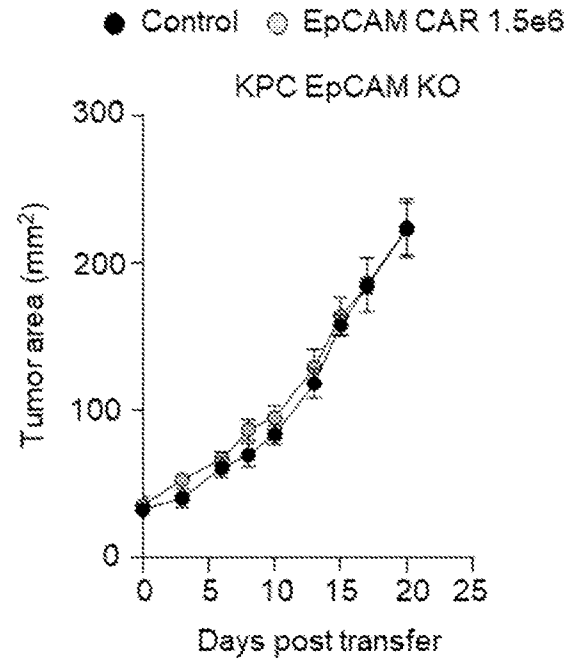
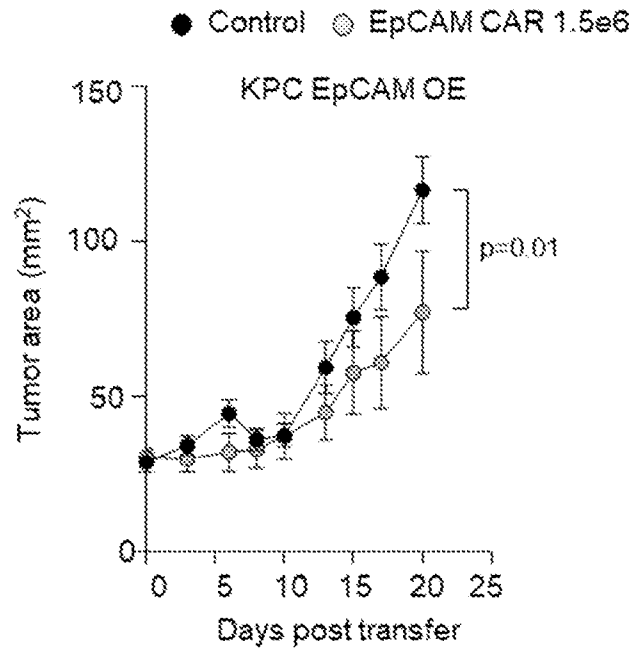


FIG. 3C



9/53

FIG. 3D

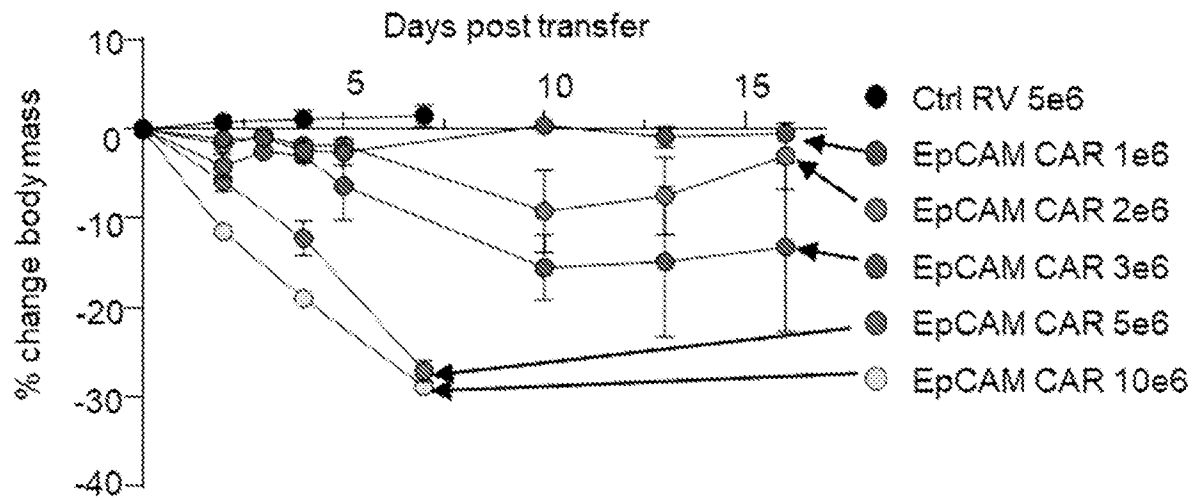
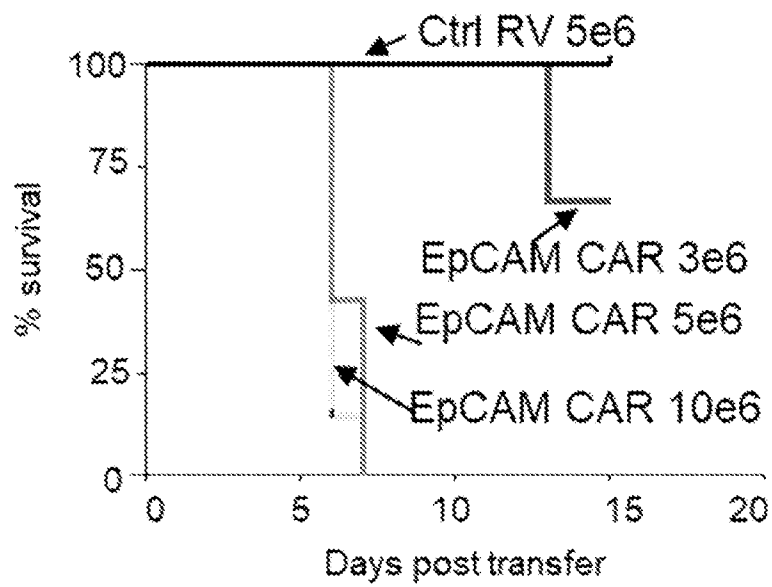
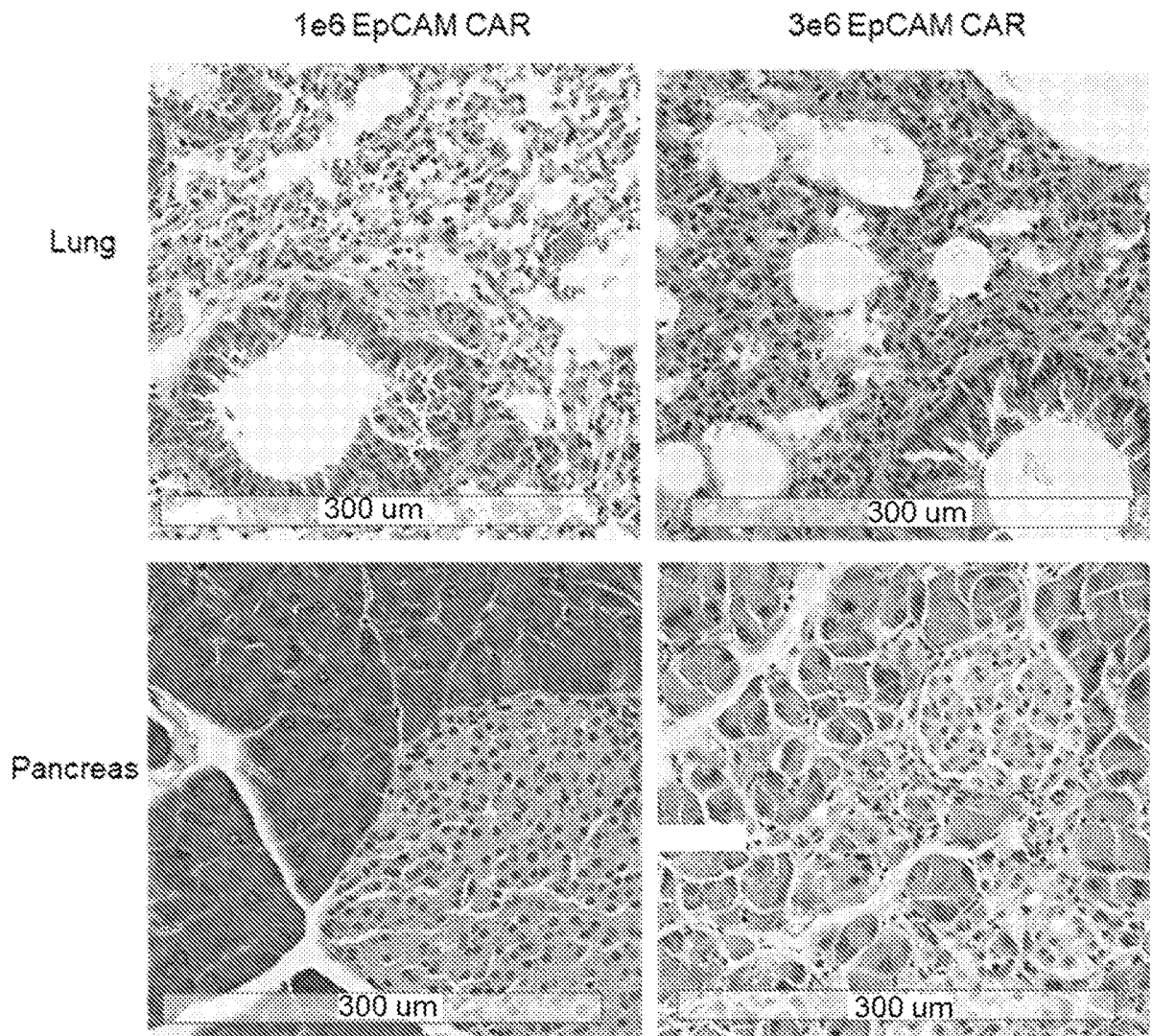


FIG. 3E



10/53

FIG. 3F



11/53

FIG. 4A

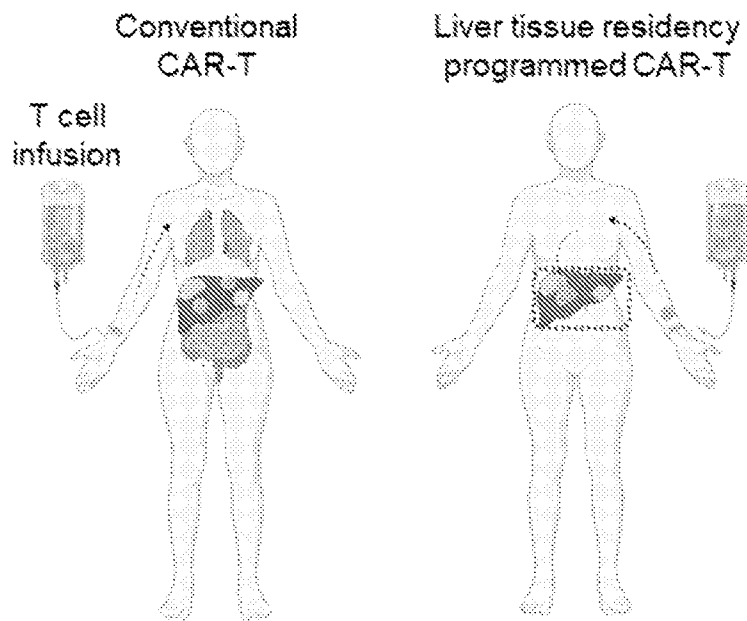


FIG. 4B

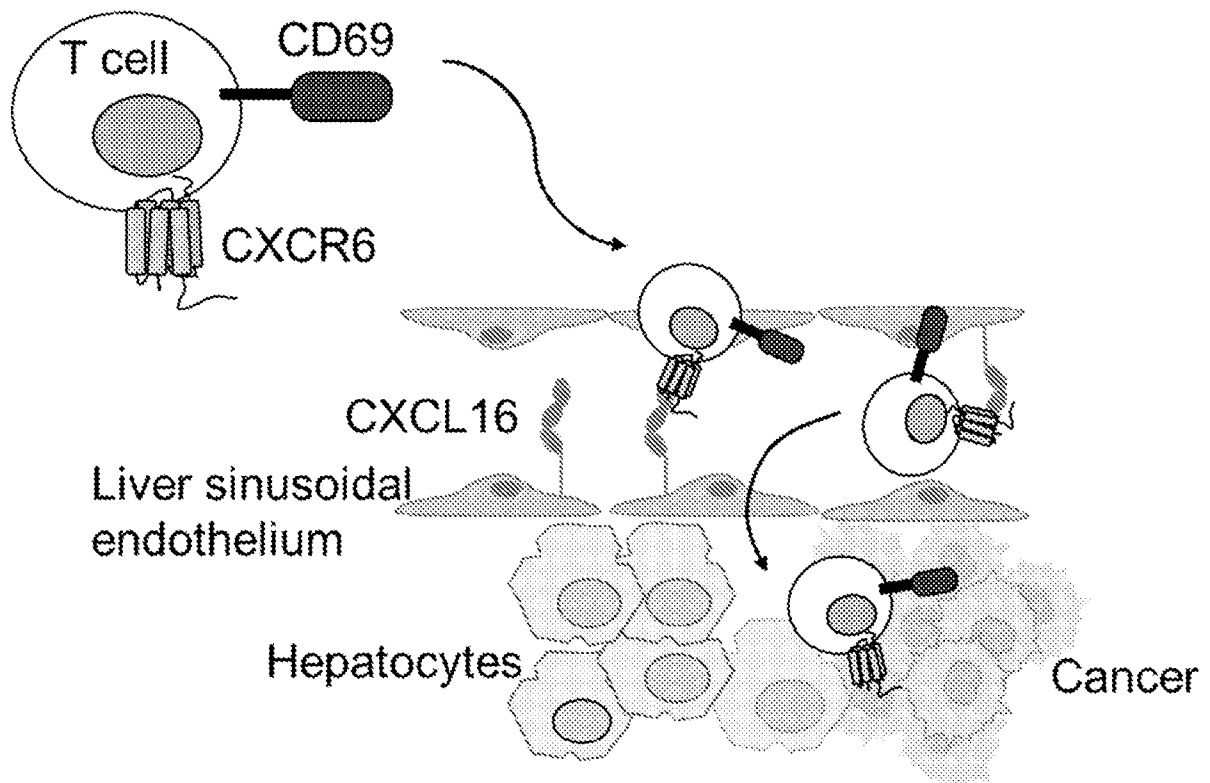


FIG. 4C

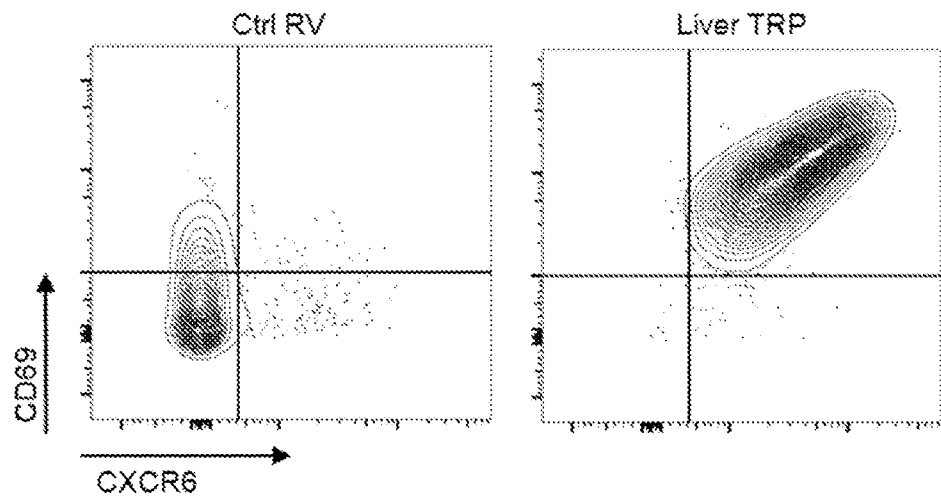
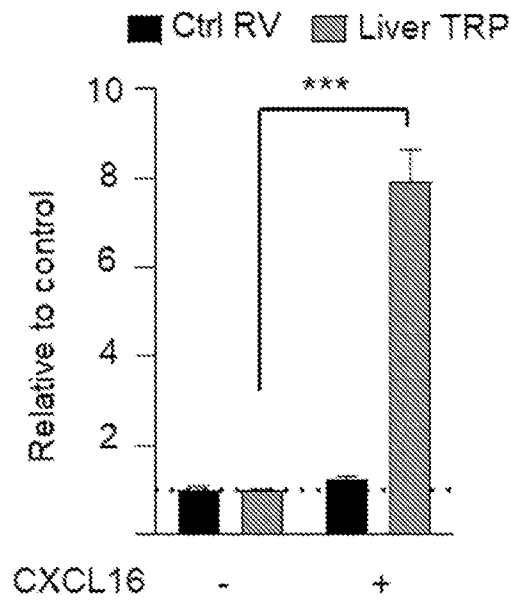


FIG. 4D



13/53

FIG. 4E

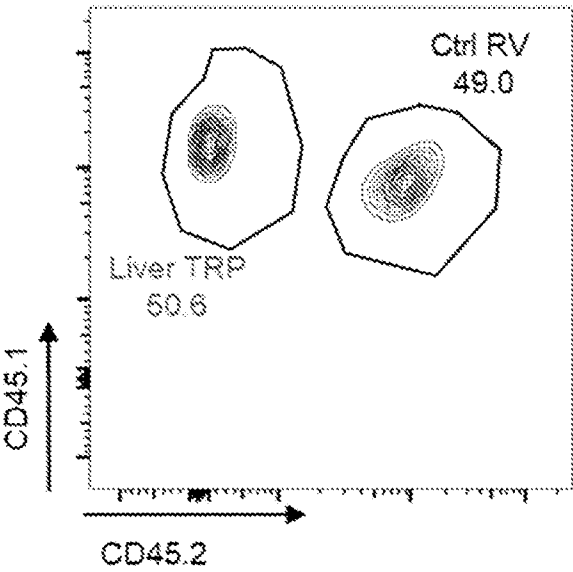
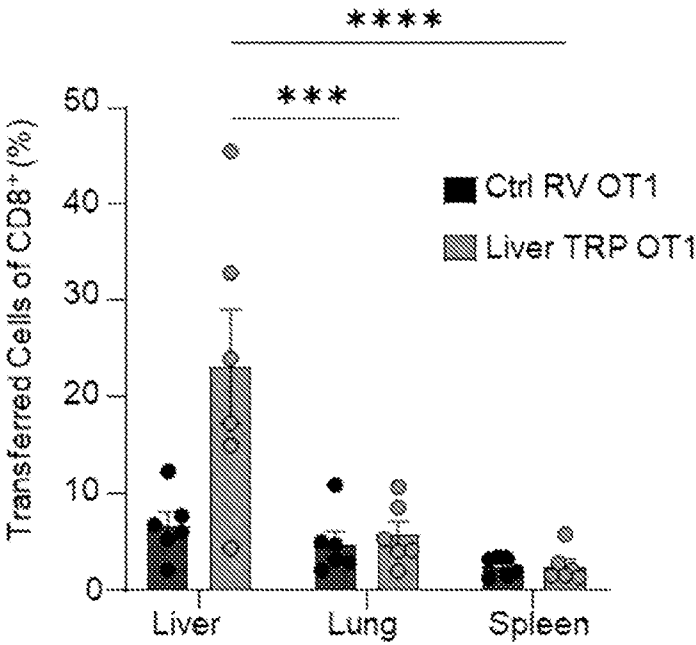


FIG. 4F



14/53

FIG. 4G

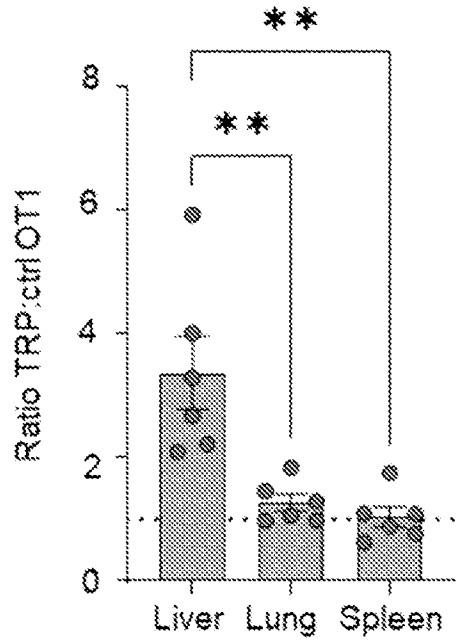


FIG. 5A

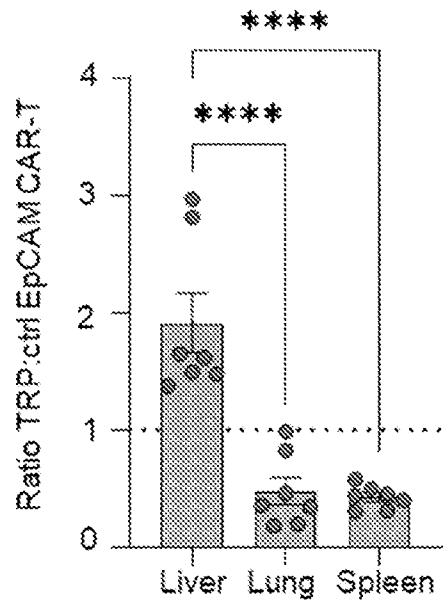


FIG. 5B

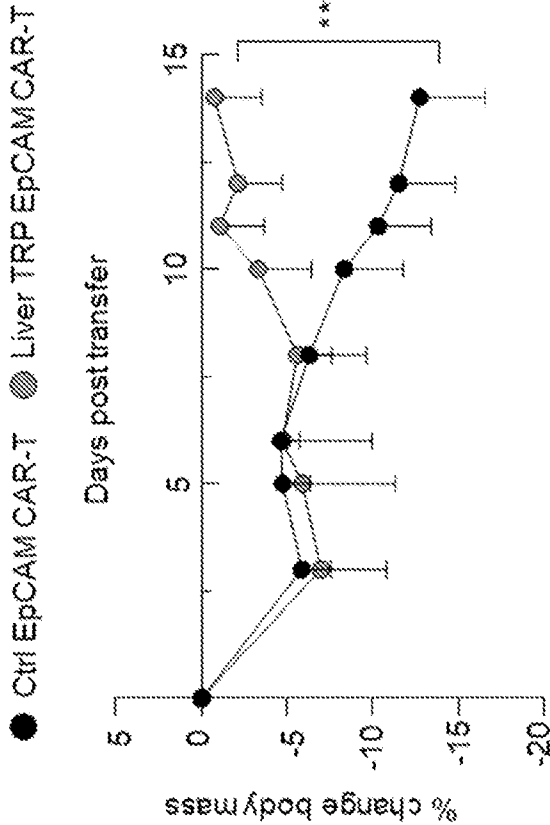


FIG. 5C

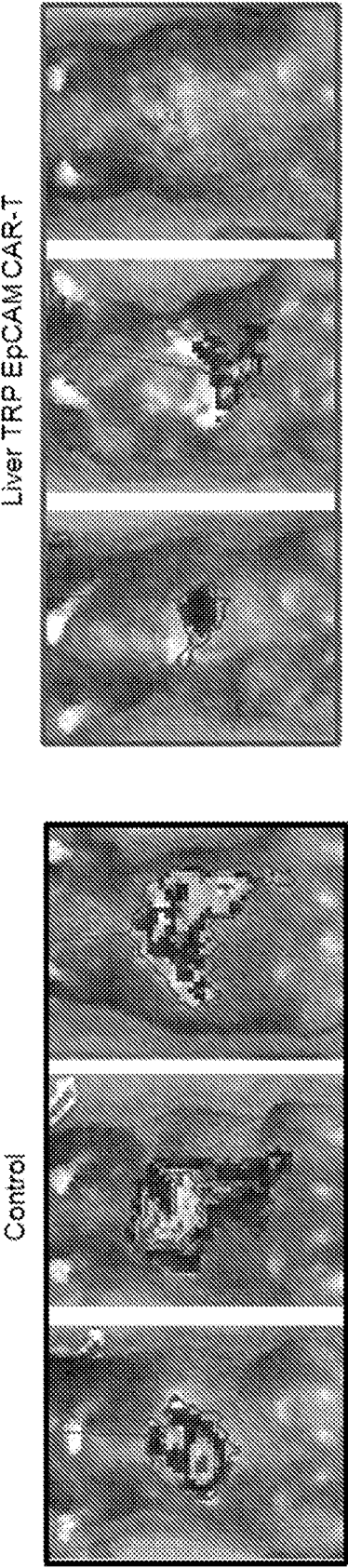


FIG. 5D

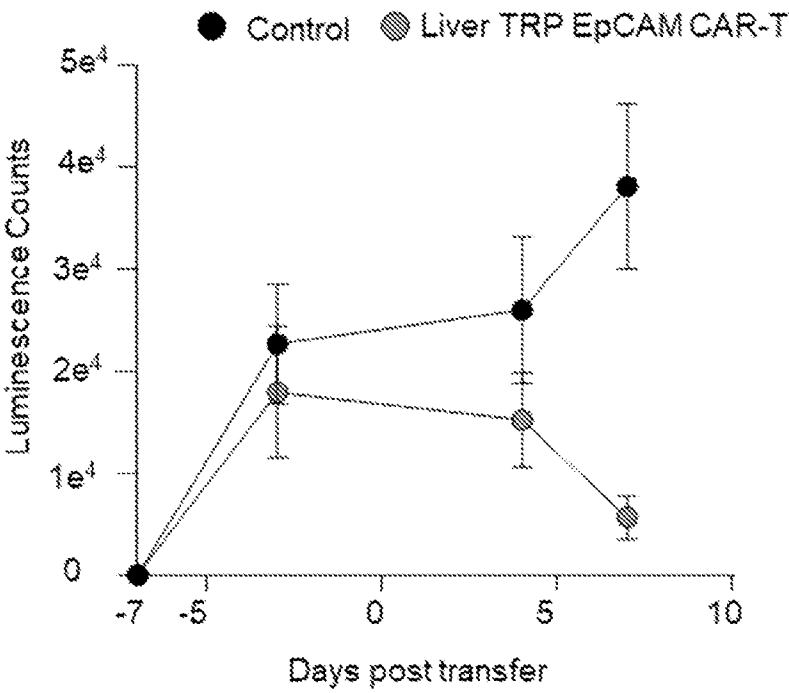


FIG. 5E

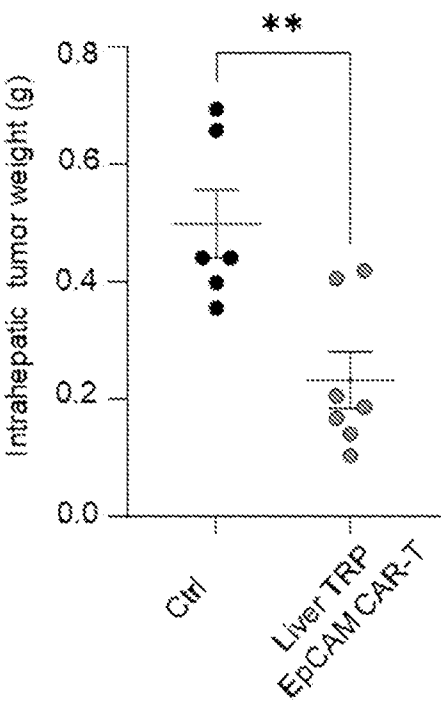


FIG. 6.

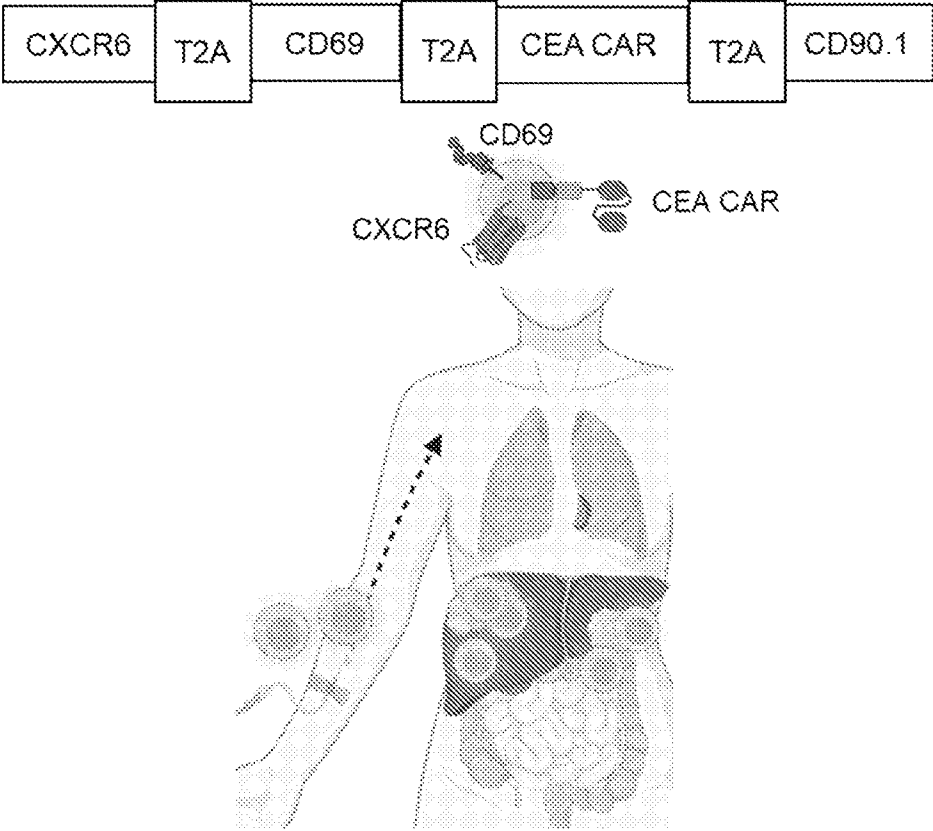
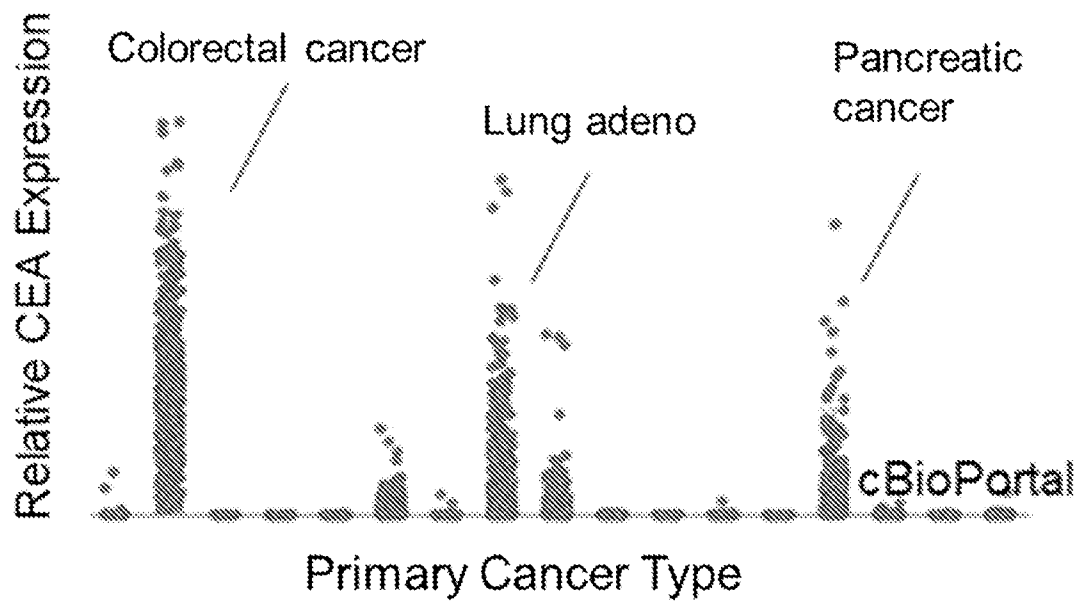


FIG. 7A



18/53

FIG. 7B

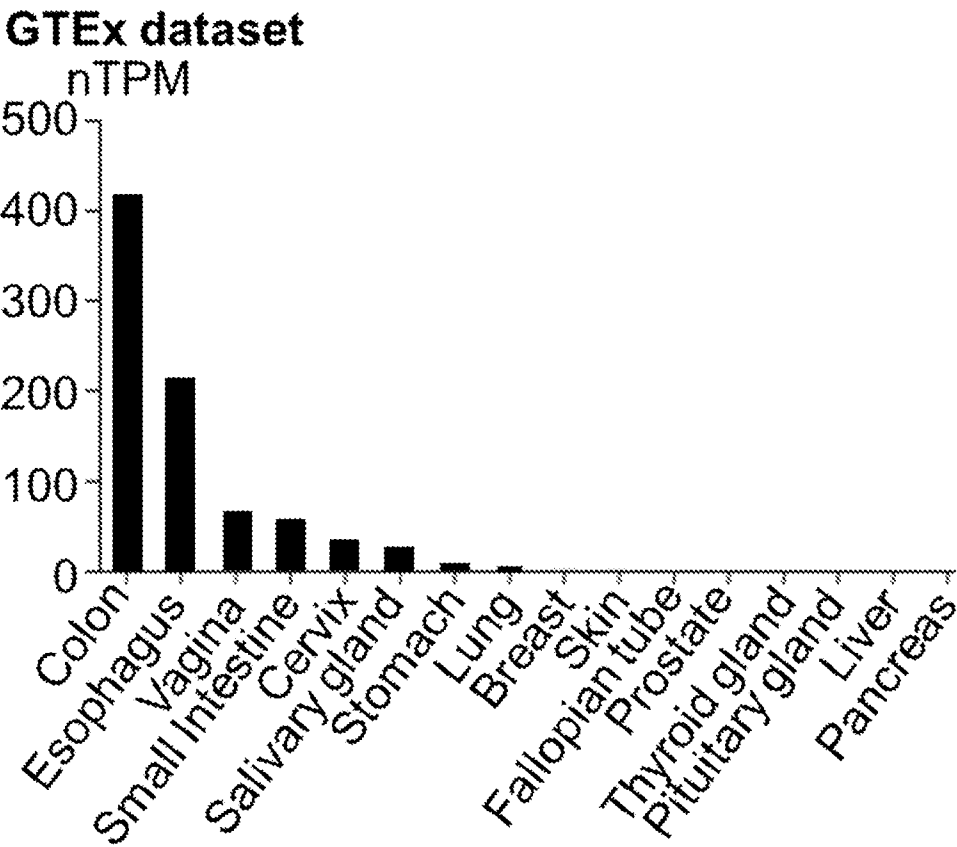
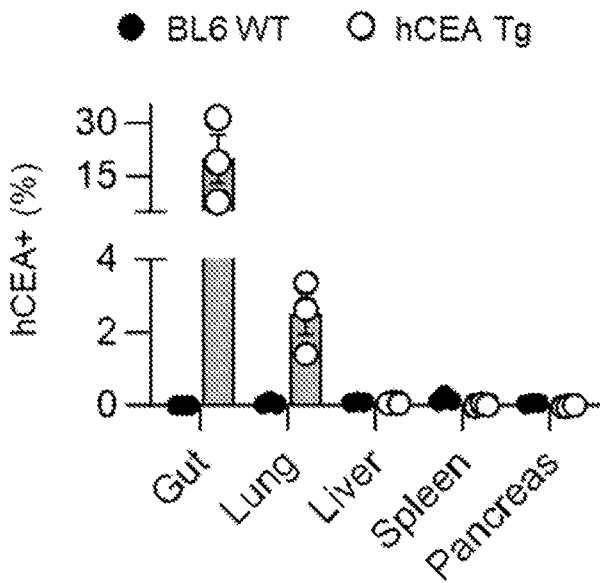


FIG. 8A



19/53

FIG. 8B

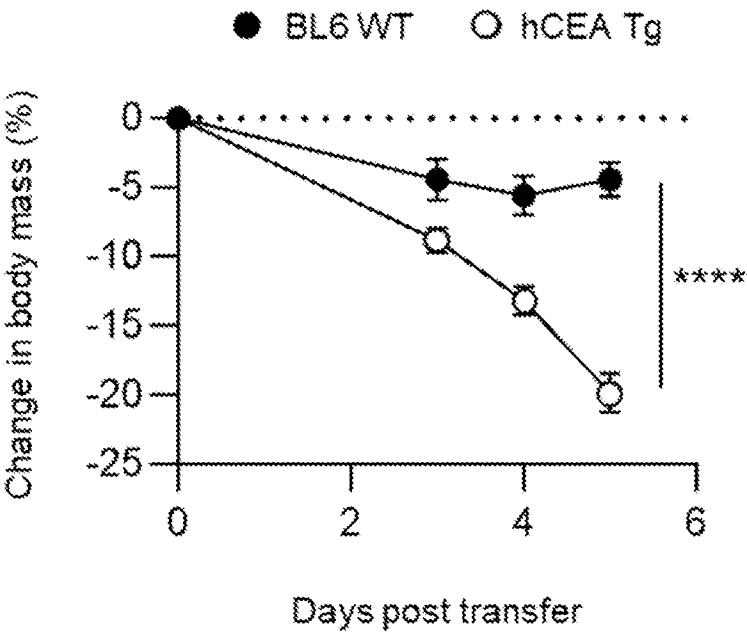
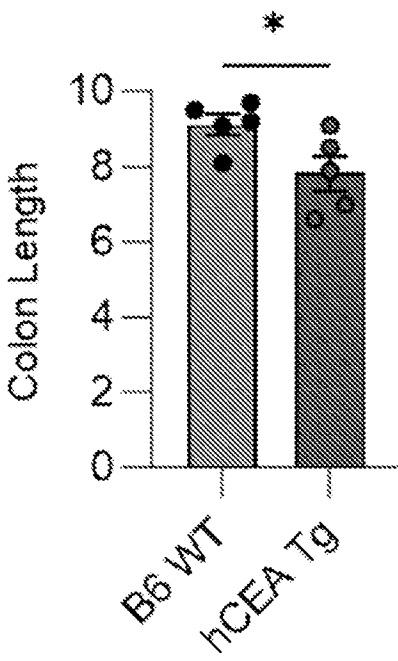


FIG. 8C



20/53

FIG. 9A

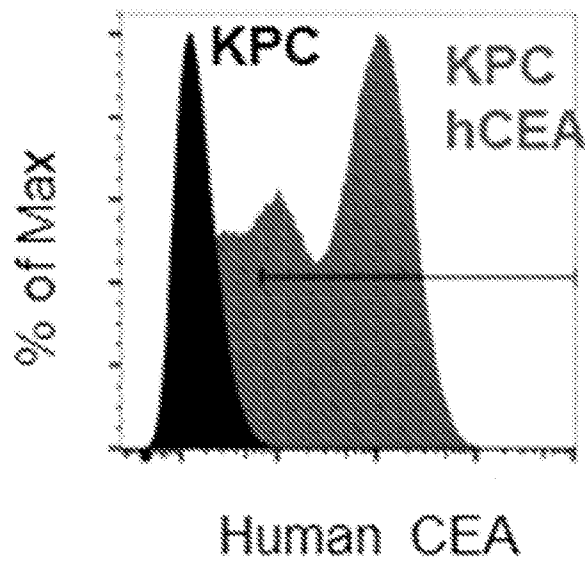
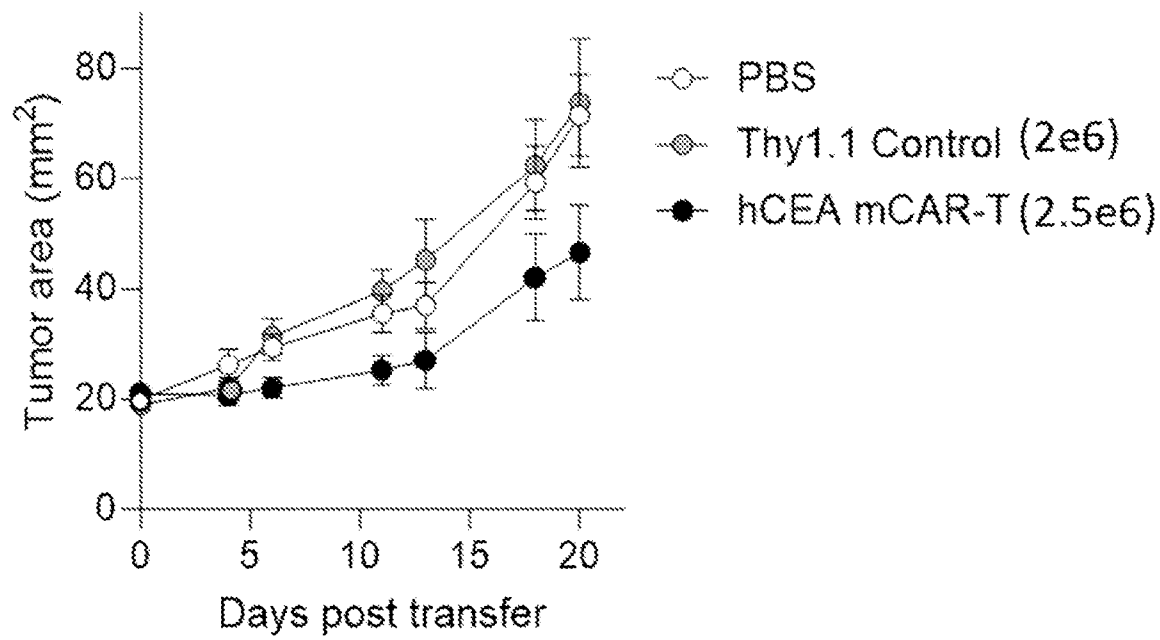


FIG. 9B



21/53

FIG. 10A

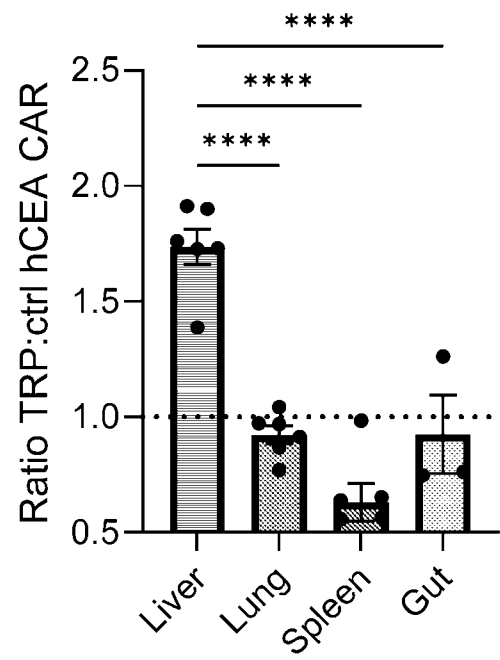


FIG. 10B

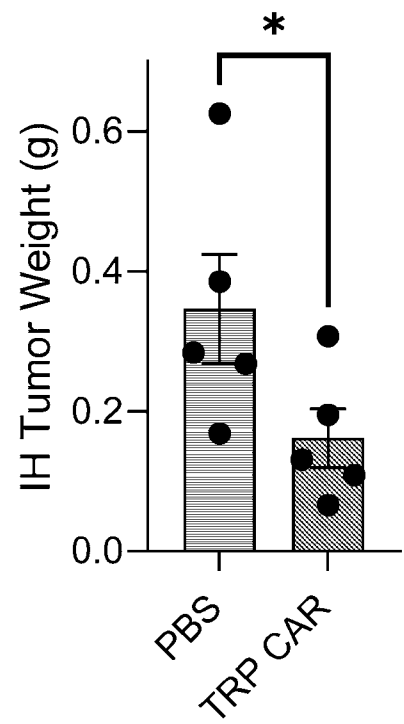


FIG. 11A

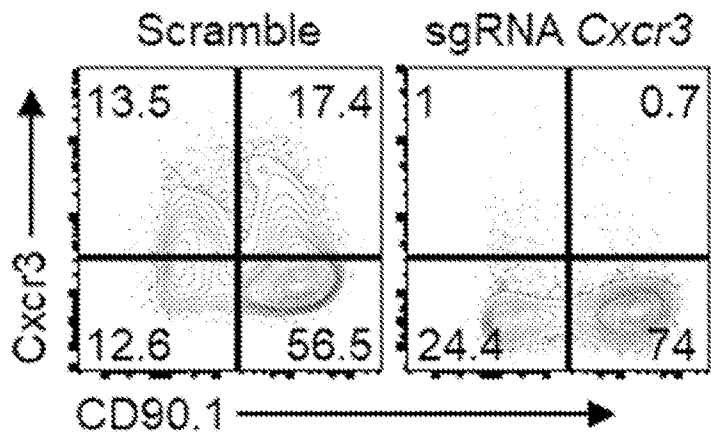


FIG. 11B

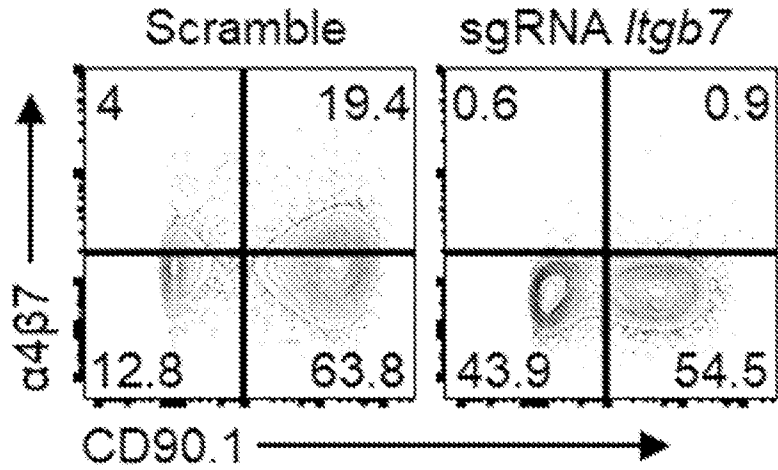


FIG. 12A

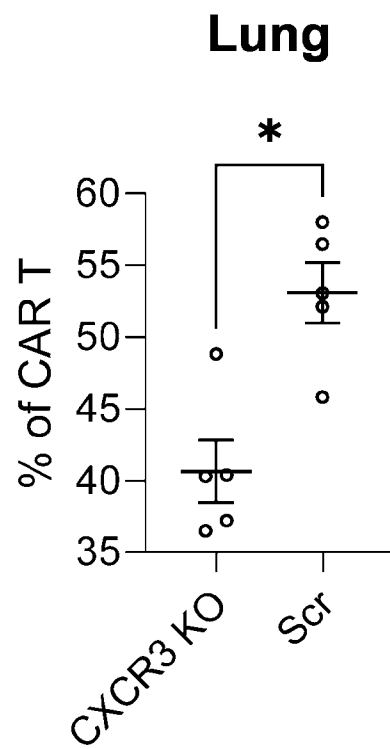


FIG. 12B

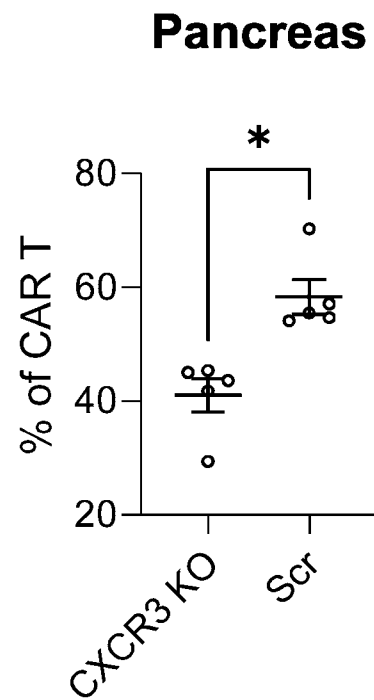
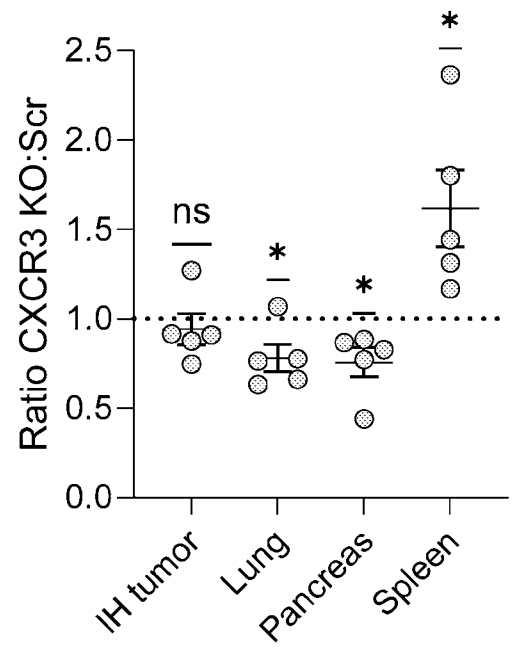


FIG. 12C



24/53

FIG. 13A

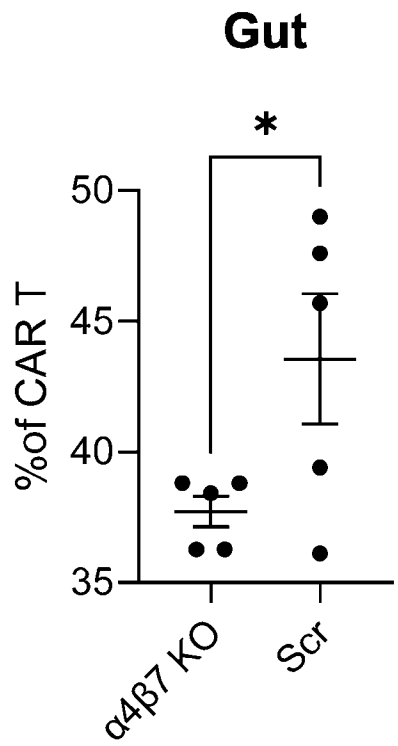
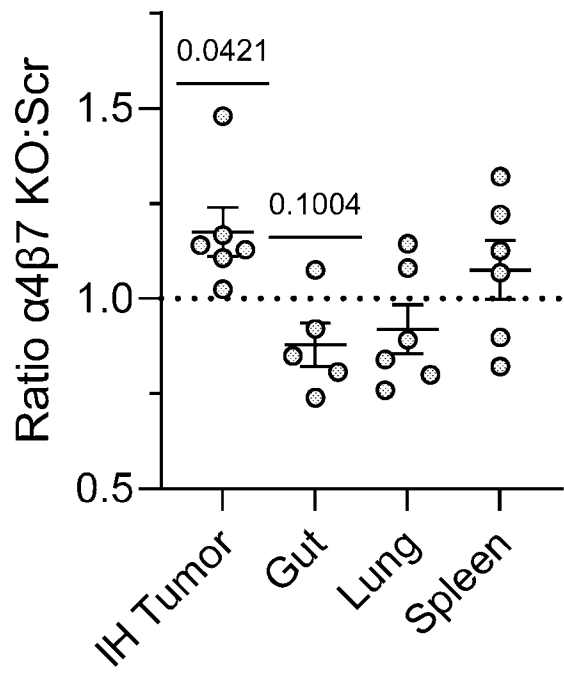


FIG. 13B



25/53

FIG. 14

Antigen Target	Sites of off-tumor toxicity	Primary Tumor	Desired site of activity	'Go here' (trafficking) Signal	Retention signal	'Don't go here' (inhibitory) signal
CEA	Colon	Colorectal	Liver	CXCR6 Exp	CD69 Exp	β 7 integrin KO
CEA	Colon	Pancreatic	Pancreas	Integrin β 2 (LFA-1) Exp, CCR1 Exp, CCR2 Exp	CD69 Exp	β 7 integrin KO
HER2	Lung	Breast, colon, gastric	Liver	CXCR6 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO
HER2	Lung	Breast	Bone	CXCR4 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO
PSA	Prostate	Prostate	Bone	CXCR4 Exp	CD69 Exp	CXCR3 KO
GPC3	Lung	Hepatocellular carcinoma	Liver	CXCR6 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO
Mesothelin	Lung	Pancreatic	Pancreas	Integrin β 2 (LFA-1) Exp, CCR1 Exp, CCR2 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO
Mesothelin	Lung	Pancreatic	Liver	CXCR6 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO
FOLR1	Lung, kidney	Ovarian, endometrial	Liver	CXCR6 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO, β 7 integrin KO
MUC1	Stomach, lung, kidney	Breast, uterine, ovarian, endometrial	Liver	CXCR6 Exp	CD69 Exp	CCR4 KO, CXCR3 KO, CCR3 KO, β 7

FIG. 14 cont'd

						integrin KO
CA19-9	Small intestine, colon	Pancreatic	Pancreas	Integrin β 2 (LFA-1) Exp, CCR1 Exp, CCR2 Exp	CD69 Exp	β 7 integrin KO
CA19-9	Small intestine, colon	Pancreatic	Liver	CXCR6 Exp	CD69 Exp	β 7 integrin KO

27/53

FIG. 15

CXCR6 Coding Sequence

ATGGCAGAGCATGACTATCACGAAGACTATGGTTTCTCTTCTTTAATGACAGCTCTCAAGA
GGAGCACCAGGATTTTCTCCAATTCTCAAAAGTATTCTTGCCATGCATGTACCTTGTGGTTT
TTGTCTGCGGCTTGGTAGGAAACAGTCTTGTGTTGGTAATTAGCATTTTTTACCATAAGCTC
CAATCTTTGACGGATGTTTTTTTGGTCAATCTCCCTCTCGCAGATCTTGTGTTTGTATGCAC
TCTCCCGTTTTGGGCATACGCTGGGATTACGAGTGGGTGTTTGGTCAAGTCATGTGCAAG
TCACTCCTGGGTATCTATACGATCAACTTTTACACGAGCATGCTCATTCTTACTTGCATTAC
AGTAGATCGGTTCATAGTGGTTGTGAAGGCGACCAAAGCATATAATCAACAAGCCAAAAGG
ATGACCTGGGGCAAGGTTACATCTCTGTTGATATGGGTTATCTCACTCTTGGTATCTTTGCC
CCAGATAATATACGGCAATGTATTTAATCTGGACAAACTCATCTGTGGCTACCATGACGAAG
CCATAAGTACCGTAGTGTGGCCACACAGATGACGCTCGGTTTCTTCCTGCCCTCCTGAC
AATGATTGTTTGCTATTCCGTAATAATTAAGACACTCCTTCATGCTGGCGGGTTCAGAAAC
ATAGATCTCTCAAGATCATTTTCTTGGTAATGGCTGTATTCCTCCTCACACAAATGCCGTTT
AACCTGATGAAATTCATTAGATCAACCCATTGGGAGTACTATGCGATGACAAGTTTCCATTA
TACAATTATGGTGACAGAAGCAATAGCGTATCTTAGGGCCTGTCTTAACCCGGTATTGTAC
GCTTTTGTCTCTCTGAAATTTAGGAAAAATTTTGAAGCTTGTAAAAGACATTGGCTGTCT
CCCGTACTTGGGCGTAAGCCATCAATGGAAAAGTTTCAGAAGATAATAGCAAGACGTTTAGT
GCTTCCCATACGTGGAGGCTACCTCCATGTTCCAACCTT (SEQ ID NO: 1)

RAKR Coding Sequence

AGGGCCAAGAGG (SEQ ID NO: 2)

T2A Coding Sequence

GAGGGCAGGGGCAGCCTGCTGACCTGCGGCGACGTGGAGGAGAACCCCGGCCCC (SEQ ID NO: 3)

CD69 Coding Sequence

ATGTCTAGCGAAAACCTGCTTTGTAGCAGAGAACTCCTCTTTGCATCCTGAATCCGGTCAGG
AAAACGATGCAACTTCACCACACTTCAGCACGCGGCATGAAGGGTCCTTCCAAGTGCCCG
TTCTTTGCGCCGTGATGAATGTGGTATTTATTACCATCCTTATAATAGCATTGATCGCGCTT
TCCGTTGGACAGTATAATTGTCCCGGACAGTACACGTTTTCAATGCCGTCTGACTCACACG
TCTCTTCTTGCAGTGAAGACTGGGTTGGGTATCAGCGGAAGTGCTACTTTATCTCTACAGT
AAAGCGGAGCTGGACCAGCGCCCAAATGCCTGCTCTGAACACGGGGCGACGCTCGCCG
TTATTGACTCAGAAAAAGATATGAACTTTCTCAAACGATACGCCGGAAGAGAGGAGCATTG
GGTAGGCCTTAAAAAGGAACCGGGTCATCCTTGGAATGGAGTAACGGTAAAGAGTTCAAT
AATTGGTTCAACGTTACAGGCTCAGACAAATGTGTTTTTCTTAAAAACACAGAGGTTAGTTC
AATGGAATGCGAAAAAAATTTGTATTGGATCTGCAATAAGCCATACAAA (SEQ ID NO: 4)

2A Coding Sequence

GAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCA (SEQ ID NO: 5)

Myc Coding Sequence

GAGCAGAAGCTGATCAGCGAGGAGGACCTG (SEQ ID NO: 6)

28/53

FIG. 15 cont'd

Extracellular Domain Spacer Coding Sequence

ATCGAGGTGATGTACCCCCCCCCCTACCTGGACAACGAGAAGAGCAACGGCACCATCATC
CACGTGAAGGGCAAGCACCTGTGCCCCAGCCCCCTGTTCCCCGGCCCCAGCAAGCCC
(SEQ ID NO: 7)

CD28 Transmembrane Domain Coding Sequence

TTCTGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTGACCGT
GGCCTTCATCATCTTCTGGGTG (SEQ ID NO: 8)

CD28 Intracellular Signaling Domain Coding Sequence

CGCAGCAAGCGCAGCCGCTGCTGCACAGCGACTACATGAACATGACCCCCCGCCGCCC
CGGCCCCACCCGCAAGCACTACCAGCCCTACGCCCCCCCCCGCGACTTCGCCGCCTACC
GCAGC (SEQ ID NO: 9)

CD3z Intracellular Signaling Domain Coding Sequence

CGCGTGAAGTTCAGCCGCAGCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCT
GTACAACGAGCTGAACCTGGGCGCGCGAGGAGTACGACGTGCTGGACAAGCGCCGCG
GCAGGGACCCCGAGATGGGCGGCAAGCCCCAGCGCCGCAAGAACCCCCAGGAGGGCCT
GTACAACGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGG
GCGAGCGCCGCGCGGCAAGGGCCACGACGGCCTGTACCAGGGCCTGAGCACCGCCAC
CAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCCGC (SEQ ID NO: 10)

IgG4 hinge-A

ESKYGPPCPPCP (SEQ ID NO: 11)

IgG4 hinge coding sequence-A

GAATCTAAGTACGGACCGCCCTGCCCCCTTGCCCT (SEQ ID NO: 12)

IgG4 hinge coding sequence-B

GAGTCTAAGTACGGACCGCCTTGCCCACCGTGCCCA (SEQ ID NO: 13)

IgG4 hinge coding sequence-C

GAGTCTAAGTACGGACCGCCCTGCCCCCTTGCCCT (SEQ ID NO: 14)

IgG4-CH2 domain -A

GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA (SEQ ID NO: 15)

IgG4-CH2 domain -B

APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA (SEQ ID NO: 16)

29/53

FIG. 15 cont'd

IgG4-CH2 domain encoding sequence-A

GGACCCAGCGTGTTCCCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACC
CCCGAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAA
CTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT
TCCAGAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAAC
GGCAAGGAATACAAGTGTAAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACC
ATCAGCAAGGCCAA (SEQ ID NO: 17)

IgG4-CH2 domain encoding sequence-B

GCCCCCCCCGTGGCCGACCCAGCGTGTTCCCTGTTCCCCCCCCAAGCCCAAGGACACCCT
GATGATCAGCCGGACCCCGAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACC
CCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG
CCCCGGGAGGAGCAGTTCCAGAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCA
CCAGGACTGGCTGAACGGCAAGGAATACAAGTGTAAAGGTGTCCAACAAGGGCCTGCCCAG
CAGCATCGAGAAAACCATCAGCAAGGCCAA (SEQ ID NO: 18)

IgG4-CH3 domain-A

KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQKSLSL (SEQ ID NO: 19)

IgG4 CH3 domain-B

KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQKSLSLSLGK (SEQ ID NO: 20)

IgG4-CH3 encoding sequence-A

AGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACC
AAGAATCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAACGGCCAGCCCCGAGAACAACCTACAAGACCACCCCCCTGTGCTGGA
CAGCGACGGCAGCTTCTTCCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGCAGG
AGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGA
AGAGCCTGTCCCTG (SEQ ID NO: 21)

IgG4 CH3 domain coding sequence-B

AGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACC
AAGAATCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAACGGCCAGCCCCGAGAACAACCTACAAGACCACCCCCCTGTGCTGGA
CAGCGACGGCAGCTTCTTCCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGCAGG
AGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGA
AGAGCCTGTCCCTGAGCCTGGGCAAG (SEQ ID NO: 22)

Intermediate Spacer (IgG4 hinge-CH3 domain)

ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQKSLSLSLGK (SEQ ID
NO: 23)

30/53

FIG. 15 cont'd

Intermediate Spacer encoding sequence (IgG4 hinge-CH3 domain)

GAATCTAAGTACGGACCGCCCTGCCCCCCTTGCCCTGGCCAGCCTCGGGAGCCCCAGGT
GTACACCCTGCCCCCTAGCCAAGAGGAGATGACCAAGAATCAGGTGTCCCTGACCTGCCT
GGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCCG
AGAACAACCTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACA
GCAGGCTGACCGTGGACAAGAGCCGGTGGCAGGAGGGCAACGTCTTTAGCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACCCCAGAAGAGCCTGTCCCTGAGCCTGGGCAAG
(SEQ ID NO: 24)

Long Spacer (IgG4 hinge-CH2 domain-CH3 domain)

ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR
EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLY
SRLTVDKSRWQEGNVFSCSVMHENHNTQKSLSL (SEQ ID NO: 25)

Long Spacer encoding sequence (IgG4 hinge-CH2 domain-CH3 domain)

GAATCTAAGTACGGACCGCCCTGCCCCCCTTGCCCTGCCCCCCCCGTGGCCGGACCCAG
CGTGTTCTGTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGT
GACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGTACG
TGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCCAGAGC
ACCTACCGGGTGGTGTCCGTGCTGACCGTGTGTCACCAGGACTGGCTGAACGGCAAGGA
ATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAA
GGCCAAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGA
TGACCAAGAATCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACCTACAAGACCACCCCCCCTGTG
CTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTG
GCAGGAGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGAGCCTGTCCCTG (SEQ ID NO: 26)

CD28 Transmembrane Domain-A

MFWWLVVVGVLACYSLLVTVAFIIFWV (SEQ ID NO: 27)

CD28 Transmembrane Domain - B

MFWWLVVVGVLACYSLLVTVAFIIFW (SEQ ID NO: 28)

CD28 Transmembrane Domain - C

FWWLVVVGVLACYSLLVTVAFIIFW (SEQ ID NO: 29)

CD28TM encoding sequence-A

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGAC
AGTGGCCTTCATCATCTTTTGGGTA (SEQ ID NO: 30)

CD28TM encoding sequence-B

ATGTTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGCTACAGCCTGCTGGTCAC
CGTGGCCTTCATCATCTTTTGGGTG (SEQ ID NO: 31)

31/53

FIG. 15 cont'd

CD28TM encoding sequence-C

ATGTTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGCTACAGCCTGCTGGTCAC
CGTGGCCTTCATCATCTTTTGG (SEQ ID NO: 32)

CD28TM encoding sequence-D

ATGTTCTGGGTGCTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGAC
AGTGGCCTTCATCATCTTTTGG (SEQ ID NO: 33)

4-1BB costimulatory domain -A

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL (SEQ ID NO: 34)

4-1BB costimulatory domain-B

VKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL (SEQ ID NO: 35)

4-1BB costimulatory domain -C

VKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC (SEQ ID NO: 36)

4-1BB signaling encoding sequence-A

AAGAGAGGACGGAAGAAGCTGCTGTATATCTTTAAGCAGCCTTTTATGAGACCTGTCCAAA
CAACCCAAGAGGAGGACGGGTGTTCTGTGCTTCCCAGAGGAGGAGGAAGGAGGGTG
GAGCTG (SEQ ID NO: 37)

4-1BB signaling encoding sequence-B

AAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAAC
TACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGA
ACTG (SEQ ID NO: 38)

4-1BB signaling coding sequence-C

GTGAAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTAC
AACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGAT
GTGAACTG (SEQ ID NO: 39)

CD28 signaling domain

RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 40)

CD28 signaling domain coding sequence

CGGAGCAAGCGGAGCAGAGGCGGCCACAGCGACTACATGAACATGACCCCCAGACGGCC
TGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCACCCAGGGACTTTGCCGCCTACC
GGTCC (SEQ ID NO: 41)

CD28/4-1BB signaling domain

RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEEGGCEL (SEQ ID NO: 42)

32/53

FIG. 15 cont'd

CD28/4-1BB signaling domain coding sequence

CGGAGCAAGCGGAGCAGAGGCGGCCACAGCGACTACATGAACATGACCCCCAGACGGCC
TGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCACCCAGGGACTTTGCCGCCTACC
GGTCCAAGAGAGGACGGAAGAAGCTGCTGTATATCTTTAAGCAGCCTTTTATGAGACCTGT
CCAAACAACCCAAGAGGAGGACGGGTGTTCTGTGCTTCCCAGAGGAGGAGGAAGGAG
GGTGCGAGCTG (SEQ ID NO: 43)

CD3 ζ signaling domain-A

GGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
NO: 44)

CD3 ζ signaling domain-B

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE
LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
45)

CD3 ζ signaling domain -C

ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY
NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
46)

CD3zeta signaling domain encoding sequence-A

GGCGGAGGGCGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCCTGCCTACCAGCAGGGCC
AGAATCAGCTGTACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATA
AGCGGAGAGGGCCGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGA
AGGCCTGTATAACGAAGTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCAT
GAAGGGCGAGCGGAGGCGGGGCAAGGGGCCACGACGGCCTGTATCAGGGCCTGTCCACC
GCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCAAGG (SEQ ID NO:
47)

CD3 ζ signaling domain encoding sequence-B

CGGGTGAAGTTCAGCAGAAGCGCCGACGCCCCCTGCCTACCAGCAGGGCCAGAATCAGCT
GTACAACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAGAG
GCCGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTAT
AACGAAGTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGA
GCGGAGGCGGGGCAAGGGGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAG
GATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCAAGG (SEQ ID NO: 48)

Thoseaasigna Virus 2A (T2A) Peptide-v1

LEGGGEGRGSLLTCGDVEENPGPR (SEQ ID NO: 49)

Thoseaasigna Virus 2A (T2A) Peptide-v2

GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 50)

33/53

FIG. 15 cont'd

Porcine Teschovirus-1 2a (P2A) Peptide

GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 51)

Equine Rhinitis A Virus (ERAV) 2A (E2A) Peptide

GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 52)

Foot-And-Mouth Disease Virus 2A (F2A) Peptide

GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 53)

T2A encoding sequence-v1CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAA
TCCCGGCCCTAGG (SEQ ID NO: 54)**T2A encoding sequence-v2**CTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAA
TCCAGGCCCTAGG (SEQ ID NO: 55)**EGFRt**MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSSISGDLHILPVAFRGD
SFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGR TKQH GQFSLAVVSLNIT
SLGLRSLKEISDGDVIIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCS
PEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPRFVENSECIQCHPECLPQAMNITCTG
RPGDNCIQCAHYIDGPHCVKTC PAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLE
GCPTNGPKIPSIATGMV GALLLLL VVALGIGLFM (SEQ ID NO: 56)**EGFRt encoding sequence**ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCTCCTGA
TCCCACGCAAAGTGTGTAAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAAT
GCTACGAATATTAACACTTCAAAAAC TGCACCTCCATCAGTGGCGATCTCCACATCCTGC
CGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAAGTGA
TATTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGTGATTGAGGCTTGGCCTGAAAAC
AGGACGGACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACAT
GGTCAGTTTTCTCTTGAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCA
AGGAGATAAGTGATGGAGATGTGATAATTT CAGGAAACAAAAATTTGTGCTATGCAAATACA
ATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAG
GTGAAAACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGC
TGCTGGGGCCCCGGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGA
ATGCGTGGACAAGTGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGA
GTGCATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACG
GGGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGAC
CTGCCCCGGCAGGAGTCATGGGAGAAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCG
GCCATGTGTGCCACCTGTGCCATCCAACTGCACCTACGGATGCACTGGGCCAGGTCTTG
AAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCC
CTCCTCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATG (SEQ ID NO: 57)

34/53

FIG. 15 cont'd

tCD19 coding sequence

ATGCCACCTCCAAGACTCCTCTTCTTCCTCCTCCTGACACCAATGGAAGTCAGGCCTG
AGGAACCTCTAGTGGTGAAGGTGGAAGAGGGAGATAACGCTGTGTTACAGTGCCTCAAGG
GAACCTCAGATGGACCCACTCAGCAGCTGACCTGGTCTCGGGAGTCTCCGCTTAAACCCT
TCCTGAAACTCAGCCTTGGACTGCCAGGTCTGGGAATCCACATGAGGCCACTGGCTATCT
GGCTGTTTCATCTTCAACGTCTCTCAACAGATGGGAGGCTTCTACCTGTGTCAGCCTGGACC
ACCTTCTGAGAAGGCATGGCAGCCTGGTTGGACAGTCAATGTGGAGGGTTCTGGTGAGCT
GTTCCGGTGAATGTTTCGGACCTAGGTGGACTGGGATGTGGTCTGAAGAACAGGTCCTC
AGAGGGACCTAGCTCTCCTTCCGGGAAGCTCATGAGCCCCAAGCTGTATGTGTGGGCCAA
AGACCGCCCTGAGATCTGGGAGGGAGAGCCTCCGTGTGTCCACCGAGGGACAGCCTGA
ACCAGAGCCTCAGCCAGGACCTCACCATGGCCCCTGGCTCCACACTCTGGCTGTCTGTG
GGGTACCCCTGACTCTGTGTCCAGGGGCCCTCTCCTGGACCCATGTGCACCCCAAG
GGGCTAAGTCATTGCTGAGCCTAGAGCTGAAGGACGATCGCCCTGCCAGAGATATGTGG
GTAATGGAGACGGGTCTGTTGTTGCCCCGGGCCACAGCTCAAGACGCTGGAAAGTATTAT
TGTCACCGTGGAACCTGACCATGTCAATCCACCTGGAGATCACTGCTCGGCCAGTACTAT
GGCACTGGCTGCTGAGGACTGGTGGCTGGAAGGTCTCAGCTGTGACTTTGGCTTATCTGA
TCTTCTGCCTGTGTTCCCTTGTGGGCATTCTTCATCTTCAAAGAGCCCTGGTCCTGAGGAG
GAAAAGATGA (SEQ ID NO: 58)

CD90.1 (THY1.1)

NPAISVALLLSVLQVSRGQKVTSLTACLVNQNLRLDCRHENNTKDNSIQHEFSLTREKRKHVLS
GTLGIPEHTYRSRVTLNQPYIKVLTLANFTTKDEGDYFCELRVSGANPMSSNKSISVYRDKLVK
CGGISLLVQNTSWMLLLLLLSLLQALDFISL (SEQ ID NO: 198)

Retro_CXCR6_CD69_myc-hCEACAR

TCGAGCTAAGCTTCGTATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCGATAAAATA
AAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGC
AAGCTAGCTTAAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAA
GTTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTG
GTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCG
CCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATG
ACCTGTGCCTTATTTGAACTAACCAATCAGTTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTG
CTCCCCGAGCTCAATAAAAGAGCCCAACAACCCCTCACTCGGCGCGCCAGTCCTCCGATAG
ACTGCGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAAGTTGCATCCGACTTGTG
GTCTCGCTGTTCCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTGACGCGGGGTCT
TTCATGGGTAAACAGTTTCTTGAAGTTGGAGAACAAACATTCTGAGGGTAGGAGTCGAATATT
AAGTAATCCTGACTCAATTAGCCACTGTTTTGAATCCACATACTCCAATACTCCTGAAATCC
ATCGATGGAGTTCATTATGGACAGCGCAGAAAGAGCTGGGGAGAAATTGTGAAATTGTTATC
CGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCT
AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAA
CCTGTCTGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTCGTAT
TGGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCGGCTGCGGCG
AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC
AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAGGCCGCGTT
GCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAACAGGCGTTTCCCCCTGGAAGCTC
CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC

35/53

FIG. 15 cont'd

GTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTA
TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAG
TGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGC
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTT
TGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTA
AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA
CCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG
CAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCG
TGGTGTACGCTCGTCGTTTGGTATGGCTTCATTACAGTCCGGTTCCTAACGATCAAGGCG
AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT
GTCAGAAGTAAGTTGGCCGAGTGTTATCACTCATGTTTATGGCAGCACTGCATAATTCTC
TTACTGTCATGCCATCCGTAAGATGCTTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTC
TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACC
GCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAAC
TCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCAACTG
ATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAT
GCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTT
AGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTA
AGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTC
TCGCGCGTTTCCGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
CAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTGCGGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTAAGTGAAGTG
CACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGC
CATTCGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGGCTCTTCGCTA
TTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAACGCCAGG
GTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCACGCTCTCCCTTATGCGACTC
CTGCATTAGGAAGCAGCCAGTAGTAGGTTGAGGCGGTTGAGCACCGCCGCGCGCAAGGA
ATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATA
CCCACGCCGAAACAAGCGCTCATGAGCCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGT
GATGTGCGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGA
TGCGTCCGGCGTAGAGGCGATTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGA
CTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAATAAAAGATTTTA
TTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTT
AAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTCAGATCA
AGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTT
CCTGCCCCGGCTCAGGGGCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAG
TTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCC
TTATTTGAACTAACCAATCAGTTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAG
CTCAATAAAAGAGCCCAACCCCTCACTCGGCGCGCCAGTCCTCCGATAGACTGCGTCG
CCCGGGTACCCGATTTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGAAGTCTGCT
GATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGCCCACCTCGGGGGTCTTTCATTTGGA
GGTTCCACCGAGATTTGGAGACCCCTGCCTAGGGACCACCGACCCCCCGCGGGGAGGT
AAGCTGGCCAGCGGTCTTTTCGTGTCTGTCTCTGTCTTTGTGCGTGTTTGTGCCGGCATCT

36/53

FIG. 15 cont'd

AATGTTTGGCGCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG
TGGAAGTACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCG
GGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGACTCTTTGGTG
CACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAACAGTTCC
CGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCGCCGCGCGTCTTGTCT
GCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAGAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTGCG
AGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCT
CTGCAGAATGGCCAACCTTTAACGTGGGATGGCCGCGAGACGGCACCTTTAACCGAGACC
TCATCACCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGACACCCAGACCAGGT
CCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTT
TGTAACCCCTAAGCCTCCGCTCCTCTTCCCTCCATCCGCCCGTCTCTCCCCCTTGAACCT
CCTCGTTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCC
CCATATGGCCATATGAGATCTTATATGGGGCACCCCGCCCTTGTAACTTCCCTGACCC
TGACATGACAAGAGTTACTAACAGCCCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTA
GTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAAGTGGACCGACC
GGTGGTACCTCACCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAA
GAACCTAGAACCTCGCTGGAAGGACCTTACACAGTCCTGCTGACCACCCCGACCGCCCT
CAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCG
GGGGTGGACCATCCTCTAGACCGCCATGGCAGAGCATGACTATCACGAAGACTATGGTTT
CTCTTCTTTTAAATGACAGCTCTCAAGAGGAGCACCAAGATTTTCTCCAATTCTCAAAAGTAT
TCTTGCCATGCATGTACCTTGTGGTTTTTGTCTGCGGCTTGGTAGGAAACAGTCTTGTGTT
GGTAATTAGCATTTTTTACCATAAGCTCCAATCTTTGACGGATGTTTTTTTGGTCAATCTCCC
TCTCGCAGATCTTGTGTTTGTATGCACTCTCCCGTTTTTGGGCATACGCTGGGATTACAGAG
TGGGTGTTTGGTCAAGTCATGTGCAAGTCACTCCTGGGTATCTATACGATCAACTTTTACAC
GAGCATGCTCATTCTTACTTGCATTACAGTAGATCGGTTTCATAGTGGTTGTGAAGGCGACC
AAAGCATATAATCAACAAGCCAAAAGGATGACCTGGGGCAAGGTTACATCTCTGTTGATAT
GGGTATCTCACTCTTGGTATCTTTGCCCCAGATAATATACGGCAATGTATTTAATCTGGAC
AAACTCATCTGTGGCTACCATGACGAAGCCATAAGTACCGTAGTGTTGGCCACACAGATGA
CGCTCGGTTTTCTTCTGCCCCCTCCTGACAATGATTGTTTGCTATTCCGTAATAATTAAGACA
CTCCTTCATGCTGGCGGGTTCAGAAACATAGATCTCTCAAGATCATTTTCTTGGTAATGGC
TGTAATCCTCCTCACACAAATGCCGTTTAACTGATGAAATTCATTAGATCAACCCATTGGG
AGTACTATGCGATGACAAGTTTCCATTATACAATTATGGTGACAGAAGCAATAGCGTATCTT
AGGGCCTGTCTTAACCCGGTATTGTACGCTTTTTGTCTCTCTGAAATTTAGGAAAAATTTTG
GAAGCTTGTAAGAGACATTGGCTGTCTCCCGTACTTGGGCGTAAGCCATCAATGGAAAAGT
TCAGAAGATAATAGCAAGACGTTTGTGCTTCCCATACGTGGAGGCTACCTCCATGTTCC
AACTTATGGGCAACAGCAGGGCCAAGAGGAGCGGCAGCGGCGAGGGCAGGGGCAGCCT
GCTGACCTGCGGCGACGTGGAGGAGAACCCCGGCCCGCCAGCATGTCTAGCGAAAACCT
GCTTTGTAGCAGAGAACTCCTCTTTCATCCTGAATCCGGTCAGGAAAACGATGCAACTTC
ACCACACTTCAGCACGCGGCATGAAGGGTCCTTCCAAGTGCCCGTTCTTTGCGCCGTGAT
GAATGTGGTATTTATTACCATCCTTATAATAGCATTGATCGCGCTTCCGTTGGACAGTATA
ATTGTCCTCGGACAGTACACGTTTTCAATGCCGTCTGACTCACACGTCTCTTCTTGCAGTGA
AGACTGGGTTGGGTATCAGCGGAAGTGCTACTTTATCTCTACAGTAAAGCGGAGCTGGAC
CAGCGCCCAAAATGCCTGCTCTGAACACGGGGCGACGCTCGCCGTTATTGACTCAGAAAA
AGATATGAACTTTCTCAAACGATACGCCGGAAGAGAGGAGCATTGGGTAGGCCTTAAAAAG
GAACCGGGTCATCCTTGGAAATGGAGTAACGGTAAAGAGTTCAATAATTGGTTCAACGTTA
CAGGCTCAGACAAATGTGTTTTTCTTAAAAACACAGAGGTTAGTTCAATGGAATGCGAAAAA
AATTTGTATTGGATCTGCAATAAGCCATACAAAGCGAATTCACGTGCCAAGCGAAGCGGAA
GCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCA
GCTAGCAACATGCTGCGCCTGCTGCTGGCCCTGAACCTGTTCCCCAGCATCCAGGTGACC

37/53

FIG. 15 cont'd

GGCGGCAGCAGCGAGCAGAAGCTGATCAGCGAGGAGGACCTGCAGACCGTGCTGACCCA
GAGCCCCAGCAGCCTGAGCGTGAGCGTGGGCGACCGCGTGACCATCACCTGCCGCGCCA
GCAGCAGCGTGACCTACATCCACTGGTACCAGCAGAAGCCCCGGCCTGGCCCCCAAGAGC
CTGATCTACGCCACCAGCAACCTGGCCAGCGGCGTGCCAGCCGCTTCAGCGGCAGCGG
CAGCGGCACCGACTACACCTTCACCATCAGCAGCCTGCAGCCCCGAGGACATCGCCACCTA
CTACTGCCAGCACTGGAGCAGCAAGCCCCCACCTTCGGCCAGGGCACCAAGGTGGAGG
TGAAGCGCACCGTGCGCGCCCCCAGCGTGTTTCATCTTCGGCAGCGGCGGCGGCGGCAG
CGGCGGCGGCGGCGGCGAGGTGCAGCTGCTGGAGAGCGGCGGCGGCGGCGCTGGTGCAGCC
CGGCGGCGAGCCTGCGCCTGAGCTGCGCCACCAGCGGCTTCACCTTCACCGACTACTACAT
GAACTGGGTGCGCCAGGCCCGCGCAAGGGCCTGGAGTGGCTGGGCTTCATCGGCAACA
AGGCCAACGGCTACACCACCGAGTACAGCGCCAGCGTGAAGGGCCGCTTCACCATCAGC
CGCGACAAGAGCAAGAGCACCCCTGTACCTGCAGATGAACACCCTGCAGGCCGAGGACAG
CGCCATCTACTACTGCACCCGCGACCGCGGCGCTGCGCTTCTACTTCGACTACTGGGGCCA
GGGCACCCTGGTGACCGTGAGCAGCGCCAGCACCAAGGGCATCGAGGTGATGTACCCCC
CCCCCTACCTGGACAACGAGAAGAGCAACGGCACCATCATCCACGTGAAGGGCAAGCACCC
TGTGCCCCAGCCCCCTGTTCCCCGGCCCCAGCAAGCCCTTCTGGGTGCTGGTGGTGGTG
GGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTGACCGTGGCCTTCATCATCTTCTGGGTG
CGCAGCAAGCGCAGCCGCGCTGCTGCACAGCGACTACATGAACATGACCCCCCGCGCGCC
CGGCCCCACCCGCAAGCACTACCAGCCCTACGCCCCCCCCCGCGACTTCGCCGCGCTACC
GCAGCCGCGTGAAGTTACAGCCGCAGCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAAC
CAGCTGTACAACGAGCTGAACCTGGGCGCGCGGAGGAGTACGACGTGCTGGACAAGCG
CCGCGGCGAGGGACCCCGAGATGGGCGGCAAGCCCCAGCGCCGCAAGAACCCCCAGGAG
GGCCTGTACAACGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCAT
GAAGGGCGAGCGCCGCGCGGCAAGGGCCACGACGGCCTGTACCAGGGCCTGAGCACC
GCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCCGCTGAGCGGC
CGCTACGTAGTCGACGGATCCC (SEQ ID NO: 59)

Retro_CXCR6_CD69_hCEACAR

TCGAGCTAAGCTTCGTATTCTGCAGTCGACGGTACCGCGGGCCCCGGGATCCGATAAAATA
AAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGC
AAGCTAGCTTAAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAA
GTTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTG
GTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCG
CCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATG
ACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCCGCGCGCTTCTG
CTCCCCGAGCTCAATAAAAGAGCCCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATAG
ACTGCGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAAGTTGCATCCGACTTGTG
GTCTCGCTGTTCCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTGACGCGGGGGTCT
TTCATGGGTAACAGTTTCTTGAAGTTGGAGAACAACATTCTGAGGGTAGGAGTCGAATATT
AAGTAATCCTGACTCAATTAGCCACTGTTTTGAATCCACATACTCCAATACTCCTGAAATCC
ATCGATGGAGTTCATTATGGACAGCGCAGAAAGAGCTGGGGAGAATTGTGAAATTGTTATC
CGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCT
AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAA
CCTGTCTGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGGCGGTTTGCAT
TGGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCGGCTGCGGCG
AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC
AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTT
GCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTC
CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCGCTTCTCCCT

38/53

FIG. 15 cont'd

TCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC
GTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA
TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAG
TGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGC
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTAAAGGATTT
TGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAATGAAGTTTA
AATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA
CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG
CAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCG
TGGTGTACGCTCGTCGTTTGGTATGGCTTCATTACGCTCCGGTTCCTAACGATCAAGGCG
AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT
GTCAGAAGTAAGTTGGCCGCGAGTGTTATCACTCATGTTATGGCAGCACTGCATAATTCTC
TTACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACC
GCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAACGTTCTTCGGGGCGAAAAAC
TCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTG
ATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAAT
GCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTATTT
AGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTA
AGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTC
TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
CAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTGCGGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTAAGTGAAGT
CACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGC
CATTCGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTA
TTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAAACGCCAGG
GTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCACGCTCTCCCTTATGCGACTC
CTGCATTAGGAAGCAGCCAGTAGTAGGTTGAGGCGGTTGAGCACCGCCGCGCAAGGA
ATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATA
CCCACGCCGAAACAAGCGCTCATGAGCCCCGAAGTGCGAGCCCGATCTTCCCCATCGGT
GATGTGCGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGA
TGCGTCCGGCGTAGAGGCGATTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGA
CTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAAATAAAGATTTTA
TTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTT
AAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTCAGATCA
AGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTT
CCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAG
TTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCC
TTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTGCGCGCTTCTGCTCCCCGAG
CTCAATAAAAGAGCCCAACCCCTCACTCGGCGCGCCAGTCCTCCGATAGACTGCGTCG
CCCGGGTACCGTATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGAAGTCTGCT
GATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGCCACCTCGGGGGTCTTTCATTTGGA
GGTCCACCGAGATTGGAGACCCCTGCCTAGGGACCACCGACCCCCCGCGGGGAGGT

39/53

FIG. 15 cont'd

AAGCTGGCCAGCGGTCGTTTCGTGTCTGTCTCTGTCTTTGTGCGTGTTTGTGCCGGCATCT
AATGTTTGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG
TGGAAGTACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCG
GGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGACTCTTTGGTG
CACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAACAGTTCC
CGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCGCCGCGCGTCTTGTCT
GCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAGAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTGCG
AGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTACCTTCTGCT
CTGCAGAATGGCCAACCTTTAACGTGCGATGGCCGCGAGACGGCACCTTTAACCGAGACC
TCATCACCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGACACCCAGACCAGGT
CCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTT
TGTAACCCCTAAGCCTCCGCTCCTCTTCCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCT
CCTCGTTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCC
CCATATGGCCATATGAGATCTTATATGGGGCACCCCGCCCCCTTGTAACCTTCCCTGACCC
TGACATGACAAGAGTTACTAACAGCCCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTA
GTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAAGTGGACCGACC
GGTGGTACCTCACCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAA
GAACCTAGAACCTCGCTGGAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCT
CAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCG
GGGGTGGACCATCCTCTAGACCGCCATGGCAGAGCATGACTATCACGAAGACTATGGTTT
CTCTTCTTTAATGACAGCTCTCAAGAGGAGCACCCAGGATTTTCTCCAATTCTCAAAAGTAT
TCTTGCCATGCATGTACCTTGTGGTTTTTGTCTGCGGCTTGGTAGGAAACAGTCTTGTGTT
GGTAATTAGCATTTTTTACCATAAGCTCCAATCTTTGACGGATGTTTTTTGGTCAATCTCCC
TCTCGCAGATCTTGTGTTTGTATGCACTCTCCCGTTTTTGGGCATACGCTGGGATTCACGAG
TGGGTGTTTGGTCAAGTCATGTGCAAGTCACTCCTGGGTATCTATACGATCAACTTTTACAC
GAGCATGCTCATTCTTACTTGCATTACAGTAGATCGGTTTCATAGTGGTTGTGAAGGCGACC
AAAGCATATAATCAACAAGCCAAAAGGATGACCTGGGGCAAGGTTACATCTCTGTTGATAT
GGGTATCTCACTCTTGGTATCTTTGCCCCAGATAATATACGGCAATGTATTTAATCTGGAC
AACTCATCTGTGGCTACCATGACGAAGCCATAAGTACCGTAGTGTTGGCCACACAGATGA
CGCTCGGTTTTCTTCTGCCCCCTCCTGACAATGATTGTTTGTATTCCGTAATAATTAAGACA
CTCCTTCATGCTGGCGGGTTCAGAAACATAGATCTCTCAAGATCATTTTCTTGGTAATGGC
TGTAATCCTCCTCACACAAATGCCGTTTAACTGATGAAATTCATTAGATCAACCCATTGGG
AGTACTATGCGATGACAAGTTTCCATTATACAATTATGGTGACAGAAGCAATAGCGTATCTT
AGGGCCTGTCTTAACCCGGTATTGTACGCTTTTGTCTCTCTGAAATTTAGGAAAAATTTTG
GAAGCTTGTAAGAGACATTGGCTGTCTCCCGTACTTGGGCGTAAGCCATCAATGGAAAAGT
TCAGAAGATAATAGCAAGACGTTTGTGCTTCCCATACGTGGAGGCTACCTCCATGTTCC
AACTTATGGGCAACAGCAGGGCCAAGAGGAGCGGCAGCGGCGAGGGCAGGGGCAGCCT
GCTGACCTGCGGCGACGTGGAGGAGAACCCCGGCCCGCCAGCATGTCTAGCGAAAACCT
GCTTTGTAGCAGAGAACTCCTCTTTCATCCTGAATCCGGTCAGGAAAACGATGCAACTTC
ACCACACTTCAGCACGCGGCATGAAGGGTCTTCCAAGTGCCCGTTCTTTGCGCCGTGAT
GAATGTGGTATTTATTACCATCCTTATAATAGCATTGATCGCGCTTTCGGTTGGACAGTATA
ATTGTCCCGGACAGTACACGTTTTCAATGCCGTCTGACTCACACGTCTCTTCTTGCAGTGA
AGACTGGGTGGGTATCAGCGGAAGTGCTACTTTATCTCTACAGTAAAGCGGAGCTGGAC
CAGCGCCCAAAATGCCTGCTCTGAACACGGGGCGACGCTCGCCGTTATTGACTCAGAAAA
AGATATGAACTTTCTCAAACGATACGCCGGAAGAGAGGAGCATTGGGTAGGCCTTAAAAAG
GAACCGGGTCATCCTTGGAAATGGAGTAACGGTAAAGAGTTCAATAATTGGTTCAACGTTA
CAGGCTCAGACAAATGTGTTTTTCTTAAAAACACAGAGGTTAGTTCAATGGAATGCGAAAAA
AATTTGTATTGGATCTGCAATAAGCCATACAAAGCGAATTCACGTGCCAAGCGAAGCGGAA
GCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCA

40/53

FIG. 15 cont'd

GCTAGCAACATGCTGCGCCTGCTGCTGGCCCTGAACCTGTTCCCCAGCATCCAGGTGACC
GGCGGCAGCAGCCAGACCGTGCTGACCCAGAGCCCCAGCAGCCTGAGCGTGAGCGTGG
GCGACCGCGTGACCATCACCTGCCGCGCCAGCAGCAGCGTGACCTACATCCACTGGTAC
CAGCAGAAGCCCCGGCCTGGCCCCCAAGAGCCTGATCTACGCCACCAGCAACCTGGCCAG
CGGCGTGCCAGCCGCTTCAGCGGCAGCGGCAGCGGCACCGACTACACCTTCACCATCA
GCAGCCTGCAGCCCCGAGGACATCGCCACCTACTACTGCCAGCACTGGAGCAGCAAGCCC
CCCACCTTCGGCCAGGGCACCAAGGTGGAGGTGAAGCGCACCGTGCCCGCCCCCAGCGT
GTTTCATCTTCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGAGGTGCAGCTG
CTGGAGAGCGGCGGCGGCGCCTGGTGACGCCCGGCGGCAGCCTGCGCCTGAGCTGCGCCA
CCAGCGGCTTCACCTTCACCGACTACTACATGAACTGGGTGCGCCAGGCCCCCCGGCAAG
GGCCTGGAGTGGCTGGGCTTCATCGGCAACAAGGCCAACGGCTACACCACCGAGTACAG
CGCCAGCGTGAAGGGCCGCTTCACCATCAGCCGCGACAAGAGCAAGAGCACCCCTGTACC
TGCAGATGAACACCCTGCAGGCCGAGGACAGCGCCATCTACTACTGCACCCGCGACCGC
GGCCTGCGCTTCTACTTTCGACTACTGGGGCCAGGGCACCTGGTGACCGTGAGCAGCGC
CAGCACCAAGGGCATCGAGGTGATGTACCCCCCCCCCTACCTGGACAACGAGAAGAGCAA
CGGCACCATCATCCACGTGAAGGGCAAGCACCTGTGCCCCAGCCCCCTGTTCCCCGGCC
CCAGCAAGCCCTTCTGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGCCTG
CTGGTGACCGTGGCCTTCATCATCTTCTGGGTGCGCAGCAAGCGCAGCCGCCTGCTGCAC
AGCGACTACATGAACATGACCCCCCGCCGCCCGGCCCCACCCGCAAGCACTACCAGCC
CTACGCCCCCCCCCGCGACTTCGCCGCTACCGCAGCCGCGTGAAAGTTCAGCCGCAGCG
CCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGC
CGCCGCGAGGAGTACGACGTGCTGGACAAGCGCCGCGGCAGGGACCCCGAGATGGGCG
GCAAGCCCCAGCGCCGCAAGAACCCCCAGGAGGGCCTGTACAACGAGCTGCAGAAGGAC
AAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGCCGCCGCGGCAAGG
GCCACGACGGCCTGTACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTG
CACATGCAGGGCCCTGCCCCCCCGCTGAGCGGCCGCTACGTAGTCGACGGATCCC (SEQ
ID NO: 60)

Lenti_MND_hs_CXCR6_CD69_myc-hCEACAR

AGCTTAATGTAGTCTTATGCAATACTCTTGTAAGTCTTGCAACATGGTAACGATGAGTTAGCA
ACATGCCTTACAAGGAGAGAAAAAGCACCGTGATGCCGATTGGTGGAAGTAAGGTGGTA
CGATCGTGCCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCACTGA
ATTGCCGCAATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAATAAACGGGTCTCTC
TGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAG
CCTCAATAAAGCTTGCCCTGAGTGCTTCAAGTAGTGTGTGCCCCGTCTGTTGTGTGACTCTG
GTAAGTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAATCTCTAGCAGTGGCGCCCCG
AACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTCGACGCAGGACTCGGCTTGCT
GAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGAC
TAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAA
TTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGGAAAGAAAAAATATAAATTA
ACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTGCGAGTTAATCCTGGCCTGTTAGAA
ACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAG
AAGAAGCTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAG
ATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCA
CCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTG
GAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCA
AGGCAAGAGAAAGAGTGGTGACAGAGAGAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCC
TTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACGGTAC
AGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGA
GGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAAT

41/53

FIG. 15 cont'd

CCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGG
AAAACATCTTGCACCACTGCTGTGCCTTGAATGCTAGTTGGAGTAATAAATCTCTGGAAC
AGATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTT
AATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGG
AATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGGTATATA
AAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAAGAATAGTTTTTGTGTACTTTCT
ATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAACCC
CGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGAC
AGATCCATTTCGATTAGTGAACGGATCTCGACGGTATCGGTTAACTTTTAAAAAGAAAAGGGG
GGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAA
CTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTATCGATCACGAGACTAGCCTCG
AGAAGCTTGATGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTATGGGGATCCG
AACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCC
GGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTA
AGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCC
TCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCC
TGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCC
CCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATC
CACGCTGTTTTGACCTCCATAGAAGACACCGACTCTAGAGGATCCACCGGTGCCACCAT
GGCAGAGCATGACTATCACGAAGACTATGGTTTCTCTTCTTTAATGACAGCTCTCAAGAG
GAGCACCAGGATTTTCTCCAATTCTCAAAAGTATTCTTGCCATGCATGTACCTTGTGGTTTT
TGTCTGCGGCTTGGTAGGAAACAGTCTTGTGTTGGTAATTAGCATTTTTTACCATAAGCTCC
AATCTTTGACGGATGTTTTTTTTGGTCAATCTCCCTCTCGCAGATCTTGTGTTTGTATGCACT
CTCCCGTTTTTGGGCATACGCTGGGATTCACGAGTGGGTGTTTGGTCAAGTCATGTGCAAGT
CACTCCTGGGTATCTATACGATCAACTTTTACACGAGCATGCTCATTCTTACTTGCATTACA
GTAGATCGGTTTCATAGTGGTTGTGAAGGCGACCAAAGCATATAATCAACAAGCCAAAAGGA
TGACCTGGGGCAAGGTTACATCTCTGTTGATATGGGTTATCTCACTCTTGGTATCTTTGCC
CAGATAATATACGGCAATGTATTTAATCTGGACAACTCATCTGTGGCTACCATGACGAAGC
CATAAGTACCGTAGTGTTGGCCACACAGATGACGCTCGGTTTCTTCCCTGCCCTCCTGACA
ATGATTGTTTGCTATTCCGTAATAATTAAGACACTCCTTCATGCTGGCGGGTTCAGAAACA
TAGATCTCTCAAGATCATTTTCTTGGTAATGGCTGTATTCTCCTCACACAAATGCCGTTTA
ACCTGATGAAATTCATTAGATCAACCCATTGGGAGTACTATGCGATGACAAGTTTCCATTAT
ACAATTATGGTGACAGAAGCAATAGCGTATCTTAGGGCCTGTCTTAACCCGGTATTGTACG
CTTTTGTCTCTCTGAAATTTAGGAAAAATTTTGGAGCTTGTAAGAGACATTGGCTGTCTC
CCGTACTTGGGCGTAAGCCATCAATGGAAAAGTTCAGAAGATAATAGCAAGACGTTTAGTG
CTTCCCATACGTGGAGGCTACCTCCATGTTCCAATTATGGGCAACAGCAGGGCCAAGA
GGAGCGGCAGCGGCGAGGGCAGGGGCAGCCTGCTGACCTGCGGCGACGTGGAGGAGAA
CCCCGGCCCCGCCAGCATGTCTAGCGAAAACCTGCTTTGTAGCAGAGAACTCCTCTTTGCAT
CCTGAATCCGGTCAGGAAAACGATGCAACTTCACCACACTTCAGCACGCGGCATGAAGGG
TCCTTCCAAGTGCCCGTTCTTTGCGCCGTGATGAATGTGGTATTTATTACCATCCTTATAAT
AGCATTGATCGCGCTTTCCGTTGGACAGTATAATTGTCCCGGACAGTACACGTTTTCAATG
CCGTCTGACTCACACGTCTCTTCTTGCACTGAAGACTGGGTTGGGTATCAGCGGAAGTGC
TACTTTATCTCTACAGTAAAGCGGAGCTGGACCAGCGCCCAAAATGCCTGCTCTGAACACG
GGGCGACGCTCGCCGTTATTGACTCAGAAAAAGATATGAACTTTCTCAAACGATACGCCGG
AAGAGAGGAGCATTGGGTAGGCCTTAAAAAGGAACCGGGTCATCCTTGGAATGGAGTAA
CGGTAAAGAGTTCAATAATTGTTTCAACGTTACAGGCTCAGACAAATGTGTTTTTCTTAAAA
ACACAGAGGTTAGTTCAATGGAATGCGAAAAAAATTTGTATTGGATCTGCAATAAGCCATAC
AAAGCGAATTCACGTGCCAAGCGAAGCGGAAGCGGAGAGGGCAGAGGAAGTCTGCTAAC
ATGCGGTGACGTGAGGAGAATCCTGGCCCAGCTAGCAACATGCTGCGCCTGCTGCTGG
CCCTGAACCTGTTCCCCAGCATCCAGGTGACCGGCGGCAGCAGCGAGCAGAAGCTGATC

42/53

FIG. 15 cont'd

AGCGAGGAGGACCTGCAGACCGTGCTGACCCAGAGCCCCAGCAGCCTGAGCGTGAGCGT
GGGCGACCGCGTGACCATCACCTGCCGCGCCAGCAGCAGCGTGACCTACATCCACTGGT
ACCAGCAGAAGCCCCGGCCTGGCCCCCAAGAGCCTGATCTACGCCACCAGCAACCTGGCC
AGCGGCGTGCCAGCCGCTTCAGCGGCAGCGGCAGCGGCACCGACTACACCTTCACCAT
CAGCAGCCTGCAGCCCCGAGGACATCGCCACCTACTACTGCCAGCACTGGAGCAGCAAGC
CCCCACCTTCGGCCAGGGCACCAAGGTGGAGGTGAAGCGCACCGTGGCCGCCCCAG
CGTGTTTCATCTTCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGAGGTGCAG
CTGCTGGAGAGCGGCGGCGGCGCTGGTGACGCCCGGCGGCAGCCTGCGCCTGAGCTGCG
CCACCAGCGGCTTCACCTTCACCGACTACTACATGAACTGGGTGCGCCAGGCCCCCGGCA
AGGGCCTGGAGTGGCTGGGCTTCATCGGCAACAAGGCCAACGGCTACACCACCGAGTAC
AGCGCCAGCGTGAAAGGGCGCTTCACCATCAGCCGCGACAAGAGCAAGAGCACCTGT
CCTGCAGATGAACACCCTGCAGGCCGAGGACAGCGCCATCTACTACTGCACCCGCGACC
GCGGCCTGCGCTTCTACTTCGACTACTGGGGCCAGGGCACCTGGTGACCGTGAGCAGC
GCCAGCACCAAGGGCATCGAGGTGATGTACCCCCCCCCCTACCTGGACAACGAGAAGAG
CAACGGCACCATCATCCACGTGAAGGGCAAGCACCTGTGCCCCAGCCCCCTGTTCCCG
GCCCCAGCAAGCCCTTCTGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGC
CTGCTGGTGACCGTGGCCTTCATCATCTTCTGGGTGCGCAGCAAGCGCAGCCGCCTGCTG
CACAGCGACTACATGAACATGACCCCCCGCCGCCCGGCCACCCGCAAGCACTACCA
GCCCTACGCCCCCCCCCGCGACTTCGCCGCCTACCGCAGCCGCGTGAAAGTTCAGCCGCA
GCGCCGACGCCCCCGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTG
GGCCGCGCGAGGAGTACGACGTGCTGGACAAGCGCCGCGGCAGGGACCCCGAGATGG
GCGGCAAGCCCCAGCGCCGCAAGAACCCCCAGGAGGGCCTGTACAACGAGCTGCAGAAG
GACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGCCGCGCGGCA
AGGGCCACGACGGCCTGTACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCC
CTGCACATGCAGGCCCTGCCCCCCCCGCTGATAAAGGGCGAATTCCAGCACACTGGCGGC
CGTTACTAGTGTGACAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTC
TTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTA
TTGCTTCCCGTATGGCTTTTCTCTCTCTTGTATAAATCCTGGTTGCTGTCTCTTTATG
AGGAGTTGTGGCCCGTTGTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAA
CCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTTCGCTTTCC
CCCTCCCTATTGCCACGGCGGAACCTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGG
GCTCGGCTGTTGGGCACTGACAATTCGCTGGTGTGTCGGGGAAGCTGACGTCTTTCCA
TGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTTCTGCTACGTCCCT
TCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTT
CCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCGCCTCCCCGCCT
GGAATTCGAGCTCGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCA
CTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATCTG
CTTTTTGCTTGTAAGTGGGTCTCTCTGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCT
AACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCCTGAGTGCTTCAAGTAGTGTG
TGCCCGTCTGTTGTGTGACTCTGGTAAGTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGG
AAAATCTCTAGCAGTAGTAGTTCATGTATCTTATTATTAGTATTTATAACTTGC AAAGAAA
TGAATATCAGAGAGTGAGAGGAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA
TAGCATCACAAATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAA
ACTCATCAATGTATCTTATCATGTCTGGCTCTAGCTATCCCGCCCCCTAACTCCGCCAGTTC
CGCCCATCTCCGCCCATGGCTGACTAATTTTTTTTTTATTTATGCAGAGGCCGAGGCCGCC
TCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTTGCG
TCGAGACGTACCCAATTGCCCCTATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTCGTT
TTACAACGTGCTGACTGGGAAAACCTGGCGTTACCCAACCTTAATCGCCTTGACGACATC
CCCCTTTGCGCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGT
TGCGCAGCCTGAATGGCGAATGGCGCGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCG

43/53

FIG. 15 cont'd

GGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCC
TTTCGCTTTCTTCCCTTCTTTCTCGCCACGTTCCGCGGCTTTCCCGTCAAGCTCTAAATC
GGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGA
TTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTTTGACG
TTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTAT
CTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGA
GCTGATTTAAACAAAAATTTAACGCGAATTTTAAACAAAATATTAACGTTTACAATTTCCCAGGT
GGCACTTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTATTTTTCTAAATACATTCAAA
TATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGA
GTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTTCGGGCATTTTGCCTTCT
GTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCAC
GAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGA
AGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTA
TTGACGCCGGGCAAGAGCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTGGTTG
AGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAG
TGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
CCGAAGGAGCTAACCCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTT
GGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAG
CAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCA
ACAATTAATAGACTGGATGGAGGCGGATAAAGTTGACAGGACCACTTCTGCGCTCGGCCCT
TCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTAT
CATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGG
GAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATT
AAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACCTTCAT
TTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAA
CGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAG
ATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGT
GGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGA
GCGCAGATACCAAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACT
CTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGG
CGATAAGTCGTGTCTTACCGGGTTGGAAGTCAAGACGATAGTTACCGGATAAGGCGCAGCG
GTCGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCG
AACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG
CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCA
GGGGGAAACGCCTGGTATCTTTATAGTCTGTCCGGTTTTCGCCACCTCTGACTTGAGCGT
CGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGC
CTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTATCCC
CTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCC
GAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAA
CCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCACGACAGGTTTCCCGAC
TGGAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCC
CAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATT
TCACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAAGG
GAACAAAAGCTGGAGCTGCA (SEQ ID NO: 61)

Lenti_MND_hs_CXCR6_CD69_hCEACAR

AGCTTAATGTAGTCTTATGCAATACTCTTGAGTCTTGCAACATGGTAACGATGAGTTAGCA
ACATGCCTTACAAGGAGAGAAAAAGCACCGTGATGCCGATTGGTGGAAGTAAGGTGGTA
CGATCGTGCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCACTGA
ATTGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAATAAACGGGTCTCTC

44/53

FIG. 15 cont'd

TGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAG
CCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCCGTCTGTTGTGTGACTCTG
GTAAGTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAATCTCTAGCAGTGGCGCCCCG
AACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTCGACGCAGGACTCGGCTTGCT
GAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGAC
TAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAA
TTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGGAAAGAAAAAATATAAATTA
ACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTTCGCAGTTAATCCTGGCCTGTTAGAA
ACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAG
AAGAAGCTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAG
ATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCA
CCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTG
GAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCACCA
AGGCAAGAGAGAAGAGTGGTGACAGAGAGAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCC
TTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACGGTAC
AGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGA
GGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAAT
CCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGG
AAACTCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAAC
AGATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTT
AATACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGG
AATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGGTATATA
AAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAAGAATAGTTTTTGTGTACTTTCT
ATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAACCC
CGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGAC
AGATCCATTTCGATTAGTGAACGGATCTCGACGGTATCGGTAACTTTTAAAGAAAAGGGG
GGATTGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAA
CTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTATCGATCACGAGACTAGCCTCG
AGAAGCTTGATGGCCGCCAGTGTGATGGATATCTGCAGAATTCGCCCTTATGGGGATCCG
AACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCC
GGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTA
AGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCCGCC
TCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCC
TGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCC
CCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATC
CACGCTGTTTTGACCTCCATAGAAGACACCGACTCTAGAGGATCCACCGGTCGCCACCAT
GGCAGAGCATGACTATCACGAAGACTATGGTTTCTCTTCTTTAATGACAGCTCTCAAGAG
GAGCACCAGGATTTTCTCCAATTCTCAAAAGTATTCTTGCCATGCATGTACCTTGTGGTTTT
TGTCTGCGGCTTGGTAGGAAACAGTCTTGTGTTGGTAATTAGCATTTTTTACCATAAGCTCC
AATCTTTGACGGATGTTTTTTTTGGTCAATCTCCCTCTCGCAGATCTTGTGTTTGTATGCACT
CTCCCGTTTTTGGGCATACGCTGGGATTCACGAGTGGGTGTTTGGTCAAGTCATGTGCAAGT
CACTCCTGGGTATCTATACGATCAACTTTTACACGAGCATGCTCATTCTTACTTGCAATTACA
GTAGATCGGTTTCATAGTGGTTGTGAAGGCGACCAAGCATATAATCAACAAGCCAAAAGGA
TGACCTGGGGCAAGGTTACATCTCTGTTGATATGGGTTATCTCACTCTTGGTATCTTTGCC
CAGATAATATACGGCAATGTATTTAATCTGGACAACTCATCTGTGGCTACCATGACGAAGC
CATAAGTACCGTAGTGTGGCCACACAGATGACGCTCGGTTTTCTCCTGCCCTCCTGACA
ATGATTGTTTGCTATTCCGTAATAATTAAGACACTCCTTCATGCTGGCGGGTTCAGAAACA
TAGATCTCTCAAGATCATTTTCTTGGTAATGGCTGTATTCTCCTCACACAAATGCCGTTTA
ACCTGATGAAATTCATTAGATCAACCCATTGGGAGTACTATGCGATGACAAGTTTCCATTAT
ACAATTATGGTGACAGAAGCAATAGCGTATCTTAGGGCCTGTCTTAACCCGGTATTGTACG

45/53

FIG. 15 cont'd

CTTTTGTCTCTCTGAAATTTAGGAAAAATTTTTGGAAGCTTGTAAGACATTGGCTGTCTC
CCGTACTTGGGCGTAAGCCATCAATGGAAAAAGTTCAGAAGATAATAGCAAGACGTTTAGTG
CTTCCCATACGTGGAGGCTACCTCCATGTTCCAACCTTATGGGCAACAGCAGGGCCAAGA
GGAGCGGCAGCGGCGAGGGCAGGGGCAGCCTGCTGACCTGCGGCGACGTGGAGGAGAA
CCCCGGCCCCGCCAGCATGTCTAGCGAAAACTGCTTTGTAGCAGAGAACTCCTCTTTGCAT
CCTGAATCCGGTCAGGAAAACGATGCAACTTCACCACACTTCAGCACGCGGCATGAAGGG
TCCTTCCAAGTGCCCGTTCTTTGCGCCGTGATGAATGTGGTATTTATTACCATCCTTATAAT
AGCATTGATCGCGCTTTCCGTTGGACAGTATAATTGTCCCGGACAGTACACGTTTTCAATG
CCGTCTGACTCACACGTCTCTTCTTGCAGTGAAGACTGGGTTGGGTATCAGCGGAAGTGC
TACTTTATCTCTACAGTAAAGCGGAGCTGGACCAGCGCCCAAATGCCTGCTCTGAACACG
GGGCGACGCTCGCCGTTATTGACTCAGAAAAAGATATGAACTTTCTCAAACGATACGCCGG
AAGAGAGGAGCATTGGGTAGGCCTTAAAAAGGAACCGGGTCATCCTTGGAATGGAGTAA
CGGTAAAGAGTTCAATAATTGTTCAACGTTACAGGCTCAGACAAATGTGTTTTTCTTAAAA
ACACAGAGGTTAGTTCAATGGAATGCGAAAAAAATTTGTATTGGATCTGCAATAAGCCATAC
AAAGCGAATTCACGTGCCAAGCGAAGCGGAAGCGGAGAGGGCAGAGGAAGTCTGCTAAC
ATGCGGTGACGTGAGGAGAATCCTGGCCCAGCTAGCAACATGCTGCGCCTGCTGCTGG
CCCTGAACCTGTTCCCCAGCATCCAGGTGACCGGCGGCAGCAGCCAGACCGTGCTGACC
CAGAGCCCCAGCAGCCTGAGCGTGAGCGTGGGCGACCGCGTGACCATCACCTGCCGCGC
CAGCAGCAGCGTGACCTACATCCACTGGTACCAGCAGAAGCCCGGCCTGGCCCCCAAGA
GCCTGATCTACGCCACCAGCAACCTGGCCAGCGGCGTGCCAGCCGCTTCAGCGGCAGC
GGCAGCGGCACCGACTACACCTTCACCATCAGCAGCCTGCAGCCCCGAGGACATCGCCAC
CTACTACTGCCAGCACTGGAGCAGCAAGCCCCCACCTTCGGCCAGGGCACCAAGGTGG
AGGTGAAGCGCACCGTGCGCGCCCCCAGCGTGTTTCATCTTCGGCAGCGGCGGCGGCGG
CAGCGGCGGCGGCGGCGGCGAGGTGCAGCTGCTGGAGAGCGGCGGCGGCGGCGGCTGGTGCA
GCCCCGGCGGCGAGCCTGCGCCTGAGCTGCGCCACCAGCGGCTTCACCTTCACCGACTACT
ACATGAACTGGGTGCGCCAGGCCCCCGGCAAGGGCCTGGAGTGGCTGGGCTTCATCGGC
AACAAAGGCCAACGGCTACACCACCGAGTACAGCGCCAGCGTGAAGGGCCGCTTCACCAT
CAGCCGCGACAAGAGCAAGAGCACCCCTGTACCTGCAGATGAACACCCTGCAGGCCGAGG
ACAGCGCCATCTACTACTGCACCCGCGACCGCGGCGCTGCGCTTCTACTTCGACTACTGGG
GCCAGGGCACCCCTGGTGACCGTGAGCAGCGCCAGCACCAAGGGCATCGAGGTGATGTAC
CCCCCCCCCTACCTGGACAACGAGAAGAGCAACGGCACCATCATCCACGTGAAGGGCAA
GCACCTGTGCCCCAGCCCCCTGTTCCCCGGCCCCAGCAAGCCCTTCTGGGTGCTGGTGG
TGGTGGGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTGACCGTGCCCTTCATCATCTTCT
GGGTGCGCAGCAAGCGCAGCCGCTGCTGCACAGCGACTACATGAACATGACCCCCCGC
CGCCCCGGCCCCACCCGCAAGCACTACCAGCCCTACGCCCCCCCCCGGCGACTTCGCCGC
CTACCGCAGCCGCGTGAAGTTCAGCCGCAGCGCCGACGCCCCCGCCTACCAGCAGGGCC
AGAACCAGCTGTACAACGAGCTGAACCTGGGCGCGCGCGAGGAGTACGACGTGCTGGAC
AAGCGCCGCGGCAGGGACCCCGAGATGGGCGGCAAGCCCCAGCGCCGCAAGAACCCCC
AGGAGGGCCTGTACAACGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATC
GGCATGAAGGGCGAGCGCCGCGCGGCAAGGGCCACGACGGCCTGTACCAGGGCCTGA
GCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCCCGCTGA
TAAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGTGACAATCAACCTCTGGATT
ACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGAT
ACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCT
TGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGG
CGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGTTTGGGGCATTGCCACCACCTG
TCAGCTCCTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACCTCATCGCC
GCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGT
GTTGTCGGGGGAAGCTGACGTCCTTTCCATGGCTGCTCGCCTGTGTTGCCACCTGGATTCT
GCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCG

46/53

FIG. 15 cont'd

CGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTC
GGATCTCCCTTTGGGCCGCTCCCCGCTGGAATTCGAGCTCGGTACCTTTAAGACCAAT
GACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAAGAAAAGGGGGGACTGGAAGG
GCTAATTCCTCCCAACGAAGACAAGATCTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAG
ACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATA
AAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAG
AGATCCCTCAGACCCTTTTAGTCAGTGTGGAATACTCTAGCAGTAGTAGTTCATGTCATCT
TATTATTCAGTATTTATAACTTGCAAAGAAATGAATATCAGAGAGTGAGAGGAACTTGTTTAT
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTT
TTCACTGCATTCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGCTCTA
GCTATCCCGCCCCCTAACTCCGCCAGTTCCGCCATTCTCCGCCCATGGCTGACTAATTT
TTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGG
AGGCTTTTTTGGAGGCCCTAGGCTTTTTGCGTCGAGACGTACCCAATTGCCCTATAGTGAGT
CGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCG
TTACCCAACCTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGA
GGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCGACG
CGCCCTGTAGCGGCGCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT
ACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTCTTCCCTTCTTCTCGCCACGT
TCGCCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGC
TTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCG
CCCTGATAGACGGTTTTTCGCCCTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTT
GTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTT
GCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTA
ACAAAAATATTAACGTTTACAATTTCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACC
CCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGA
TAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCT
TATTCCTTTTTTTCGGGCATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAG
TAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAG
CGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAA
GTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCG
CGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTAC
GGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCG
GCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACA
TGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAA
ACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAA
CTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAA
AGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCT
GGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCC
CTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGA
CAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACT
CATATATACTTTAGATTGATTTAAAACTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCT
TTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACC
CCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTG
CAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGGCCGGATCAAGAGCTACCAACTC
TTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAATACTGTCCTTCTAGTGTA
GCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTA
ATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCA
AGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACA
GCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA
AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCTG

47/53

FIG. 15 cont'd

GAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTG
TCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA
GCCTATGGA AAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTT
TGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTG
AGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAG
GAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATAA
TGCAGCTGGCAGCAGAGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAAT
GTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGT
TGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGC
CAAGCGCGCAATTAACCTCACTAAAGGGAACAAAAGCTGGAGCTGCA (SEQ ID NO: 62)

CEA CAR

QTVLTQSPSSLVSVGDRVITICRASSSVTYIHWWYQQKPLAPKSLIYATSNLASGVPSRFGSGS
GSGTDYFTFISSLQPEDIATYYCQHWSSKPPTFGQGTKEVEKRTVAAPSVFIFGSGGGSGGG
GSEVQLLESGGGLVQPGGSLRLSCATSGFTFTDYYMNWVRQAPGKGLEWLGFIGNKANGYTT
EYSASVKGRFTISRDKSKSTLYLQMNLTQAEDSAIYYCTRDRGLRFYFDYWGGQTLTVTVSSAS
TKGIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFWWLVVVGGLVACYSLLVTVAFIG
FWRSKRSLRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ
NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 200)

CEA CAR coding sequence

CAGACCGTGCTGACCCAGAGCCCCAGCAGCCTGAGCGTGAGCGTGGGCGACCGCGTGAC
CATCACCTGCCGCGCCAGCAGCAGCGTGACCTACATCCACTGGTACCAGCAGAAGCCCG
GCCTGGCCCCCAAGAGCCTGATCTACGCCACCAGCAACCTGGCCAGCGGCGTGCCAGC
CGCTTCAGCGGCAGCGGCAGCGGCACCGACTACACCTTCACCATCAGCAGCCTGCAGCC
CGAGGACATCGCCACCTACTACTGCCAGCACTGGAGCAGCAAGCCCCCACCTTCGGCCA
GGGCACCAAGGTGGAGGTGAAGCGCACCGTGCCGCGCCCCAGCGTGTTTCATCTTCGGCA
GCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGAGGTGCAGCTGCTGGAGAGCGGCG
GCGGCCTGGTGAGCCCGGCGGCGAGCCTGCGCCTGAGCTGCGCCACCAGCGGCTTCAC
CTTCACCGACTACTACATGAACTGGGTGCGCCAGGCCCGCGCAAGGGCCTGGAGTGGC
TGGGCTTCATCGGCAACAAGGCCAACGGCTACACCACCGAGTACAGCGCCAGCGTGAAG
GGCCGCTTCACCATCAGCCGCGACAAGAGCAAGAGCACCTGTACCTGCAGATGAACACC
CTGCAGGCGGAGGACAGCGCCATCTACTACTGCACCCGCGACCGCGGCCTGCGCTTCTA
CTTCGACTACTGGGGCCAGGGCACCTGGTGACCGTGAGCAGCGCCAGCACCAAGGGCA
TCGAGGTGATGTACCCCCCCCCCTACCTGGACAACGAGAAGAGCAACGGCACCATCATCC
ACGTGAAGGGCAAGCACCTGTGCCCCAGCCCCCTGTTCCCCGGCCCCAGCAAGCCCTTC
TGGGTGCTGGTGGTGGTGGGCGGCGTGCTGGCCTGCTACAGCCTGCTGGTGACCGTGGC
CTTCATCATCTTCTGGGTGCGCAGCAAGCGCAGCCGCCTGCTGCACAGCGACTACATGAA
CATGACCCCCCGCGCGCCCCGCCCCACCCGCAAGCACTACCAGCCCTACGCCCCCCCCC
GCGACTTCGCCGCCTACCGCAGCCGCGTGAAATTTCAGCCGCGAGCGCCGACGCCCCCGCC
TACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCGCGCGGAGGAGTA
CGACGTGCTGGACAAGCGCCGCGGCAGGGACCCCGAGATGGGCGGCAAGCCCCAGCGC
CGCAAGAACCCCCAGGAGGGCCTGTACAACGAGCTGCAGAAGGACAAGATGGCCGAGGC
CTACAGCGAGATCGGCATGAAGGGCGAGCGCCGCGCGGCAAGGGCCACGACGGCCTG
TACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCT
GCCCCCCCCG (SEQ ID NO: 201)

48/53

FIG. 15 cont'd

Example anti-EpCAM CAR (G8.8 VL – GS linker – G8.8 VH-mCD28 ECD-mCD28 TMD-mCD28 ICD-mCD3z ICD

MGVPTQLLGLLLWITDAICDIQMTQSPASLSASLGETVSIECLASEGISNDLAWYQQKSGKSPQ
LLIYATSRLLQDGVPSRFSGSGSGTRYSLKISGMQPEDEADYFCQQSYKYPWTFGGGTKLELKG
SGGGGSGGGGSEVQLAESGGGLVQPGRSMKLSCAASGFTFSNFPMAWWRQAPTGLEWWA
TISTSGGSTYYRDSVKGRFTISRDNASTLYLQMNLSRSEDATYYCTRTLILRVFYFDYWGQ
GVMVTVSSIEFMYPYLDNERSNGTIIHIKEKHLCHTQSSPKLFWALVVVAGVLFCYGLLVTV
LCVIWTNSRRNRGGQSDYMNMTPRRPLTRKPYQPYAPARDFAAAYRPRAKFSRSAETAANLQ
DPNQLYNELNLGRREEYDVLEKKRARDPEMGGKQQRNRNPQEGVYNALQKDKMAEAYSEIG
TKGERRRGKGHDLGLYQGLSTATKDTYDALHMQLAPR (SEQ ID NO: 199)

EpCAM CAR coding sequence

TCGAGCTAAGCTTCGTATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCGATAAAATA
AAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGC
AAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAA
GTTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTG
GTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCG
CCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGA
CCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGC
TCCCCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGCGCGCCAGTCCCTCCGATAGA
CTGCGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAAGTTGCATCCGACTTGTG
GTCTCGCTGTTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTGACGCGGGGGTCT
TTCATGGGTAACAGTTTCTTGAAGTTGGAGAACAACTTCTGAGGGTAGGAGTCGAATATT
AAGTAATCCTGACTCAATTAGCCACTGTTTTGAATCCACATACTCCAATACTCCTGAAATCC
ATCGATGGAGTTCATTATGGACAGCGCAGAAAGAGCTGGGGAGAAATTGTGAAATTGTTATC
CGCTCACAAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCT
AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAA
CCTGTGCTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTAT
TGGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCGGCTGCGGGC
AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC
AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTT
GCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTC
CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC
GTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCCGACCGCTGCGCCTTA
TCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAG
TGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGC
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTT
TGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTA
AATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTGCTGTA
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA
CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG
CAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCACACGTTGTTGCCATTGCTACAGGCATCG
TGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCG

49/53

FIG. 15 cont'd

AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT
GTCAGAAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTC
TACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACC
GCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGAAAACGTTCTTCGGGGCGAAAAC
TCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTG
ATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAT
GCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTT
AGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTA
AGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTTCGTC
TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
CAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTGCGGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTACTGAGAGTG
CACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGC
CATTCGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTA
TTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAACGCCAGG
GTTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGCCACGCTCTCCCTTATGCGACTC
CTGCATTAGGAAGCAGCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCGCGCAAGGA
ATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATA
CCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGCGGAGCCCGATCTTCCCCATCGGT
GATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGA
TGCGTCCGGCGTAGAGGCGATTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGA
CTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAAATAAAGATTTTA
TTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTT
AAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTCAGATCA
AGGTTAGGAACAGAGAGACAGCAGAAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTT
CCTGCCCCGGCTCAGGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAG
TTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCC
TTATTTGAACTAACCAATCAGTTCGTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAG
CTCAATAAAAGAGCCCAACCCCTCACTCGGCGCGCCAGTCCTCCGATAGACTGCGTGC
CCCGGGTACCCGTATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGAAGTCTGCT
GATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGCCACCTCGGGGGTCTTTTCAATTTGA
GGTTCACCGAGATTTGGAGACCCCTGCCTAGGGACCAACCGACCCCCCGCCGGGAGGT
AAGCTGGCCAGCGGTGTTTTCTGTGTCTGTCTGTCTTTGTGCGTGTGTGTGCCGGCATCT
AATGTTTGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG
TGGAAGTACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCG
GGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTTGACTCTTTGGT
CACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCC
CGCCTCCGTCTGAATTTTTGCTTTTCGTTTTGGGACCGAAGCCGCGCCGCGCGTCTTGTCT
GCTGCAGCATCGTTCTGTGTTGTCTGTCTGACTGTGTTTCTGTATTTGTCTGAGAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTGCG
AGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTACCTTCTGCT
CTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAACCGAGACC
TCATCACCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGACACCCAGACCAGGT
CCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTT
TGTACACCCTAAGCCTCCGCCTCCTCTTCTCCATCCGCCCCGTCTCTCCCCCTTGAACCT
CCTCGTTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCC
CCATATGGCCATATGAGATCTTATATGGGGCACCCCCGCCCCCTTGAACTTCCCTGACCC
TGACATGACAAGAGTTACTAACAGCCCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTA

50/53

FIG. 15 cont'd

GTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACCTGGACCGACC
GGTGGTACCTCACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAA
GAACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCT
CAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCG
GGGGTGGACCATCCTCTAGACCGCCATGGGCGTGCCACCCAGCTGCTGGGCCTGCTGC
TGCTGTGGATCACCAGCGCCATCTGCGAGCAGAAGCTGATCAGCGAGGAGGACCTGGAC
ATCCAGATGACCCAGAGCCCCGCCAGCCTGAGCGCCAGCCTGGGCGAGACCGTGAGCAT
CGAGTGCCTGGCCAGCGAGGGCATCAGCAACGACCTGGCCTGGTACCAGCAGAAGAGCG
GCAAGAGCCCCCAGCTGCTGATCTACGCCACCAGCAGGCTGCAGGACGGCGTGCCACG
AGGTTACGCGGCAGCGGCAGCGGCACCAGGTACAGCCTGAAGATCAGCGGCATGCAGCC
CGAGGACGAGGCCGACTACTTCTGCCAGCAGAGCTACAAGTACCCCTGGACCTTCGGCG
GCGGCACCAAGCTGGAGCTGAAGGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCA
GCGAGGTGCAGCTGGCCGAGAGCGGCGGCGGCGCTGGTGCAGCCCGGCAGGAGCATGAA
GCTGAGCTGCGCCGCCAGCGGCTTACCTTCAGCAACTTCCCAATGGCCTGGGTGAGGC
AGGCCCCCACCAGGGCCTGGAGTGGGTGGCCACCATCAGCACCAGCGGCGGCAGCAC
CTACTACAGGGACAGCGTGAAGGGCAGGTTACCATCAGCAGGGACAACGCCAAGAGCA
CCCTGTACCTGCAGATGAACAGCCTGAGGAGCGAGGACACCGCCACCTACTACTGCACCA
GGACCCTGTACATCCTGAGGGTGTCTACTTCGACTACTGGGGCCAGGGCGTGATGGTGA
CCGTGAGCAGCATCGAGTTCATGTACCCCCCCCCCTACCTGGACAACGAGAGGAGCAACG
GCACCATCATCCACATCAAGGAGAAGCACCTGTGCCACACCCAGAGCAGCCCCAAGCTGT
TCTGGGGCCCTGGTGGTGGTGGCCGGCGTGCTGTTCTGCTACGGCCTGCTGGTGACCGTG
GCCCTGTGCGTGATCTGGACCAACAGCAGGAGGAACAGGGGCGGCCAGAGCGACTACAT
GAACATGACCCCCAGGAGGCCCGGCCTGACCAGGAAGCCCTACCAGCCCTACGCCCCCG
CCAGGGACTTCGCCGCCTACAGGCCCAGGGCCAAAGTTCAGCAGGAGCGCCGAGACCGCC
GCCAACCTGCAGGACCCCCAACAGCTGTACAACGAGCTGAACCTGGGCAGGAGGGAGGA
GTACGACGTGCTGGAGAAGAAGAGGGCCAGGGACCCCGAGATGGGCGGCAAGCAGCAG
AGGAGGAGGAACCCCCAGGAGGGCGTGTAACGCCCTGCAGAAGGACAAGATGGCCGA
GGCCTACAGCGAGATCGGCACCAAGGGCGAGAGGAGGAGGGGCAAGGGCCACGACGGC
CTGTACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGAC
CCTGGCCCCCAGGGCGAATTCAGTGCCAAGCGAAGCGGAAGCGGAGAGGGGCAGAGGA
AGTCTGCTAACATGCGGTGACGTGAGGAGAATCCTGGCCCAGCTAGCTGAGCGGCCGC
TACGTAGTCGACGGATCCC (SEQ ID NO: 202)

EpCAM CAR + Liver tissue residency program (TRP)

TCGAGCTAAGCTTCGTATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCGATAAAATA
AAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGC
AAGCTAGCTTAAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAA
GTTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTG
GTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCG
CCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATG
ACCCTGTGCCTTATTTGAACCTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTG
CTCCCCGAGCTCAATAAAAGAGCCCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATAG
ACTGCGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAAGTTGCATCCGACTTGTG
GTCTCGCTGTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTGACGGGGGTCT
TTCATGGGTAACAGTTTCTTGAAGTTGGAGAACAACATTCTGAGGGTAGGAGTCGAATATT
AAGTAATCCTGACTCAATTAGCCACTGTTTTGAATCCACATACTCCAATACTCCTGAAATCC
ATCGATGGAGTTCATTATGGACAGCGCAGAAAGAGCTGGGGAGAATTGTGAAATTGTTATC
CGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCT
AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAA
CCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGGCGGTTTGCAT

51/53

FIG. 15 cont'd

TGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTGTTCCGGCTGCGGCG
AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC
AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTT
GCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTC
CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT
TCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC
GTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTA
TCCGGTAAGTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAG
TGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGC
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTT
TGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTAA
AATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA
CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG
CAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCCGCCAGTTAATAGTTTTCGCAACGTTGTTGCCATTGCTACAGGCATCG
TGGTGTACGCTCGTCGTTTGGTATGGCTTCATTTCAGCTCCGGTTCCTAACGATCAAGGCG
AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTT
GTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTC
TACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTC
TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACC
GCGCCACATAGCAGAACTTTAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAAAC
TCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTG
ATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAT
GCCGCAAAAAAGGGAATAAGGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTT
AGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTA
AGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTTCGTC
TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
CAGTTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTGCGGGGCTGGCTTAAGTATGCGGCATCAGAGCAGATTGTACTGAGAGTG
CACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGC
CATTCGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTA
TTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAACGCCAGG
GTTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGCCACGCTCTCCCTTATGCGACTC
CTGCATTAGGAAGCAGCCAGTAGTAGGTTGAGGCGGTTGAGCACCGCCGCGCGCAAGGA
ATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATA
CCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGCGAGCCCGATCTTCCCCATCGGT
GATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGA
TGCGTCCGGCGTAGAGGCGATTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGA
CTCAACAATATCACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAAATAAAGATTTTA
TTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTT
AAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTCAGATCA
AGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTT
CCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAG

52/53

FIG. 15 cont'd

TTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCC
TTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTGCGCGCTTCTGCTCCCCGAG
CTCAATAAAAGAGCCCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATAGACTGCGTCG
CCCGGGTACCCGTATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGA CTGCT
GATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGCCACCTCGGGGGTCTTTTATTTTGA
GGTTCCACCGAGATTTGGAGACCCCTGCCTAGGGACCACCGACCCCCCGCCGGGAGGT
AAGCTGGCCAGCGGTCTGTTTCTGTCTGTCTCTGCTTTTGTGCGTGTGTTGTGCCGGCATCT
AATGTTTTCGCGCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG
TGGA ACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCG
GGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTTGGACTCTTTGGTG
CACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAACAGTTCC
CGCCTCCGTCTGAATTTTTGCTTTTCGTTTTGGGACCGAAGCCGCGCCGCGCGTCTTGTCT
GCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAGAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTGCG
AGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTACCTTCTGCT
CTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAACCGAGACC
TCATCACCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGACACCCAGACCAGGT
CCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTT
TGTACACCCTAAGCCTCCGCCTCCTCTTCTCCATCCGCCCGCTCTCTCCCCCTTGAACCT
CCTCGTTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCC
CCATATGGCCATATGAGATCTTATATGGGGCACCCCGCCCCCTTGTAACTTCCCTGACCC
TGACATGACAAGAGTTACTAACAGCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTA
GTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACCTGGACCGACC
GGTGGTACCTCACCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAA
GAACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCT
CAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCG
GGGGTGGACCATCCTCTAGACCGCCATGGACAGCGAGAACTGCAGCATCACCGAGAACA
GCAGCAGCCACCTGGAGAGGGGCCAGAAGGACCACGGCACCAGCATCCACTTCGAGAAG
CACCACGAGGGGCAGCATCCAGGTGAGCATCCCCTGGGCCGTGCTGATCGTGGTGCTGAT
CACCAGCCTGATCATCGCCCTGATCGCCCTGAACGTGGGCAAGTACAACCTGCCCCGGCCT
GTACGAGAAGCTGGAGAGCAGCGACCACACGTGGCCACCTGCAAGAACGAGTGGATCA
GCTACAAGAGGACCTGCTACTTCTTACGACCAACCAAGAGCTGGGCCCTGGCCCAGA
GGAGCTGCAGCGAGGACGCCGCCACCTGGCCGTGATCGACAGCGAGAAGGACATGACC
TTCCTGAAGAGGTACAGCGGCGAGCTGGAGCACTGGATCGGCCTGAAGAACGAGGCCAA
CCAGACCTGGAAGTGGGCCAACGGCAAGGAGTTCAACAGCTGGTTCAACCTGACCGGCA
GCGGCAGGTGCGTGAGCGTGAACCACAAGAACGTGACCGCCGTGGACTGCGAGGCCAAC
TTCCACTGGGTGTGCAGCAAGCCCAGCAGGATGGGCAACAGCAGGGCCAAGAGGAGCGG
CAGCGGCGAGGGCAGGGGCAGCCTGCTGACCTGCGGCGACGTGGAGGAGAACCCCGGC
CCCGCCAGCATGGACGACGGCCACCAGGAGAGCGCCCTGTACGACGGCCACTACGAGG
GCGACTTCTGGCTGTTCAACAACAGCAGCGACAACAGCCAGGAGAACAAGAGGTTTCTGA
AGTTCAAGGAGGTGTTCCCTGCCCTGCGTGTACCTGGTGGTGTTCGTGTTCCGGCCTGCTGG
GCAACAGCCTGGTGCTGATCATCTACATCTTCTACCAGAAGCTGAGGACCCTGACCGACG
TGTTCCCTGCTGAACCTGCCCTGGCCGACCTGGTGTTCGTGTGCACCCTGCCCTTCTGGG
CCTACGCCGGCACCTACGAGTGGGTGTTCCGGCACCGTGATGTGCAAGACCCTGAGGGGC
ATGTACACCATGAACTTCTACGTGAGCATGCTGACCCTGACCTGCATCACCGTGGACAGGT
TCATCGTGGTGGTGCAGGCCACCAAGGCCCTTCAACAGGCAGGCCAAGTGGAAGATCTGG
GGCCAGGTGATCTGCCTGCTGATCTGGGTGGTGAGCCTGCTGGTGAGCCTGCCCCAGAT
CATCTACGGCCACGTGCAGGACATCGACAAGCTGATCTGCCAGTACCACAGCGAGGAGAT
CAGCACTATGGTGCTGGTGATCCAGATGACCCTGGGCTTCTTCTGCCCTGCTGACCAT
GATCCTGTGCTACAGCGGCATCATCAAGACCCTGCTGCACGCCAGGAACTTCCAGAAGCA

53/53

FIG. 15 cont'd

CAAGAGCCTGAAGATCATCTTCCTGGTGGTGGCCGTGTTCCCTGCTGACCCAGACCCCCTT
CAACCTGGCCATGCTGATCCAGAGCACCAGCTGGGAGTACTACACCATCACCAGCTTCAA
GTACGCCATCGTGGTGACCGAGGCCATCGCCTACTTCAGGGCCTGCCTGAACCCCGTGCT
GTACGCCTTCGTGGGCCTGAAGTTCAGGAAGAACGTGTGGAAGCTGATGAAGGACATCGG
CTGCCTGAGCCACCTGGGCGTGAGCAGCCAGTGGAAGAGCAGCGAGGACAGCAGCAAGA
CCTGCAGCGCCAGCCACAACGTGGAGACCACCAGCATGTTCCAGCTGGCGAATTCACGTG
CCAAGCGAAGCGGAAGCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTGAG
GAGAATCCTGGCCCAGCTAGCATGGGCGTGCCCAACCAGCTGCTGGGCCTGCTGCTGCT
GTGGATCACCGACGCCATCTGCGAGCAGAAGCTGATCAGCGAGGAGGACCTGGACATCC
AGATGACCCAGAGCCCCGCCAGCCTGAGCGCCAGCCTGGGCGAGACCGTGAGCATCGAG
TGCCTGGCCAGCGAGGGCATCAGCAACGACCTGGCCTGGTACCAGCAGAAGAGCGGCAA
GAGCCCCCAGCTGCTGATCTACGCCACCAGCAGGCTGCAGGACGGCGTGCCCAGCAGGT
TCAGCGGCAGCGGCAGCGGCACCAGGTACAGCCTGAAGATCAGCGGCATGCAGCCCGAG
GACGAGGCCGACTACTTCTGCCAGCAGAGCTACAAGTACCCCTGGACCTTCGGCGGCGG
CACCAAGCTGGAGCTGAAGGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGCGA
GGTGCAGCTGGCCGAGAGCGGCGGCGGCGCCTGGTGCAGCCCGGCAGGAGCATGAAGCTG
AGCTGCGCCGCCAGCGGCTTCACCTTCAGCAACTTCCCAATGGCCTGGGTGAGGCAGGC
CCCCACCAAGGGCCTGGAGTGGGTGGCCACCATCAGCACCAGCGGCGGCAGCACCTACT
ACAGGGACAGCGTGAAGGGCAGGTTCAACCATCAGCAGGGACAACGCCAAGAGCACCTG
TACCTGCAGATGAACAGCCTGAGGAGCGAGGACACCGCCACCTACTACTGCACCAGGACC
CTGTACATCCTGAGGGTGTTCCTACTTCGACTACTGGGGCCAGGGCGTGATGGTGACCGTG
AGCAGCATCGAGTTCATGTACCCCCCCCCCTACCTGGACAACGAGAGGAGCAACGGCACC
ATCATCCACATCAAGGAGAAGCACCTGTGCCACACCCAGAGCAGCCCCAAGCTGTTCTGG
GCCCTGGTGGTGGTGGCCGGCGTGCTGTTCTGCTACGGCCTGCTGGTGACCGTGGCCCT
GTGCGTGATCTGGACCAACAGCAGGAGGAACAGGGGCGGCCAGAGCGACTACATGAACA
TGACCCCCAGGAGGCCCGGCCTGACCAGGAAGCCCTACCAGCCCTACGCCCCCGCCAGG
GACTTCGCCCGCCTACAGGGCCAGGGCCAAAGTTCAGCAGGAGCGCCGAGACCGCCGCCAA
CCTGCAGGACCCCAACCAGCTGTACAACGAGCTGAACCTGGGCAGGAGGGAGGAGTACG
ACGTGCTGGAGAAGAAGAGGGGCCAGGGACCCCGAGATGGGCGGCAAGCAGCAGAGGAG
GAGGAACCCCCAGGAGGGCGTGTAACGCCCTGCAGAAGGACAAGATGGCCGAGGCCT
ACAGCGAGATCGGCACCAAGGGCGAGAGGAGGAGGGGCAAGGGGCCACGACGGCCTGTA
CCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGACCCTGG
CCCCAGGGCGGCCGCTACGTAGTCGACGGATCCC (SEQ ID NO: 203)