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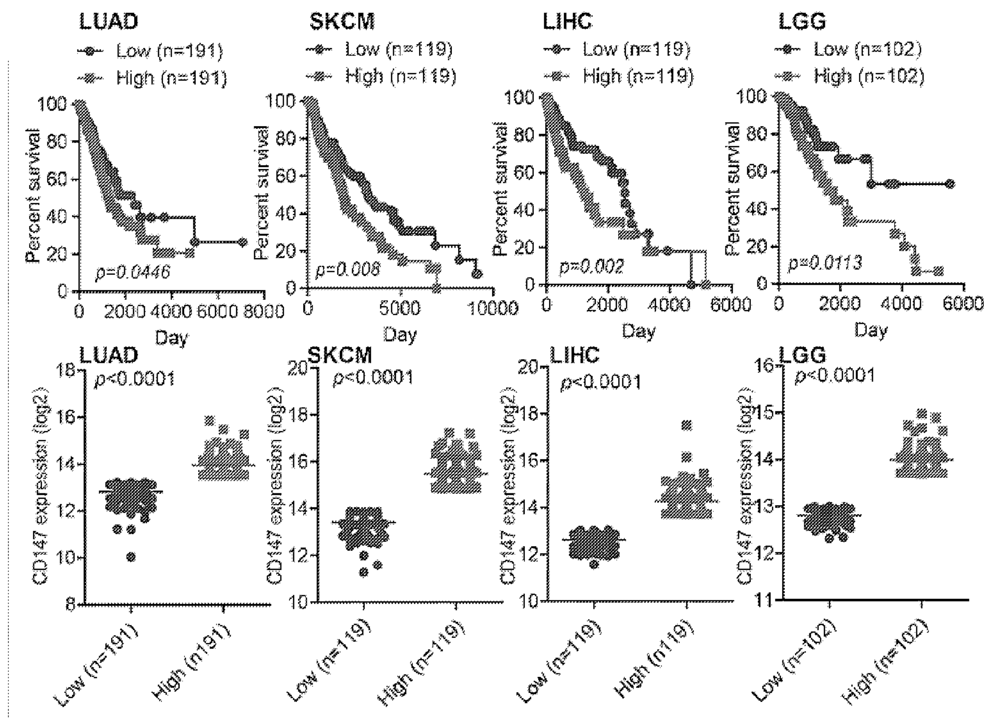
(54) Titre : RECEPTEURS ANTIGENIQUES CHIMERIQUES CD147 ET PROCEDES D'UTILISATION
(54) Title: CD147 CHIMERIC ANTIGEN RECEPTORS AND METHODS OF USE

FIG. 1A



(57) Abrégé/Abstract:
Modified single chain variable fragments (scFv) that specifically bind CD147 are provided. Also provided are chimeric antigen receptors (CARs) including the modified CD147 scFv, nucleic acids encoding the CARs, vectors including the nucleic acids encoding the CARs, and immune cells expressing the CARs. Methods of treating a subject with cancer including administering to the subject an immune cell expressing a disclosed CD147-CAR are also provided.

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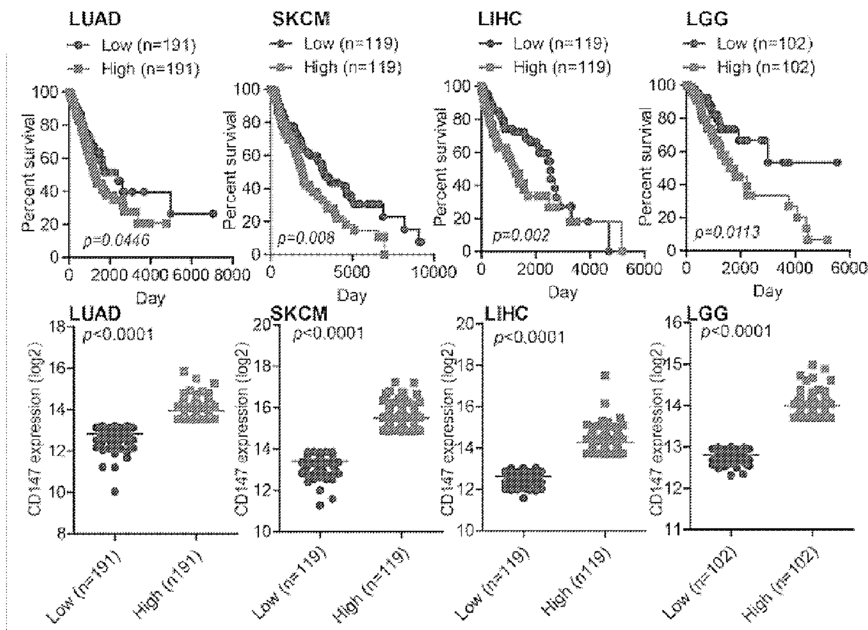
(54) **Title:** CD147 CHIMERIC ANTIGEN RECEPTORS AND METHODS OF USE

FIG. 1A



(57) **Abstract:** Modified single chain variable fragments (scFv) that specifically bind CD147 are provided. Also provided are chimeric antigen receptors (CARs) including the modified CD147 scFv, nucleic acids encoding the CARs, vectors including the nucleic acids encoding the CARs, and immune cells expressing the CARs. Methods of treating a subject with cancer including administering to the subject an immune cell expressing a disclosed CD147-CAR are also provided.

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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))*
— *of inventorship (Rule 4.17(iv))*

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CD147 CHIMERIC ANTIGEN RECEPTORS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Patent Application No. 62/819,403, filed March 15, 2019, which is incorporated herein by reference in its entirety.

FIELD

This disclosure related to immunotherapies, particularly chimeric antigen receptors targeting 10 CD147 and their use for treating cancer.

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BACKGROUND

Liver cancer is the second most common cause of cancer-related death worldwide. The burden of liver cancer is projected to be over 1 million cases by 2030. Liver cancer ranks fifth in terms of global 20 cases and second in terms of deaths for males. More than half a million patients die from hepatocellular carcinoma (HCC) each year.

Primary liver cancer includes hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (iCCA), fibrolamellar carcinoma, and hepatoblastoma. HCC and iCCA are the most common primary liver cancers, which account for more than 99% of primary liver cancer cases. HCC alone (nearly 25 800,000 new cases per year) accounts for 90% of all cases of primary liver cancer. Currently, there is no effective therapy available to treat HCC. Sorafenib (CheckMate-040, a multi-kinase inhibitor widely used for advanced HCC patients with low efficacy and severe side effects) is a first-line standard systemic agent for HCC. Currently, PD-1 blockade Opdivo (Nivolumab) has been approved by the US Food and Drug Administration (FDA) as a second line treatment strategy for patients with HCC who 30 have been previously treated with Sorafenib. Clinical trials testing PD-1 blockade as a first-line treatment for HCC are underway. Meanwhile, various clinical trials using PD-1 or PD-L1 blockades in combination with other interventions are ongoing as well. For example, a study evaluating anti-PD-1 antibody in combination with anti-CTLA-4 antibody in patients with resectable and potentially resectable HCC is being tested in clinical trials (NCT03222076).

35 Chimeric antigen receptor (CAR)-modified T cell therapy has become a promising immunotherapeutic strategy for the treatment of various blood cancers. Despite recent advances in CAR-modified T cell immunotherapy in blood cancers, high costs and severe toxicity have hindered its widespread use. Meanwhile, CAR-T cells face additional challenges during the targeting of solid tumors,

such as maintaining durable proliferation and persistence in the tumor microenvironment. An additional challenge for CAR-mediated immunotherapy for liver cancer is to find an effective target.

SUMMARY

5 CD147 is expressed on different cell types (*e.g.*, hematopoietic, epithelial, and endothelial cells) at varying levels. However, CD147 is significantly upregulated in disease states, such as in HCC, breast cancer, bladder cancer, colorectal cancer, ovarian cancer, melanoma, and osteosarcoma. CARs that specifically target cells expressing CD147 are provided. These CARs can be used in immunotherapy of cancers expressing or overexpressing CD147.

10 Disclosed herein are modified single-chain variable fragments (scFvs) that specifically bind CD147. In some embodiments, the scFv has an amino acid sequence that includes the variable heavy chain (VH) domain complementarity determining region 1 (CDR1), CDR2 and CDR3 amino acid sequences of SEQ ID NO: 8 and the variable light chain (VL) domain CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 9. In some examples, the scFv has at least 90% sequence identity to the 15 amino acid sequence of SEQ ID NO: 2 or includes or consists of the amino acid sequence of SEQ ID NO: 2. Also provided are nucleic acids that encode the modified CD147 scFv, such as a nucleic acid with at least 90% sequence identity to the nucleic acid molecule of SEQ ID NO: 1, or include or consist of the nucleic acid sequence of SEQ ID NO: 1 and vectors including the nucleic acid sequence. In additional embodiments, provided are vectors encoding the modified CD147 scFv (such as SEQ ID NO: 20 1), which further comprise an inducible promoter or enhancer nucleic acid molecule operably linked to the CD147 scFv nucleic acid molecule. In some examples, the enhancer nucleic acid is a Gal4 upstream activation sequence (UAS) that is operably linked to a nucleic acid encoding the CD147 scFv. In another example, the vector is a synNotch construct, for example a vector including the nucleic acid sequence of the modified CD147 scFv nucleic acid molecule (*e.g.*, SEQ ID NO: 1) linked to synNotch and a Gal4-25 VP64 encoding sequence (*e.g.*, SEQ ID NO: 17).

Also provided are CARs that include a modified CD147 scFv provided herein, a hinge domain, a transmembrane domain, an intracellular domain comprising one or more co-stimulatory molecule intracellular domains and an intracellular signaling domain. In one embodiment, the CD147-CAR includes a modified CD147 scFv provided herein, an IgG1 hinge domain, a CD28 transmembrane 30 domain, CD28 and 4-1BB co-stimulatory domains, and a CD3ζ signaling domain. In some examples, the CD147-CAR includes an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 5 or includes or consists of the amino acid sequence of SEQ ID NO: 5.

In some embodiment, the CD147-CAR further includes an inducible suicide molecule, such as caspase 9. In some examples, expression of the suicide molecule is induced by tetracycline, doxycycline, 35 or rapamycin. In one example, the CD147-CAR with an inducible suicide gene includes an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 7 or includes or consists of the amino acid sequence of SEQ ID NO: 7. The CD147-CAR may further include a cytokine receptor

intracellular domain, such as an interleukin-15 receptor intracellular domain (*e.g.*, SEQ ID NO: 12), an interleukin-12 receptor intracellular domain or an interleukin 18 receptor intracellular domain.

Also provided are nucleic acids encoding the CD147-CARs disclosed herein, and vectors including the nucleic acids (such as a viral vector). In some examples, the CD147-CAR is encoded by a nucleic acid sequence with at least 90% identity to the nucleic acid sequence of SEQ ID NO: 4 or SEQ ID NO: 6. In other examples, the CD147-CAR is encoded by a nucleic acid that includes or consists of the nucleic acid sequence of SEQ ID NO: 4 or SEQ ID NO: 6.

In additional embodiments, provided are vectors encoding a CD147-CAR (such as SEQ ID NO: 4 or SEQ ID NO: 6), further comprising an inducible promoter or enhancer nucleic acid molecule operably linked to the CD147-CAR nucleic acid molecule. In some examples, the enhancer nucleic acid is a Gal4 upstream activation sequence (UAS) that is operably linked to a nucleic acid encoding the CD147-CAR (*e.g.*, SEQ ID NO: 14). In one example, the vector includes the CD147-CAR in a synNotch construct, for example a vector including the nucleic acid sequence of SEQ ID NO: 15. In other examples, the CD147-CAR nucleic acid molecule (*e.g.*, SEQ ID NO: 1) is linked to synNotch and a Gal4-VP64 encoding sequence (*e.g.*, SEQ ID NO: 17).

Also provided are T cells, natural killer (NK) cells, natural killer T (NKT) cells, double negative T (DNT) cells ($CD3^{+}CD4^{-}CD8^{-}$), neutrophils, or macrophages expressing the disclosed scFvs and/or CARs, such as T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages comprising a nucleic acid encoding a disclosed CD147 scFv or CD147-CAR or a vector encoding a disclosed CD147 scFv or CD147-CAR. In some examples, the NK cells are NK-92 or NK-92MI cells. Methods of producing cells expressing the CARs, including but not limited to CD147-CAR-NK cells, CD147-CAR-T cells, or CD147-CAR-macrophages are provided. These methods include transducing or transfecting T cell, NK cells, NKT cells, DNT cells, neutrophils, or macrophages with a vector encoding a disclosed CAR.

In further embodiments, provided are T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages expressing a CD147-CAR operably linked to an activator of the inducible promoter or enhancer element. In some examples, the T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages further express a nucleic acid encoding an anti-GPC3 specific binding agent (such as an anti-GPC3 scFv) operably linked to an activator of the inducible promoter or enhancer element. In other embodiments, provided are T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages expressing a CD147 scFv operably linked to an inducible promoter or enhancer element and further comprising a nucleic acid molecule encoding an anti-GPC3 chimeric antigen receptor operably linked to the inducible promoter or enhancer.

Disclosed herein are methods for treating a subject with cancer, for example by administering a CAR-expressing NK cell, T cell, NKT cell, DNT cell, neutrophil, or macrophage disclosed herein (*e.g.*, CD147-CAR NK cell, CD147-CAR-T cell, or CD147-CAR macrophage) to the subject. In some examples, the subject has a cancer that expresses CD147. In particular non-limiting examples, the subject has hepatocellular carcinoma, neuroblastoma, breast cancer, pancreatic cancer, leukemia,

lymphoma, multiple myeloma, colorectal cancer, lung cancer, melanoma, renal cell carcinoma, sarcoma, or nasopharyngeal carcinoma.

The foregoing and other features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

5

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1E show CD147 overexpression in hepatocellular carcinoma cells. FIG. 1A shows prognostic value of the CD147 upregulated expression for overall survival of human cancer patients from TCGA datasets. Survival curves (top panel) of different patient populations based on relative CD147
10 high and low expression (bottom panel). The data of LUAD (Lung adenocarcinoma), SKCM (Skin Cutaneous Melanoma), LIHC (Liver hepatocellular carcinoma), and LGG (Brain Lower Grade Glioma) were collected for analysis. FIG. 1B shows comparison of CD147 expression between normal tissue (NT) and tumor sample (TP) in multiple cancer types from TCGA datasets. Data represent the mean ± SEM of three separate experiments. Unpaired Student's t test were employed. *p<0.05, **p<0.01,
15 ***p<0.001 and n.s (no significant difference). According to the TCGA database, the full name of each cancer type is: BRCA (Breast invasive carcinoma), CHOL (Cholangiocarcinoma), GBM (Glioblastoma multiforme), LGG (Brain Lower Grade Glioma), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), KIPAN Pan-kidney cohort (KICH+KIRC+KIRP), KIRP (Kidney renal papillary cell carcinoma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma),
20 LUSC (Lung squamous cell carcinoma), PRAD (Prostate adenocarcinoma), UCEC (Uterine Corpus Endometrial Carcinoma). FIG. 1C shows Western blot analysis of CD147 in HCC cell lines. 1×10^6 cells of various cell lines were lysed in 200 µl RIPA buffer and mixed with 50 µl 5X SDS loading buffer before loading onto an SDS-PAGE independently. Mouse anti-Human CD147 (HIM6, Mouse IgG1) was used for Western blot analysis. Anti-GAPDH was used as a loading control. FIG. 1D shows CD147 in
25 HCC cell lines (SK-Hep1 and HepG2). SK-Hep1 and HepG2 (1×10^6 cells) were stained with 2 µg FITC-mouse anti-human CD147 (anti-CD147) or 2 µg FITC-isotype mouse IgG1 (Isotype, Kappa). After incubation and washing, samples were analyzed by flow cytometry. Number represents mean fluorescence intensity (MFI) of each sample. FIG. 1E is a series of panels showing histopathology analysis of CD147 antigen expression on human HCC tumor isolated from PDX mouse model.
30 Representative H&E (top row) and CD147 IHC staining (middle row) of tumor samples from different patient-derived xenograft (PDX) mice treated with PBS, NK-92MI, and CD147-CAR NK-92MI, respectively. Bottom row shows IHC staining without the primary antibody. Scale bars represent 50 µm. Data are representative of three independent experiments.

FIGS. 2A-2F show design of CD147-CAR and phenotyping of CAR-modified NK-92MI cells.
35 FIG. 2A shows the schematic design of a CD147-specific CAR based on the SFG retroviral vector. The construct includes a CD147-specific single chain antibody fragment (modified scFv, from clone 5F6, mIgG1), a human IgG1 CH2CH3 hinge region and CD28 transmembrane region, followed by the intracellular domains of co-stimulatory CD28, 4-1BB and intracellular domain of CD3ζ. FIG. 2B shows

flow cytometric analysis of CAR expression and CD56 on the surface of parental NK-92MI and CD147-CAR-NK-92MI. Data are representative of at least three experiments. FIG. 2C shows Western blot analysis of CAR expression in parental NK-92MI and CD147-CAR-NK-92MI cells by anti-human CD3ζ-specific antibody for detection of endogenous CD147 and CD147-CAR fusion protein. FIG. 2D shows NK activation and inhibition markers in parental NK-92MI, CAR-CD19 (4-1BB)-NK-92MI, and CD147-CAR-NK-92MI cells. Each data represents at least three or four experiments. Number in the flow graph represents mean fluorescence intensity (MFI) of each sample. FIG. 2E shows flow cytometric analysis of expression of CARs on CD19-CAR-NK-92MI and CD147-CAR-NK-92MI using goat anti-human IgG (H+L). Wild type NK-92MI cells were used as control. FIG. 2F shows flow cytometric analysis of expression of CD147 on NK-92MI, CD19-CAR-NK-92MI, and CD147-CAR-NK-92MI. FIG. 2G shows overlaid flow cytometric profile of CD147 expression levels on NK-92MI, CD19-CAR-NK-92MI, and CD147-CAR-NK-92MI. Data are representative of two independent experiments.

FIGS. 3A-3D show that CD107a degranulation and cytokine production in CD147-CAR-NK-92MI cells is stimulated with its sensitive target cells. FIG. 3A shows representative flow cytometric data illustrating CD107a degranulation on NK-92MI, CD19-4-1BB-CAR, CD19-CD28-CAR and CD147-CAR after 10 hours with medium (control), SK-Hep1, and HepG2. The ratio of effector and target is 1:1.2. Cells were gated for CD56 positive subsets for quantifying surface CD107a expression. FIG. 3B shows quantitative data for percentage of surface CD107a expression on CD147-CAR-NK-92MI cells upon different stimulations, as indicated. Cytokine TNF-alpha (FIG. 3C) and IFN-gamma (FIG. 3D) production by CD147-CAR-NK-92MI, CD19-4-1BB-CAR-NK-92MI, CD19-CD28-CAR-NK-92MI, and wild-type NK-92MI stimulated by different conditions. The NK-92MI cells were co-cultured with SK-Hep1 cells at an effector/target ratio of 1:1 or medium for 12 hours. Phorbol-12-myristate-13-acetate (PMA)/ionomycin (IONO) were used for a positive control. The ratios of cytokine release were calculated by the following equation: sample value/average of positive control value × 100 (%). Data were pooled from at least three or four experiments.

FIGS. 4A-4C show CD147-CAR-NK-92MI killing of two HCC cell lines. FIG. 4A is representative flow cytometric staining of surface CD147 molecules on Huh7 (left) and HCO2 (right) cell lines. FIG. 4B is a graph showing cytotoxicity of CD147-CAR-NK-92MI measured by a standard 4-hr ^{51}Cr release assay. CD147-positive Huh7 cells were used as the CD147-CAR-NK-92MI susceptible target cells. Wild type NK-92MI was used as control. FIG. 4C is a graph showing cytotoxicity of CD147-CAR-NK-92MI measured by a standard 4-hr ^{51}Cr release assay. CD147-positive HCO2 cells were used as the CD147-CAR-NK-92MI susceptible target cells. Wild type NK-92MI was used as control. Data represent the mean ± SEM from three independent experiments. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

FIGS. 5A-5G show activation of CD147-CAR-NK-92MI cells upon CD147 positive target cell stimulation. FIG. 5A shows representative data showing the percentage of surface CD107a expression on CD147-CAR-NK-92MI cells upon different stimulation, as indicated. CD147-CAR-NK-92MI cells

were stimulated with SK-Hep1 or HepG2 cells for 4 hours. To block the interaction between CD147-CAR and CD147 molecules, 5 μg mouse-anti-human CD147 (HIM6) was added into the mixture of effector and target cells. As a control, 5 μg Isotype-Mouse IgG (IgG) or PBS (Vehicle control group) was used, as indicated. FIGS. 5B and 5C are graphs showing quantitative data for the percentage of surface CD107a staining on CD147-CAR-NK-92MI cells stimulated with CD147 positive SK-Hep1 (FIG. 5B) and CD147 positive HepG2 (FIG. 5C) cell lines. Wild type NK-92MI cells alone and CD147-CAR-NK-92MI cells alone were used as the control, as indicated. FIG. 5D shows representative data showing the percentage of surface CD107a expression on CD147-CAR-NK-92MI cells stimulated with CD147 positive wild type (WT) SK-Hep1 cell line (top panel) and CD147-knockout ($CD147^{-/-}$) SK-Hep1 cell line (middle panel). The culture medium only group was used as a control. Naive NK-92MI and 4-1BB-CD19-CAR (CD19-CAR) was used as the control effector cell. FIG. 5E shows quantitative data for the percentage of surface CD107a staining on CD147-CAR-NK-92MI cells stimulated with CD147 positive SK-Hep1 (WT) and CD147-Knock out SK-Hep1 ($CD147^{-/-}$) cell lines, respectively. FIG. 5F shows representative data showing the percentage of surface CD107a expression on CD147-CAR-NK-92MI cells stimulated with a CD147 positive wild type (WT) HepG2 cell line (top panel) and a CD147-knockout HepG2 cell line (middle panel). The culture medium only group was used as a control. Naive and CD19-4-1BB-CAR-NK-92MI (CD19-CAR) was used as the control effector cell. FIG. 5G shows quantitative data for the percentage of surface CD107a staining on CD147-CAR-NK-92MI cells stimulated with CD147 positive HepG2 (WT) and CD147-Knock out HepG2 ($CD147^{-/-}$) cell lines, respectively. The ratio of effector and target is 1:1.2. CD147-CAR-NK-92MI cells were gated by CD56 antibody surface staining. NK degranulation was measured by the CD107a surface staining by flow cytometry. Data represent the mean ± SEM of three separate experiments. *p<0.05, **p<0.01, and ***p<0.001.

FIG. 6 shows that mouse-anti-human CD147 (HIM6) does not affect the cytotoxicity of CD19-CAR-NK cells. Cytotoxicity of CD19-CAR-NK-92MI was measured using the FFLuc report system assay. Briefly, Daudi-FFLuc cells ($1x10^4$) were pre-seeded in Matrigel (BD) treated 96-well optical-bottom microplate overnight. Effector cells (CD19-CAR-NK-92MI) at two different effector/target ratios (5:1 and 1:1, as indicated) were co-cultured for 6 hours. The luminescence signal was quantified by a microplate reader and the percentage of specific lysis was calculated. Data are pooled from three independent experiments. Error bars show ± SEM (stand error of the mean). *p <0.05, **p < 0.01, and ***p < 0.001.

FIGS. 7A and 7B show representative images of CD147-CAR-NK-92MI killing activities. Effector cells ($1x10^4$) CD147-CAR-NK-92MI and NK-92MI were co-cultured for 12 hours with target cells FFLuc-GFP-SK-Hep1(FIG. 7A, top) ($1x10^4$) and FFLuc-GFP-HepG2 (FIG. 7A, bottom) in a 96-well optical-bottom microplate. Conventional fluorescent microscopy detected GFP fluorescence (top lane) and brightfield (bottom lane) was used to visualize CD147-CAR-NK-92MI killing activities at the same setting. The GFP fluorescence intensity was quantified by ImageJ software (NIH) (FIG. 7B).

Quantitative mean fluorescence intensity (MFI) of GFP was plotted by Graph prism 5 software (GraphPad Software, San Diego, CA, USA).

FIGS. 8A-8N show CD147-CAR-T and -NK cells specifically kill CD147-positive tumor cells *in vitro*. FIG. 8A shows cytotoxicity of primary CD147-CAR-T cells measured by FFLuc reporter assays. CD147-positive FFLuc-GFP-SK-Hep1 were used as the CD147-CAR-T susceptible target cells. Kappa-CAR T cells were used as control groups for each experiment. FIGS. 8B and 8C show significantly decreased cytotoxicity of CD147-CAR-T cells using knockout-CD147 FFLuc-GFP-SK-Hep1 cell line and HepG2 cell line by FFLuc reporter assays. Data represent the mean ± SEM from three independent experiments. FIG. 8D shows cytotoxicity of primary CD147-CAR-NK cells measured by the 4-h standard ^{51}Cr release assays. CD147-positive FFLuc-GFP-SK-Hep1 were used as the CD147-CAR-T susceptible target cells. Kappa-CAR T cells were used as a control group for each experiment. FIGS. 8E and 8F demonstrate significantly decreased cytotoxicity of primary CD147-CAR-NK cells using knockout-CD147 FFLuc-GFP-SK-Hep1 cell line and HepG2 cell line by the 4-h standard ^{51}Cr release assays. Data represent the mean ± SEM from three independent experiments. FIG. 8G shows that anti-NKG2D antibody blocked primary CD147-CAR-NK naturally killing to FFLuc-GFP-SK-Hep1. Primary CD147-CAR-NK cells in different ratios were co-cultured with FFLuc-GFP-SK-Hep1, knockout-CD147 FFLuc-GFP-SK-Hep1 cell line, or knockout-CD147 FFLuc-GFP-SK-Hep1 cell lines with 5μg anti-NKG2D for 4 hours. FFLuc reporter assays were used. Data represent the mean ± SEM from three independent experiments. FIG. 8H shows cytotoxicity of CD147-CAR-NK-92MI to the SK-Hep1 was measured by a standard 4-hr ^{51}Cr release assay. Effector cells (CD147-CAR-NK-92MI and NK-92MI) were co-cultured with target cells at 1×10^4 per well FFLuc-GFP-SK-Hep1. Four hours later, the supernatants were collected and the released ^{51}Cr was measured with a gamma counter. FIGS. 8I and 8J are FFLuc reporter system assay for specific killing of FFLuc-GFP-SK-Hep1 and FFLuc-GFP-HepG2 cell lines by CD147-CAR-NK-92MI. Effector cells (CD147-CAR-NK-92MI and NK-92MI) were co-cultured with 1×10^4 FFLuc-GFP-SK-Hep1 (FIG. 8I) or FFLuc-GFP-HepG2 (FIG. 8J) target cells per well in a 96-well optical-bottom microplate for 6 hours. Luminescent signals were measured by microplate reader after incubated with D-Luciferin for 5 minutes to calculate cytotoxicity of NK cells. The control groups used were wild type NK-92MI incubated with CD147-positive FFLuc-GFP-SK-Hep1 or CD147-positive FFLuc-GFP-HepG2. FIGS. 8K and 8L demonstrate decreased cytotoxicity of CD147-CAR-NK-92MI cells using knockout-CD147 FFLuc-GFP-SK-Hep1 (FIG. 8K) and knockout-CD147-FFLuc-GFP-HepG2 (FIG. 8L) cell lines by FFLuc reporter system assay. Effector cells (CD147-CAR-NK-92MI and NK-92MI) were co-cultured with 1×10^4 wild-type or CD147 knockout target cells per well in a 96-well optical-bottom microplate for 6 hours. Luminescent signals were measured by microplate reader after incubated with D-Luciferin for 5 minutes to calculate cytotoxicity of NK cells. FIGS. 8M and 8N are graphs showing that anti-CD147 (clone, HIM6) inhibited the CD147-CAR-NK-92MI specific lysis effect against and FFLuc-GFP-SK-Hep1 (FIG. 8M) and FFLuc-GFP-HepG2 (FIG. 8N). Effector cells (CD147-CAR-NK-92MI and NK-92MI) were co-cultured with 1×10^4 FFLuc-GFP-SK-Hep1 or FFLuc-GFP-HepG2 target cells per well in a 96-well optical-bottom microplate for 6 hours.

Luminescent signals were measured by microplate reader after incubated with D-Luciferin for 5 minutes to calculate cytotoxicity of NK cells. Data represent the mean ± SEM from three independent experiments. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

FIGS. 9A and 9B show verification of knockout-CD147 SK-Hep1 and HepG2 cell lines by flow cytometry and western-blot. FIG. 9A shows staining of surface CD147 molecules on wild-type (wt) SK-Hep1 and $CD147^{-/-}$-SK-Hep1 cell lines (top), as well as wild-type (wt) HepG2 and $CD147^{-/-}$-HepG2 cell lines (bottom). FIG. 9B shows a Western blot analysis of CD147 molecules on wild-type (wt) SK-Hep1 and $CD147^{-/-}$-SK-Hep1 cell lines, as well as on wild-type (wt) HepG2 and $CD147^{-/-}$-HepG2 cell lines. GAPDH was used as a loading control (bottom).

FIGS. 10A-10C show CD147-CAR-NK-T cells specifically kill CD147-positive tumor cells. Cytotoxicity of CD147-CAR-T cells was measured by a FFLuc report system assay. CD147-positive FFLuc-EGFP-Hep-G2 (FIG. 10A) and CD147-positive FFLuc-EGFP-SK-Hep1 (FIG. 10B) were used as the CD147-CAR-T susceptible target cells. Kappa-CAR T cells were used as control groups for each experiment. FIG. 10C shows significantly decreased cytotoxicity of CD147-CAR-T cells using knockout-CD147 FFLuc-GFP-SK-Hep1 cell line by FFLuc report system assay. Briefly effector cells (CD147-CAR-T cells) were co-cultured with target cells FFLuc-EGFP-SK-Hep1 or CD147 knockout FFLuc-EGFP-SK-Hep1 ($1x10^4$) in a 96-well optical-bottom microplate for 6 hours. Cytotoxicity of CD147-CAR-T cells was measured by the luminescence signal read by a microplate reader. Data represent the mean ± SEM from three independent experiments. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

FIGS. 11A-11D show that CD147-CAR-T-92MI cells control progression of HCC in a xenograft mouse model. FIG. 11A is a diagram of experimental design of HCC xenograft model. Briefly, NSG mice were subcutaneous injected with 4×10^6 SK-Hep1 cells premixed with equal volume Matrigel (Day 0). Mice were monitored tumor burden (achieved nearly 50 mm^2) and randomly grouped on day 4. At day 5 (D5), mice were injected (i.v.) with one dose of 1×10^7 effector CD147-CAR-T (Group #1) cells with 2×10^4 IU IL-2. The control groups were injected with vehicle (PBS) control only (Group #2). At day 7, 9, and 16, identical treatments in each group were administrated. FIG. 11B shows quantification of tumor burden of SK-Hep1 xenografts treated with CD147-CAR-T and PBS (vehicle control group), respectively. All results are mean ± SEM. The difference for each group was analyzed by two-way ANOVA analysis. FIG. 11C is quantitative body weight of each group was assessed at the indicated time points. FIG. 11D shows Kaplan-Meier survival curves of tumor-bearing mice after treatment with CD147-CAR-T cells and PBS (vehicle control group). The p-value was analyzed by log-rank (Mantel-Cox) Test.

FIGS. 12A-12D show the antitumor efficacy of CD147-CAR-NK-92-MI cells against HCC in a mouse xenograft model. FIG. 12A is a diagram of experimental design for anti-tumor efficacy of primary CD147-CAR-NK in HCC xenograft model. After tumor implantation for 5 days (day 5), the mice were injected (i.v.) with one dose 1×10^7 effector primary CD147-CAR-NK cells with 2×10^4 IU IL-2. The control groups were injected with the same number of non-transduced primary NK cells with 2×10^4 IU IL-2 (Group #2) or PBS only (Group #3). At day 5, 7, 9, 16, and 18, identical treatments in

each group were administrated, as indicated. FIG. 12B shows quantitative tumor burden of HCC xenograft mice treated with primary CD147-CAR-NK, non-transduced primary NK, and PBS (vehicle control group), respectively. All results are mean ± SEM. The difference for each group was analyzed by two-way ANOVA analysis. FIG. 12C shows quantitative body weights of each group were assessed at the indicated time points. FIG. 12D is Kaplan-Meier survival curves of tumor-bearing mice after treatment with primary CD147-CAR-NK, parental primary NK groups, and PBS (vehicle control group). P-value analysis by log-rank (Mantel-Cox) Test. The difference for each group was analyzed by two-way ANOVA analysis. The $*p < 0.05$, $**p < 0.01$, and $*** p < 0.001$ are indicated as in comparison of the CD147-CAR-modified cells treated groups with the control groups.

FIG. 13 shows comparable anti-HCC tumor activity between irradiated CD147-CAR-NK-92MI and non-irradiated CD147-CAR-NK-92MI cells in killing HCC cell lines *in vitro*. Cytotoxicity of irradiated and non-irradiated CD147-CAR-NK-92MI was measured by the standard 4-hr ^{51}Cr release assay. CD147-positive wild type-HepG2 tumor cell (experimental group, left panel) or CD147-knockout (CD147KO, right panel) HepG2 tumor cell lines were used as the CD147-CAR-NK-92MI susceptible target cells. Irradiated and non-irradiated wild type NK-92MI cells were used as effector cell control groups. Data are representative of three independent experiments. All data are presented as the mean ± SEM.

FIGS. 14A-14D show comparable anti-HCC tumor activity between irradiated CD147-CAR-NK-92MI and non-irradiated CD147-CAR-NK-92MI cells in control of HCC progression in xenograft mouse model. FIG. 14A is a diagram of experimental design of HCC xenograft model. NSG mice were injected (s.c.) with 2×10^6 SK-Hep1 cells premixed with equal volume Matrigel (Day 0). One day before the treatment (at day 4), tumor burden was determined (achieved nearly 50 mm^2) and mice randomly grouped. At day 5 (D5) mice were injected (i.v.) with one dose of 1×10^7 effector non-irradiated CD147-CAR-NK-92MI (Group #1) cells with 2×10^4 IU IL-2. The control groups were injected with the same number of irradiated CD147-CAR-NK-92MI with 2×10^4 IU IL-2 (Group #2) in PBS or vehicle control only (Group #3). At days 7, 9, 16, and 18, identical treatments in each group were administrated. FIG. 14B shows quantification of tumor burden of SK-Hep1 xenografts treated with CD147-CAR-NK-92MI and PBS (vehicle control group), respectively. All results are mean ± SEM. The difference for each group was analyzed by two-way ANOVA analysis. FIG. 14C is a graph of quantitative body weight of each group was assessed at the indicated time points. FIG. 14D shows Kaplan-Meier survival curves of tumor-bearing mice after treatment with CD147-CAR-NK-92MI cells and PBS (vehicle control group). The p-value was analyzed by log-rank (Mantel-Cox) Test. Data are representative of two independent experiments. All data are presented as the mean ± SEM.

FIGS. 15A-15D show that CD147-CAR-NK-92MI cells control progression of HCC in a xenograft mouse model. FIG. 15A is a diagram of experimental design of HCC xenograft model. Briefly, NSG mice were subcutaneous injected with 4×10^6 SK-Hep1 cells premixed with equal volume Matrigel (Day -7). At Day 0 (the day before day 1), tumor burden was determined (around 50 mm^2) and mice were randomly grouped. At day 1 (D1) mice were injected (i.v.) with one dose of 1×10^7 effector

CD147-CAR-NK-92MI (Group #1) cells with 2×104 IU IL-2. The control groups were injected with the same number of NK-92MI with 2×10^4 IU IL-2 (Group #2) in PBS or vehicle control only (Group #3). At day 3 and 5, identical treatments in each group were administered. FIG. 15B shows quantification of tumor burden of SK-Hep1 xenografts treated with CD147-CAR-NK-92MI, parental NK-92MI cells
5 (control group), and PBS (vehicle control group), respectively. All results are mean ± SEM. The difference for each group was analyzed by two-way ANOVA analysis. The $*p < 0.05$, $**p < 0.01$, and $***\ p <0.001$ are indicated as in comparison of the CD147-CAR-treated group with the NK-92MI-treated group. The $+\ p < 0.05$, $++\ p < 0.01$, and $+++\ p < 0.001$ are indicated in comparison of the CD147-CAR-treated group with the vehicle control-treated group. FIG. 15C shows quantitative body weight of
10 each group was assessed at the indicated time points and FIG. 15D shows Kaplan-Meier survival curves of tumor-bearing mice after treatment with CD147-CAR-NK-92MI cells, parental NK-92MI group, and PBS (vehicle control group). The p-value was analyzed by log-rank (Mantel-Cox) Test.

FIGS. 16A-16D show that CD147-CAR-NK-92MI cells control progression of HCC in a PDX mouse model. FIG. 16A is a diagram of experimental design for anti-tumor efficacy of CD147-CAR-
15 NK-92MI in a liver PDX model generated by The Jackson Laboratory. The patient-derived xenograft liver cancer mice were purchased from The Jackson Laboratory. After tumor implantation for 4 weeks (day 1), tumor burden was determined (around 50 mm^2) and mice were randomly grouped. Then indicated mice were injected (i.v.) with one dose of 5×106 effector CD147-CAR-NK-92MI cells with 2×10^4 IU IL-2. The control groups were injected with the same number of NK-92MI cells with 2×10^4 IU
20 IL-2 (Group #2) in PBS or PBS only (Group #3). At day 6, 8, 11, 15, 18, 22, and 26, identical treatments in each group were administrated, as indicated. FIG. 16B is quantitative tumor burden of PDX mice treated with CD147-CAR-NK-92MI cells, parental NK-92MI cells (control group), and PBS (vehicle control group), respectively. All results are mean ± SEM. The difference for each group was analyzed by two-way ANOVA analysis. The $*p < 0.05$, $**p < 0.01$, and $***\ p <0.001$ are indicated as in
25 comparison of the CD147-CAR-treated group with the NK-92MI-treated group. The $+p < 0.05$, $++p < 0.01$, and $+++p < 0.001$ are indicated in comparison of the CD147-CAR-treated group with the vehicle control-treated group. FIG. 16C shows quantitative body weights of each group were assessed at the indicated time points. FIG. 16D is Kaplan-Meier survival curves of tumor-bearing mice after treatment with CD147-CAR- NK-92MI cells, parental NK-92MI groups, and PBS (vehicle control group). P-value
30 analysis by log-rank (Mantel-Cox) Test.

FIGS. 17A-17D show killing of CD147-positive HCC cells by CD147-CAR-T cells. FFLuc reporter system assay for specific killing of FFLuc-EGFP-HepG2 by CD147-CAR-T cells (FIG. 17A). The control group used the wild type kappa-CAR-T cells incubating with CD147-positive FFLuc-EGFP-HepG2. FIG. 17B shows decreased cytotoxicity of CD147-CAR-T cells using knockout-CD147 FFLuc-
35 GFP-HepG1 by FFLuc report system assay. FIG. 17C shows cytotoxicity of CD147-CAR-T cells measured by a standard 4-hr ^{51}Cr release assay. FIG. 17D shows significantly decreased cytotoxicity of CD147-CAR-T cells using knockout-CD147 FFLuc-GFP-HepG1 by a standard 4-hr ^{51}Cr release assay.

FIGS. 18A and 18B show CD107a degranulation by CD147 CAR-T or -NK cells. FIG. 18A shows representative flow cytometric data illustrating CD107a degranulation on CD147-CAR-T cells after 10 hours with medium (control), SK-N-SH tumor cells. Cells were gated for CD56 positive subsets for quantifying surface CD107a expression. FIG. 18B shows quantitative data for percentage of surface
5 CD107a expression on CD147-CAR-NK-92MI cells upon different stimulations, as indicated. Data are pooled from at least three or four experiments.

FIG. 19 shows cytotoxicity of CD147-CAR-NK-92MI cells to DaoY cells *in vitro*.

FIG. 20 is an alignment showing an optimized CD147 scFv nucleic acid sequence (SEQ ID NO: 1) compared to the original scFv sequence (SEQ ID NO: 3), and a consensus sequence (SEQ ID NO: 13).

10 FIGS. 21A-21E show patient derived primary CD147-CAR-NK cells specifically kill CD147-positive tumor cells *in vitro*. FIGS. 21A is representative H&E (top) and IHC (bottom) staining of liver samples from different stages of HCC patients. FIG. 21C is a diagram of experimental design of HCC sample acquisition from different areas of liver cancer tissues. Briefly, three regions of interest (tumor zone, adjacent zone, and non-tumor zone) were obtained. Primary NK cells were isolated from these
15 zones (illustrated in FIG. 21B). FIG. 21D is flow cytometry analysis of CD147-CAR positive primary NK cells from different zones of liver tissues. FIG. 21E shows cytotoxicity of primary CD147-CAR-NK cells measured by 4-h standard ^{51}Cr release assays.

FIGS. 22A and 22B are representative flow cytometric analysis of CD147 expression on different types of cells (FIG. 22A) and cytotoxicity of CD147-CAR-NK-92MI measured by a standard 4-
20 hr ^{51}Cr release assay against target cells with different CD147 expression levels (FIG. 22B). Data are representative of two independent experiments. All data are presented as the mean ± SEM.

FIGS. 23A-23H demonstrate that SynNotch GPC3-inducible CD147-CAR T cells selectively target GPC3+CD147+ HepG2 cells but not GPC3+CD147- or GPC3-CD147+ HepG2 cells. FIG. 23A is a schematic design of GPC3-Gal4VP64-synNotch receptor in SFG retroviral vector and CD147-CAR
25 based on the pHR lentiviral vector. The SFG retroviral vector contains eGFP, which can be used as a marker for selecting GPC3-Gal4VP64-synNotch positive cells. The pHR construct included the CD147-specific single chain antibody fragment (clone, 5F6), a human IgG1 CH2CH3 hinge region and CD28 transmembrane region, followed by the intracellular domains of co-stimulatory CD28, 4-1BB, and the intracellular domain of CD3ζ. The pHR lentiviral vector contains mCherry, which can be used as a
30 marker for selecting CD147-CAR positive cells. FIG. 23B is a schematic design of 'logic-gated' GPC3-synNotch and CD147-CAR showing induced cytotoxicity when both antigens are co-expressed, but not when they are separately expressed on bystander or healthy cells. FIGS. 23C and 23D are schematic experimental design of GPC3-synNotch-GFP and CD147-CAR-mCherry vectors co-transduced T cells (FIG. 23C) and representative flow cytometric analysis of GPC3-synNotch-GFP and CD147-CAR-
35 mCherry expression (FIG. 23D). FIG. 23E is a schematic experimental design of GPC3-synNotch-GFP and CD147-CAR-mCherry vectors co-transduced into T cells, priming by GPC3^{high}CD147^{low} HepG2 cell line, and followed by CD147-CAR expression analysis among different subsets of transduced T cells, including mCherry positive only, GFP positive only, GFP and mCherry double positive, and GFP and

mCherry double negative subsets. FIG. 23F shows representative flow cytometric analysis of CD147-CAR expression on the surface of different subsets of transduced T cells. Both mean fluorescence intensity (MFI) and percentage of CD147-CAR are displayed in each representative flow cytometric chart. FIG. 23G is a representative flow cytometric analysis of CD147 and GPC3 expression on HepG2 tumor cell lines. FIG. 23H shows quantitative analysis of surface CD107a expression on different subsets of transduced T cells after 'primed and triggered' protocol by different HepG2 tumor cell lines for 2 hours. Data are representative of two independent experiments.

FIG. 24 shows that gamma secretase inhibitor (MK-0752, a Notch signaling inhibitor) specifically blocks the GPC3-SynNotch inducible CD147-CAR expression in the GPC3-SynNotch-eGFP+ and CD147-CAR-mCherry+ primary T cell subset, but not in other subsets of primary T cells. Representative flow cytometric analysis of CD147-CAR expression among different subsets of primary T cells (middle). Transduced T cells were treated with DMSO (0.3%; control), MK-0752 (10 μM), TAPI-1 (10 μM), GI254023X (10 μM), and a combination of MK-0752 + TAPI-1 + GI254023X, respectively. Meanwhile, these cells were primed in the presence of CD147KO GPC3high HepG2 cells. CD147-CAR expression on the surface of different subsets of transduced T cells was analyzed by flow cytometry. Both mean fluorescence intensity (MFI) and percentage of CD147-CAR are displayed in each representative flow cytometric chart. Data are representative of two independent experiments. All data are presented as the mean ± SEM.

FIGS. 25A-25H shows SynNotch CD147-inducible GPC3-CAR T cells selectively target GPC3+CD147+ HepG2 cells, but not GPC3+CD147- or GPC3-CD147+ HepG2 cells. FIG. 25A is a schematic design of Myc-CD147-Gal4VP64-SynNotch receptor in the SFG retroviral vector and GPC3-CAR based on the pHR lentiviral vector. The SFG retroviral vector contains a Myc-tag, which can be used as a marker for selecting CD147-Gal4VP64-SynNotch positive cells. The pHR construct consisted of the GPC3-specific single chain antibody fragment (scFv, clone 5F6, mIgG1), a human IgG1 CH2CH3 hinge region and CD28 transmembrane region, followed by the intracellular domains of co-stimulatory CD28, 4-1BB, and the intracellular domain of CD3ζ. The pHR lentiviral vector contains mCherry, which can be used as a marker for selecting cells with GPC3-CAR positive cells. FIG. 25B is a schematic design of 'Logic-gated' CD147-SynNotch and GPC3-CAR showing induced cytotoxicity when both antigens are co-expressed, but not activated when they are separately expressed on bystander or healthy cells. FIGS. 25C and 25D are schematic experimental design of Myc-CD147-SynNotch and GPC3-CAR-mCherry vectors co-transduced T cells (FIG. 25C) and Representative flow cytometric analysis of Myc-CD147-SynNotch and GPC3-CAR-mCherry expression (FIG. 25D). FIG. 25E is a schematic experimental design of Myc-CD147-SynNotch and GPC3-CAR-mCherry vectors co-transduced in T cells, priming by GPC3^{high}CD147^{low} HepG2 cell line, and followed by GPC3-CAR expression analysis among different subsets of transduced T cells, including mCherry positive only, GFP positive only, GFP and mCherry double positive, and GFP and mCherry double negative subsets. FIG. 25F is a representative flow cytometric analysis of GPC3-CAR expression on the surface of different subsets of transduced T cells. Both mean fluorescence intensity (MFI) and percentage of GPC3-CAR are displayed

in each representative flow cytometric chart. FIG. 25G is a representative flow cytometric analysis of surface CD107a expression on different subsets of transduced T cells after 'primed and triggered' protocol by different HepG2 tumor cell lines. FIG. 25H shows quantitative analysis of surface CD107a expression on different subsets of transduced T cells after 'primed and triggered' protocol by different HepG2 tumor cell lines. Fold-change of CD107a MFI was calculated as follows: [(MFIsample – MFIprimed only) / MFIprimed only]. Data are representative of two independent experiments. All data are presented as the mean ± SEM. $*p<0.05$, $**p<0.01$, and $***p<0.001$.

FIGS. 26A-26D show that SynNotch GPC3- inducible CD147-CAR T cells selectively kill $GPC3^{high}CD147^{high}$ HepG2 cells but not $CD147^{knockout}GPC3^{high}$ HepG2 cells. FIGS. 26A and 26B are representative flow cytometric analysis of CD3, CD56, GPC3-synNotch-GFP, and CD147-CAR-mCherry expression. Primary PBMCs were transduced with CD147-CAR-mCherry lentivirus. These mCherry positive T cells were sorted using flow cytometry, followed by a secondary transduction with GPC3-synNotch-GFP retrovirus. Representative flow cytometric analysis of CD3 and CD56 (FIG. 26A) and GPC3-synNotch-GFP and CD147-CAR-mCherry expression (FIG. 26B) are displayed, respectively. FIG. 26C is a graph of cytotoxicity of primary GPC3-synNotch-GFP-CD147-CAR-mCherry T cells against HepG2-$CD147^{high}$-$GPC3^{high}$ and HepG2-$CD147^{knockout}$-$GPC3^{high}$ measured by 7-hour FFluc reporter assays. FIG. 26D is a graph showing cytotoxicity of primary GPC3-synNotch-GFP-CD147-CAR-mCherry T cells against HepG2-$CD147^{high}$-$GPC3^{high}$ and HepG2-$CD147^{knockout}$-$GPC3^{high}$ measured by 7-hour Cr-51 release assays. Data are representative of two independent experiments.

SEQUENCE LISTING

Any nucleic acid and amino acid sequences listed herein or in the accompanying Sequence Listing are shown using standard letter abbreviations for nucleotide bases and amino acids, as defined in 37 C.F.R. § 1.822. In at least some cases, only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NO: 1 is a nucleic acid sequence encoding a modified CD147 scFv. Nucleotides 349-409 are a linker sequence.

SEQ ID NO: 2 is the amino acid sequence of the modified CD147 scFv.

SEQ ID NO: 3 is the nucleic acid sequence encoding the starting CD147 scFv.

SEQ ID NO: 4 is the nucleic acid sequence encoding a CD147-CAR. Signal peptide: nucleotides 1-57; VH domain: nucleotides 58-411; Linker sequence: nucleotides 412-468; VL domain: nucleotides 469-792; CD28 TM domain-41BB intracellular domain-CD3ζ domain: nucleotides 793-2202.

SEQ ID NO: 5 is the amino acid sequence of the CD147-CAR. Signal peptide: amino acids 1-19; VH domain: amino acids 20-137; Linker sequence: amino acids 138-156; VL domain: amino acids 157-264; CD28 TM domain-41BB intracellular domain-CD3ζ domain: amino acids 269-734.

SEQ ID NO: 6 is the nucleic acid sequence encoding CD14-CAR with inducible caspase 9. The iCaspase9 sequence is nucleotides 355-1200.

SEQ ID NO: 7 is the amino acid sequence of CD147-iCaspase 9-CAR. Amino acids 119-400 are iCaspase 9.

SEQ ID NO: 8 is the amino acid sequence of anti-CD147 VH CDR domains.

SEQ ID NO: 9 is the amino acid sequence of anti-CD147 VL CDR domains.

5 SEQ ID NOs: 10 and 11 are guide RNAs targeting CD147 used to generate CD147 knock out cell lines.

SEQ ID NO: 12 is an exemplary IL-15 receptor intracellular domain.

SEQ ID NO: 13 is a consensus nucleic acid sequence of an optimized CD147 scFv nucleic acid sequence (SEQ ID NO: 1) compared to the original scFv sequence (SEQ ID NO: 3).

10 SEQ ID NO: 14 is a nucleic acid sequence of a Gal4UAS CD147-CAR construct.

SEQ ID NO: 15 is the nucleic acid sequence of a pHR_Gal4UAS-CD147-CAR-pGK_mCherry vector.

SEQ ID NO: 16 is the nucleic acid sequence of a GPC3-CAR.

SEQ ID NO: 17 is a nucleic acid encoding a GAL4-VP64 activator.

15 SEQ ID NOs: 18-35 are the nucleic acid sequences of primers used for plasmid construction.

DETAILED DESCRIPTION

Disclosed herein are immune cells (including T cells and NK cells) expressing a novel CD147-targeting CAR. The biological properties of the CD147 antigen allow CD147-CAR-NK cells and
20 CD147-CAR-T cells to produce potent antitumor activity against hepatocellular carcinoma *in vitro* and *in vivo*. In addition, CD147-CAR-NK cells are also capable of killing human neuroblastoma cells *in vitro*.

Since CD147 is also expressed on several organs with varying expression levels, CD147-CAR modified immune cells may potentially exhibit an "on-target, off-tumor" toxicity. Disclosed herein are constructs and methods for addressing this potential toxicity, including "suicide genes" (such as an
25 inducible caspase 9) and combination treatments, such as CAR-expressing cells that are only activated upon binding of two antigens, such as CD147 and GPC3.

I. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of
30 common terms in molecular biology may be found in *Lewin's Genes X*, ed. Krebs *et al.*, Jones and Bartlett Publishers, 2009 (ISBN 0763766321); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and George P. Rédei, E*ncyclopedic Dictionary of Genetics,*
35 *Genomics, Proteomics and Informatics,* 3rd Edition, Springer, 2008 (ISBN: 1402067534), and other similar references.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular

terms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. "Comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

5 Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety, as are the GenBank Accession numbers. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and 10 examples are illustrative only and not intended to be limiting.

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Antibody: A polypeptide ligand comprising at least one variable region that recognizes and binds (such as specifically recognizes and specifically binds) an epitope of an antigen. Mammalian 15 immunoglobulin molecules are composed of a heavy (H) chain and a light (L) chain, each of which has a variable region, termed the variable heavy (V_H) region and the variable light (V_L) region, respectively. Together, the V_H region and the V_L region are responsible for binding the antigen recognized by the antibody. There are five main heavy chain classes (or isotypes) of mammalian immunoglobulin, which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

20 Antibody variable regions contain "framework" regions and hypervariable regions, known as "complementarity determining regions" or "CDRs." The CDRs are primarily responsible for binding to an epitope of an antigen. The framework regions of an antibody serve to position and align the CDRs in three-dimensional space. The amino acid sequence boundaries of a given CDR can be readily determined using any of a number of well-known numbering schemes, including those described by 25 Kabat *et al.* (*Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991; the "Kabat" numbering scheme), Chothia *et al.* (see Chothia and Lesk, *J Mol Biol* 196:901-917, 1987; Chothia *et al.*, *Nature* 342:877, 1989; and Al-Lazikani *et al.*, (JMB 273,927-948, 1997; the "Chothia" numbering scheme), and the ImMunoGeneTics (IMGT) database (see, Lefranc, *Nucleic Acids Res* 29:207-9, 2001; the "IMGT" numbering scheme). The Kabat and IMGT databases are 30 maintained online.

A single-chain antibody (scFv) is a genetically engineered molecule containing the V_H and V_L domains of one or more antibody(ies) linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, for example, Bird *et al.*, *Science*, 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci.*, 85:5879-5883, 1988; Ahmad *et al.*, *Clin. Dev. Immunol.*, 2012, doi:10.1155/2012/980250; 35 Marbry, *IDrugs*, 13:543-549, 2010). The intramolecular orientation of the V_H-domain and the V_L-domain in a scFv, is typically not decisive for scFvs. Thus, scFvs with both possible arrangements (V_H-domain-linker domain-V_L-domain; V_L-domain-linker domain-V_H-domain) may be used. In a dsFv the V_H and V_L have been mutated to introduce a disulfide bond to stabilize the association of the chains.

Diabodies also are included, which are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger *et al.*, *Proc. Natl. Acad.*
5 *Sci.,* 90:6444-6448, 1993; Poljak *et al.*, *Structure,* 2:1121-1123, 1994).

Antibodies also include genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies) and heteroconjugate antibodies (such as bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

10 **Cancer**: A malignant tumor characterized by abnormal or uncontrolled cell growth. Other features often associated with cancer include metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels and suppression or aggravation of inflammatory or immunological response, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc. "Metastatic disease" refers to cancer cells that have left the original
15 tumor site and migrated to other parts of the body, for example via the bloodstream or lymph system.

CD147: Also known as basigin (BSG), extracellular matrix metalloproteinase inhibitor (EMMPRIN or EMPRIN). A transmembrane glycoprotein with multiple functions in normal cell function and disease (Hahn *et al., J. Leukocyte Biol.* 98:33-48, 2015). CD147 is important in immune cells for T cell activation and proliferation, as well as cell migration, adhesion, and invasion (Hahn *et al.,*
20 *J. Leukocyte Biol.* 98:33-48, 2015). CD147 is expressed on different cell types (*e.g.,* hematopoietic, epithelial, and endothelial cells) at varying levels (Liao *et al., Mol. Cell Biol.* 31:2591-2604, 2011) and may be significantly upregulated in disease states, such as in HCC.

CD147 sequences are publicly available. For example, GenBank Accession Nos. NM_198590, NM_198591, NM_001728, NM_198589, and NM_001322243 disclose human CD147 nucleic acid
25 sequences, and GenBank Accession Nos. NP_940992, NP_940993, NP_001719, NP_940991, and NP_001309172 disclose human CD147 amino acid sequences. Similarly, NM_009768 and NM_001077184 disclose mouse CD147 nucleic acid sequences and GenBank Accession Nos. NP_033898 and NP_001070652 disclose mouse CD147 amino acid sequences. All of these sequences are incorporated by reference as present in GenBank on March 15, 2019.

30 **Chimeric antigen receptor (CAR):** A chimeric molecule that includes an antigen-binding portion (such as a single domain antibody or scFv) and a signaling domain, such as a signaling domain from a T cell receptor (*e.g.* CD3ζ). Typically, CARs include an antigen-binding portion, a transmembrane domain, and an intracellular domain. The intracellular domain typically includes a signaling chain having an immunoreceptor tyrosine-based activation motif (ITAM), such as CD3ζ or
35 FcεRIγ. In some instances, the intracellular domain also includes the intracellular portion of at least one additional co-stimulatory domain, such as CD28, 4-1BB (CD137), ICOS, OX40 (CD134), CD27 and/or DAP10.

Complementarity determining region (CDR): A region of hypervariable amino acid sequence that defines the binding affinity and specificity of an antibody. The light and heavy chains of a mammalian immunoglobulin each have three CDRs, designated VL-CDR1, VL-CDR2, VL-CDR3 and VH-CDR1, VH-CDR2, VH-CDR3, respectively.

5 **Glypican-3 (GPC3):** A cell surface heparan sulfate proteoglycan that binds to and inhibits CD26 activity. GPC3 can induce apoptosis in some cell types. GPC3 is expressed by some tumors, including hepatocellular carcinoma, melanoma, ovarian clear-cell carcinoma, yolk sac tumors, neuroblastoma, hepatoblastoma, and Wilms tumor.

GPC3 sequences are publicly available. For example, GenBank Accession Nos. 10 NM_001164619, NM_001164618, NM_004484, and NM_001164617 disclose exemplary human GPC3 nucleic acid sequences, and GenBank Accession Nos. NP_001158091, NP_001158090, NP_004475, and NP_001158089 disclose exemplary human GPC3 amino acid sequences. Similarly, GenBank Accession No. NM_016697 discloses an exemplary mouse GPC3 nucleic acid sequence and GenBank Accession No. NP_057906 discloses an exemplary mouse GPC3 amino acid sequence. Each of these sequences are 15 incorporated by reference as present in GenBank on February 27, 2020.

Isolated: An “isolated” biological component, such as a nucleic acid, protein (including antibodies) or organelle, has been substantially separated or purified away from other biological components in the environment (such as a cell) in which the component naturally occurs, *i.e.*, other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and 20 proteins that have been “isolated” include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

Liver cancer: Hepatocellular carcinoma (HCC) is the most common type of primary malignancy of the liver, which often occurs in patients with viral hepatitis (*e.g.*, hepatitis B or hepatitis 25 C), toxin exposure, or hepatic cirrhosis (sometimes caused by alcoholism). Other types of liver cancer include intrahepatic cholangiocarcinoma (iCCA), fibrolamellar carcinoma, and hepatoblastoma.

Natural Killer (NK) cells: Cells of the immune system that kill target cells in the absence of a specific antigenic stimulus and without restriction according to MHC class. Target cells can be tumor cells or cells harboring viruses. NK cells are characterized by the presence of CD56 and the absence of 30 CD3 surface markers. NK cells typically comprise approximately 10 to 15% of the mononuclear cell fraction in normal peripheral blood. Historically, NK cells were first identified by their ability to lyse certain tumor cells without prior immunization or activation. NK cells are thought to provide a “back up” protective mechanism against viruses and tumors that might escape the CTL response by down-regulating MHC class I presentation. In addition to being involved in direct cytotoxic killing, NK cells 35 also serve a role in cytokine production, which can be important to control cancer and infection.

In some examples, a **“modified NK cell”** is a NK cell transduced or transfected with a heterologous nucleic acid (such as one or more of the nucleic acids or vectors disclosed herein) or

expressing one or more heterologous proteins. The terms “modified NK cell” and “transduced NK cell” are used interchangeably in some examples herein.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified protein or nucleic acid preparation is one in which the protein or nucleic acid is more enriched than the protein or nucleic acid is in its natural environment (*e.g.*, within a cell). In one embodiment, a preparation is purified such that the protein or nucleic acid represents at least 50% of the total protein or nucleic acid content of the preparation. Substantial purification denotes purification from other proteins or cellular components. A substantially purified protein or nucleic acid is at least 60%, 70%, 80%, 90%, 95% or 98% pure. Thus, in one specific, non-limiting example, a substantially purified protein or nucleic acid is 90% free of other components.

Recombinant: A nucleic acid or protein that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence (*e.g.*, a “chimeric” sequence). This artificial combination can be accomplished by chemical synthesis or by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques.

Subject: A living multi-cellular vertebrate organism, a category that includes both human and veterinary subjects, including human and non-human mammals.

T cell: A white blood cell (lymphocyte) that is an important mediator of the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that carries a marker on its surface known as “cluster of differentiation 4” (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. $CD8^+$ T cells carry the “cluster of differentiation 8” (CD8) marker. In one embodiment, a $CD8^+$ T cell is a cytotoxic T lymphocyte (CTL). In another embodiment, a $CD8^+$ cell is a suppressor T cell.

Activated T cells can be detected by an increase in cell proliferation and/or expression of or secretion of one or more cytokines (such as IL-2, IL-4, IL-6, IFNγ, or TNFα). Activation of CD8+ T cells can also be detected by an increase in cytolytic activity in response to an antigen.

In some examples, a **“modified T cell”** is a T cell transduced or transfected with a heterologous nucleic acid (such as one or more of the nucleic acids or vectors disclosed herein) or expressing one or more heterologous proteins. The terms “modified T cell” and “transduced T cell” are used interchangeably in some examples herein.

Transduced or Transformed: A transformed cell is a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. As used herein, the terms transduction and transformation encompass all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, the use of plasmid vectors, and introduction of DNA by electroporation, lipofection, and particle gun acceleration.

Treating or ameliorating a disease: “Treating” refers to a therapeutic intervention that decreases or inhibits a sign or symptom of a disease or pathological condition after it has begun to

develop, such as a reduction in tumor size or tumor burden. "Ameliorating" refers to the reduction in the number or severity of signs or symptoms of a disease, such as cancer.

Vector: A nucleic acid molecule that can be introduced into a host cell (for example, by transfection or transduction), thereby producing a transformed host cell. Recombinant DNA vectors are
5 vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses. A replication deficient viral vector is a vector that requires complementation of one or more regions of the viral genome
10 required for replication due to a deficiency in at least one replication-essential gene function.

II. Overview of Several Embodiments

Disclosed herein are CD147-specific binding agents, including a modified CD147 scFv. Also disclosed are chimeric antigen receptors (CARs) that encode the CD147-specific binding agent fused to a
15 hinge region, a transmembrane domain and an intracellular domain including a co-stimulatory domain and an intracellular signaling domain. In some examples, the co-stimulatory domain is from CD28 and/or 4-1BB and the signaling domain is from CD3ζ. Also provided are nucleic acids encoding the CD147-specific binding agents and the CD147-CARs, and vectors including the nucleic acids.

Also provided herein are modified immune cells (such as T cells, NK cells, NKT cells, DNT
20 cells, neutrophils, or macrophages) that express the CD147-CARs. In some embodiments, the modified immune cells express one or more additional CARs, such as a CAR targeting hepatitis virus (for example, hepatitis B or hepatitis C). In other embodiments, the modified immune cells express an inducible CD147-CAR and a construct that induces expression of the CD147-CAR, such as an anti-GPC3 SynNotch construct that drives expression of an inducer of the CD147-CAR. In further
25 embodiments, the modified immune cells express an inducible GPC3-CAR and a construct that induces expression of the GPC3-CAR, such as an anti-CD147 SynNotch construct that drives expression of an inducer of the GPC3-CAR. In particular examples, the CD147-CAR and/or the anti-CD147 SynNotch construct include the modified CD147 scFv disclosed herein.

Also provided are methods of treating a cancer that expresses CD147 in a subject. In some
30 embodiments, the method includes administering to the subject an effective amount of a modified immune cell (such as a T cell, NK cell, NKT cell, DNT cell, neutrophil, or macrophage) comprising a nucleic acid encoding a CD147-CAR. In other embodiments, the method includes administering to the subject an effective amount of a modified immune cell (such as a T cell, NK cell, NKT cell, DNT cell, neutrophil, or macrophage) comprising a nucleic acid encoding an inducible CD147-CAR and a nucleic
35 acid for an anti-GPC3 SynNotch that expresses an inducer of the CD147-CAR expression. In still other embodiments, the method includes administering to the subject an effective amount of a modified immune cell (such as a T cell, NK cell, NKT cell, DNT cell, neutrophil, or macrophage) comprising a nucleic acid encoding a GPC3-CAR and an anti-CD147 SynNotch construct that expresses an inducer of

the GPC3-CAR expression. In some examples, the modified immune cells are autologous. In other examples, the immune cells are allogeneic. In some specific examples, the subject has hepatocellular carcinoma or neuroblastoma.

5 **III. CD147 Specific Binding Agents**

Disclosed herein is a CD147 binding agent, that in some examples is used as the targeting portion of a chimeric antigen receptor. In some embodiments, the CD147 binding agent is a CD147 scFv that is a modified fragment encoding the CD147-specific scFv from the 5F6 clone (U.S. Pat. No. 8,618,264). FIG. 20 shows an alignment of the starting CD147 scFv nucleic acid sequence (SEQ ID NO: 10 3) and the modified scFv nucleic acid sequence (SEQ ID NO: 1). In some examples, the modified CD147-specific scFv binds to high-expressing CD147 cells.

In some embodiments, the CD147-specific binding agent is a single domain antibody (such as an scFv) that specifically binds CD147 and includes the CDR sequences provided in Table 1. In some examples, the scFv specifically binds CD147 and includes an amino acid sequence comprising the 15 variable heavy chain (VH) domain complementarity determining region 1 (CDR1), CDR2 and CDR3 amino acid sequences of SEQ ID NO: 8 and the variable light chain (VL) domain CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 9. In some embodiments, the scFv includes the CDR amino acid sequences provided in Table 1 and has at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 20 99% sequence identity) to the amino acid sequence of SEQ ID NO: 1. In other embodiments, the scFv includes or consists of the amino acid sequence of SEQ ID NO: 1.

Table 1. Location of the CDRs in the CD147 scFv sequence (determined using Kabat numbering scheme)

CDR	Nucleic Acid Sequence (SEQ ID NO: 1)	Amino Acid Sequence (SEQ ID NO: 2)
VH CDR1	GGCTTCACCTTCAGCAACTAC (nt 76-96)	GFTFSNY (aa 26-32)
VH CDR2	AGACTGAAGTCCTACAACTACGCC (nt 154-177)	RLKSYNYA (aa 52-59)
VH CDR3	GATGGCAGCGAC (nt 301-312)	DGSD (aa 101-104)
VL CDR1	AAGGCCTCCCAGTCCGTGAGCAACGATGTGGCC (nt 478-510)	KASQSVSNDVA (aa 155-165)
VL CDR2	TACGCCAGCAACAGGTACACA (nt 556-576)	YASNRYT (aa 181-187)
VL CDR3	CAGCAGGACTACTCCAGCCCCTACACC (nt 673-699)	QQDYSSPYT (aa 220-228)

In additional examples, the scFv is encoded by a nucleic acid including the CDR sequences provided in Table 1 and has at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity) to the nucleotide sequence of SEQ ID NO: 2. In other embodiments, the scFv is encoded by a nucleic acid that includes or consists of the nucleotide sequence of SEQ ID NO: 2.

Also provided are vectors that include a nucleic acid encoding the CD147-specific binding agents described above. In some examples, the vector includes a nucleic acid with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity) to SEQ ID NO: 1, for example, encoding an amino acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity) to SEQ ID NO: 2.

In further examples, provided herein are nucleic acids encoding an CD147-specific binding agent (such as a nucleic acid with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity to SEQ ID NO: 1) that is operably linked to a nucleic acid encoding Gal4-VP64 (*e.g.*, SEQ ID NO: 17). In some examples, the nucleic acid is part of a SynNotch construct. When CD147 binds to the protein encoded by this construct, Gal4-VP64 is activated, and can induce expression of a construct with a Gal4-responsive element (such as Gal4UAS). An exemplary vector encoding an anti-CD147 scFv SynNotch inducer construct is shown in FIG. 25A.

IV. CD147 Chimeric Antigen Receptors

Provided herein are CD147-CARs that include the CD147-specific binding agent described in Section III above. In some embodiments, the CAR includes an antigen binding domain including a CD147-specific scFv (such as SEQ ID NO: 2), a hinge domain, a transmembrane domain, and an intracellular domain including at least one co-stimulatory domain and an intracellular signaling domain.

In some embodiments, the antigen binding domain is a CD147-specific scFv, for example having an amino acid sequence with at least 90% sequence identity (for example, 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% identity) to SEQ ID NO: 2 or including or consisting of the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the hinge domain is an IgG hinge region. In one example, the hinge domain is an IgG1 hinge. Other hinge domains can be used, such as hinge regions from other immunoglobulins (for example, IgG4 or IgD) or a hinge region from CD8, CD28, or CD40.

In additional embodiments, the transmembrane domain is a CD28 transmembrane domain. In one example, the transmembrane domain is from CD28. The transmembrane domain can also be from other T cell proteins, such as CD8, CD4, CD3ζ., CD40, OX40L, 41BBL, ICOS, ICOS-L, CD80, CD86,

ICAM-1, LFA-1, ICAM-1, CD56, CTLA-4, PD-1, TIM-3, NKP30, NKP44, NKP40, NKP46, B7-H3, PD-L1, PD-2, and CD70.

In further embodiments, the intracellular domain includes one or more intracellular regions from a co-stimulatory molecule, or a portion thereof. Exemplary co-stimulatory molecules include CD28, 4-1BB, CD8, CD40, OX-40, ICOS, CD27, and DAP10, OX40-L, 4-1BBL, ICOS-L, CD80, CD86, ICAM-1, LFA-1, CD56, CTLA-4, PD-1, TIM-3, NKP30, NKP44, NKP40, NKP46, B7-H3, PD-L1, PD-2, and CD70. In particular examples, the co-stimulatory domain is from CD28 and/or 4-1BB. In one example, the co-stimulatory domain includes domains from both CD28 and 4-1BB. The intracellular domain also includes an intracellular signaling domain from CD3ζ. In other examples, the intracellular signaling domain is from DAP10, DAP12, PDK, or FcεRIγ. In one example, the intracellular signaling domain is from CD3ζ.

In some embodiments, the CD147-CAR also includes a signal sequence, which is located N-terminal to the scFv domain. In some examples, the signal sequence is a IgG signal sequence or a GM-CSF signal sequence. In one example, the signal sequence is amino acids 1-19 of SEQ ID NO: 5.

In particular embodiments, the CD147-CAR includes an amino acid sequence with at least 90% sequence identity (for example, 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% identity) to SEQ ID NO: 5. In some examples, the CD147-CAR includes or consists of the amino acid sequence of SEQ ID NO: 5.

In additional embodiments, the CD147-CAR further includes an inducible gene that can be used to eliminate CD147-CAR expressing cells (*e.g.*, a "suicide" gene). The inducible gene can be activated in the event of off target side effects (or on target/off tumor effects), such as cytokine release syndrome ("cytokine storm"). In some examples, expression of the suicide gene is inducible by a small molecule, such as tetracycline or doxycycline (a "TET ON" system) or rapamycin. See, *e.g.*, Gargett *et al., Front. Pharmacol.* 5:235, 2014; Stavrou *et al., Mol. Ther.* 6:1266-1276, 2018. In other examples, the suicide gene is inducible by a Fas domain inducible system. In some examples, the inducible suicide domain is located N-terminal or C-terminal to the antigen binding domain of the CAR, while in other examples, the inducible suicide domain is located C-terminal to the CD3ζ domain of the CAR. The inducible suicide domain is separated from the CAR by a self-cleaving peptides (such as a P2A peptide or T2A peptide). In some embodiments, the inducible suicide domain includes Caspase 9, such as amino acids 119-400 of SEQ ID NO: 7. In some examples, a CD147-CAR including a tetracycline-inducible Caspase 9 includes an amino acid sequence with at least 90% sequence identity (for example, 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% identity) to SEQ ID NO: 7. In other examples, the CD147-CAR including a tetracycline-inducible Caspase 9 includes or consists of the amino acid sequence of SEQ ID NO: 7.

In other embodiments, the CD147-CAR further includes a domain that increases survival or persistence of a modified immune cell expressing the CAR. In some examples, the domain is an intracellular domain from a cytokine receptor, for example, an intracellular domain from interleukin (IL) receptor 15 (*e.g.*, SEQ ID NO: 12), IL-12 receptor, or IL-18 receptor. In other examples, the domain is

an intracellular domain a growth factor receptor, such as an intracellular domain from CD40, NKG2D, NKP40, or NKP46. In some examples, the domain is located C-terminal to the CD3ζ domain of the CAR.

In some examples, the CD147-CAR further includes one or more additional antigen binding
5 domains that specifically bind to an antigen that is co-expressed with CD147 on tumor cells. In some non-limiting examples, the CD147-CAR includes at least one additional antigen binding domain that specifically binds to a liver cancer antigen, such as one or more of glypican-3, alpha-fetoprotein, or mucin-1. Additional tumor antigens can be selected based on the type of cancer being treated.

Also provided are nucleic acids encoding the CD147-CARs disclosed herein. In some
10 embodiments, the nucleic acid encodes a CAR including a CD147-specific scFv, such as a nucleic acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity) to SEQ ID NO: 1 or includes or consists of the nucleic acid sequence of SEQ ID NO: 1. In some examples, the CD147-specific CAR nucleic acid also encodes an IgG hinge domain, a CD28 transmembrane
15 domain, CD28 and 4-1BB co-stimulatory domains, and a CD3ζ domain. In one example, the CD147-specific CAR is encoded by a nucleic acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity) to SEQ ID NO: 4 or includes or consists of the nucleic acid sequence of SEQ ID NO: 4. In other examples, the CD147-specific CAR nucleic acid also encodes an inducible
20 Caspase 9 domain, for example a nucleic acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity) to SEQ ID NO: 6 or includes or consists of the nucleic acid sequence of SEQ ID NO: 6.

Also provided are functional variants of the CARs or the domains thereof described herein,
25 which retain the biological activity of the CAR of which it is a variant or retains the biological activity of the particular domain. The functional variant can be at least about 80%, about 85%, about 90%, about 91 %, about 92%, about 93%, about 94%, about 95%, about 96%), about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent CAR or domain. Substitutions can be made, for example, in one or more of the extracellular targeting domain, hinge domain, transmembrane domain,
30 and intracellular domains.

In some examples, the functional variant includes the amino acid sequence of the parent CAR or domain with at least one conservative amino acid substitution (such as up to 10 conservative amino acid substitutions, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative substitutions). In other examples, the functional variant includes the amino acid sequence of the parent CAR or domain with at least one
35 non-conservative amino acid substitution (such as up to 10 non-conservative amino acid substitutions, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-conservative substitutions). In this case, the non-conservative amino acid substitution does not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional

variant, such that the biological activity of the functional variant is increased as compared to the parent CAR or domain.

The CARs or domains thereof can in some examples, include one or more synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids include, for example, aminocyclohexane carboxylic acid, norleucine, a-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4- aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1 ,2,3,4- tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, oc-aminocycloheptane carboxylic acid, -(2-amino-2-norbornane)-carboxylic acid, γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine. The CARs may be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, *e.g.*, a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

In some embodiments, a nucleic acid molecule encoding a disclosed CAR is included in an expression vector (such as a viral vector) for expression in a host cell, such as a T cell or NK cell. In some examples, the expression vector includes a promoter operably linked to the nucleic acid molecule encoding the CAR. Additional expression control sequences, such as one or more enhancers, transcription and/or translation terminators, and initiation sequences can also be included in the expression vector. In some embodiments, a nucleic acid encoding a CD147-CAR provided herein is included in a viral vector. Examples of suitable virus vectors include retrovirus (*e.g.*, MoMLV or lentivirus), adenovirus, adeno-associated virus, vaccinia virus, and fowlpox vectors. In specific examples, the CD147-CAR encoding nucleic acid is included in a MoMLV vector, such as an SFG retroviral vector or a pHAGE-CPPT lentiviral vector. In other examples, the vector may be a DNA vector.

In some embodiments, the vector further includes an upstream activation sequence (UAS) that permits inducible expression of the CD147-CAR. In one non-limiting example, the UAS is a Gal4 UAS, which is activated by Gal4. However, one of skill in the art can identify other trans-activation systems that could be utilized. In one example, a Gal4UAS CD147-CAR nucleic acid includes a nucleic acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity) to SEQ ID NO: 14. An exemplary vector encoding a Gal4UAS CD147-CAR construct is shown in FIG. 23A, such as pHR_Gal4UAS-CD147-CAR-pGK_mCherry. In some examples, the vector includes a nucleic acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity) to SEQ

ID NO: 15. In some examples, the vector includes a selectable marker (such as mCherry in SEQ ID NO: 15), but in other examples, the selectable marker is not included in the vector.

In some examples, the vector further includes a nucleic acid sequence encoding at least one additional CAR. In some examples, the additional CAR is specific to an additional tumor antigen, for example, to increase specificity of targeting of the CD147-CAR to tumor cells expressing or overexpressing CD147. In some examples, the vector includes a nucleic acid encoding one or more CARs including an antigen binding domain that specifically binds to a liver cancer antigen, such as one or more of glypican-3, alpha-fetoprotein, or mucin-1. Additional tumor antigens/CARs can be selected based on the type of cancer being treated. In other examples, the vector includes a nucleic acid encoding a CAR encoding a CAR including an antigen binding domain specific for hepatitis B or hepatitis C. In some examples, the additional CAR binds to a HBV envelope protein or hepatitis B surface antigen (HBsAg). In other examples, the additional CAR binds to HCV E2 glycoprotein. In some examples, the one or more additional CARs are included in the vector with the CD147-CAR, for example, separated by a self-cleaving peptide, such as a P2A peptide sequence.

V. Cells Expressing CD147 CARs or CD147-Specific Binding Agents

Also provided herein are cells (for example, immune cells) that express the disclosed CD147-CARs or CD147-specific binding agents. In particular embodiments, the cells include T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages. In some embodiments, the cells are T cells, NK cells, or macrophages expressing a CD147-CAR.

In some examples, the cells further express a GPC3-specific binding agent, such as an anti-GPC3 scFV. In particular examples, the cells express an anti-GPC3 scFV operably linked to a nucleic acid encoding Gal4-VP64 (*e.g.*, a SynNotch construct). When GPC3 binds to the protein encoded by this construct, Gal4-VP64 is activated, and can induce expression of a construct with a Gal4-responsive element (such as Gal4UAS). An exemplary vector encoding an anti-GPC3 scFv SynNotch inducer construct is shown in FIG. 23A. Thus, in some examples, the T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages express an inducible CD147-CAR (*e.g.*, SEQ ID NO: 14) and an anti-GPC3 binding agent construct that induces expression of the inducible CD147-CAR.

In other embodiments, the cells are T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages expressing a CD147-specific binding agent, such as a CD147 scFv (for example, SEQ ID NO: 1) and a GPC3-CAR. In some examples, the GPC3-CAR includes a nucleic acid sequence with at least 90% sequence identity (for example, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identity) to SEQ ID NO: 16. In particular examples, the cells express an anti-CD147 scFV operably linked to a nucleic acid encoding Gal4-VP64 (*e.g.*, a SynNotch construct). When CD147 binds to the protein encoded by this construct, Gal4-VP64 is activated, and can induce expression of a construct with a Gal4-responsive element (such as Gal4UAS). Exemplary vectors encoding an anti-CD147 scFv SynNotch inducer construct and an anti-GPC3-CAR are shown in FIG. 25A. Thus, in some examples, the T cells, NK cells, NKT cells, DNT

cells, neutrophils, macrophages express an inducible GPC3-CAR (*e.g.*, SEQ ID NO: 16) and an anti-CD147 binding agent construct that induces expression of the inducible GPC3-CAR.

In some examples, the immune cells are transduced or transfected with one or more expression vectors including one or more nucleic acids, including nucleic acids encoding a CD147-CAR, an inducible GPC3-CAR, an inducible CD147-CAR, a CD147-specific binding agent operably linked to an inducer, a GPC3-specific binding agent operably linked to an inducer, or any combination of two or more thereof. In other examples, the vector (or a DNA encoding the construct) may be introduced by contacting the cells with a nanoparticle including the vector or DNA. In some examples, the cells are irradiated following transduction or transfection (*e.g.*, treated with γ-irradiation, such as at a dose of at least 1,000, at least 2,000, at least 3,000, at least 5,000, at least 7,000, at least 8,000, at least 9,000, at least 10,000, at least 11,000, at least 12,000, or at least 15,000 or about 1,000-15,000, 2,000-12,000, 1,000-5,000, 5,000-10,000, or 8,000-12,000, or about 10,000 Rad), for example, prior to administering to a subject.

In some examples, the transduced or transfected cells are isolated T cells (such as a primary T cell or T cells obtained from a subject), isolated NK cells (such as a primary NK cell or NK cells obtained from a subject), isolated NKT cells, isolated DNT cells, isolated neutrophils, or isolated macrophages (such as a primary macrophage or macrophages obtained from a subject). In some examples, the T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages are obtained from peripheral blood, umbilical cord blood, lymph node tissue, bone marrow, or tumor tissue. In some examples, T cells, NK cells, NKT cells, or DNT cells are also enriched, purified, and/or expanded from a sample from a subject, for example before and/or after transduction with one or more of the disclosed expression vectors.

In one non-limiting embodiment, the cell is an NK-92 cell. NK-92 cells are a NK cell line derived from a patient with non-Hodgkin's lymphoma (*e.g.*, ATCC® CRL-2407™). This cell line has properties of activated NK cells (see, *e.g.*, Gong *et al., Leukemia* 8:652-658, 1994). In another embodiment, the cell is an NK-92MI cell (*e.g.*, ATCC® CRL-2408™). The NK-92MI cell line is an interleukin-2 (IL-2) independent NK cell line, derived from NK-92, which stably expresses human IL-2 (see, *e.g.*, Tam *et al., Hum. Gene Ther.* 10:1359-1373, 1999). NK-92 or NK-92MI cells expressing a CAR (such as a CD147-CAR and/or other nucleic acids disclosed herein) can be used herein as an "off the shelf" immunotherapy, since autologous NK cells do not have to be produced for each subject. Other NK cell lines that can be used with the CD147-CARs (or other nucleic acids) described herein include NKL, KHYG-1, and YTS cells.

NK-92-mediated immunotherapy is now undergoing phase I/II clinical trials (Arai et al., 2008; Tonn et al., 2013). Commonly, NK-92 cells must be irradiated prior to infusion to prevent permanent engraftment. The amount of irradiation required is around 10 Gy. The dose of irradiated NK-92 infusion can be up to 10^{10} NK92 cells/m^2. Importantly, irradiated NK-92 cells have been shown to be safe for infusion in patients, as demonstrated by several NK-92 clinical trials (NCT00900809, NCT00990717, NCT00995137, and NCT01974479).

In some non-limiting embodiments, immune cells are transduced with a vector encoding a CD147-CAR. Following transduction, cells expressing the CD147-CAR can be detected and/or enriched, for example, by flow cytometry using a labeled antibody that binds to CD147. In some examples, the transduced cells (such as NK cells or T cells) are expanded, for example, by cell culture for a period of time following transduction. In some examples, some or all of the modified cells are cryopreserved for later use.

VI. Methods of Immunotherapy

Provided are methods of treating cancer (such as a cancer expressing or overexpressing CD147) in a subject with a CD147-CAR disclosed herein. In some embodiments, the methods include administering to the subject a composition including a modified T cell, NK cell, NKT cell, DNT cell, neutrophil, or macrophage expressing a CD147-CAR (for example, transduced with a vector encoding the CAR) and a pharmaceutically acceptable carrier. In other examples, the methods include administering to the subject a pharmaceutical composition including an expression vector encoding a CD147-CAR and a pharmaceutically acceptable carrier.

Also provided are methods of treating cancer (such as a cancer expressing or overexpressing CD147) in a subject with an inducible CD147-CAR and a GPC3-specific binding agent linked to expression of an inducer of the inducible CD147-CAR disclosed herein. In some embodiments, the methods include administering to the subject a composition including a modified T cell, NK cell, NKT cell, DNT cell, neutrophil, or macrophage expressing an inducible CD147-CAR and a GPC3-specific binding agent linked to an inducer (for example, transduced with one or more vectors encoding the CD147-CAR and the GPC3-specific binding agent) and a pharmaceutically acceptable carrier. In other examples, the methods include administering to the subject one or more expression vectors encoding the inducible CD147-CAR (*e.g.*, SEQ ID NO: 15) and the GPC3-specific binding agent linked to the inducer and a pharmaceutically acceptable carrier.

Also provided are methods of treating cancer (such as a cancer expressing or overexpressing CD147) in a subject with an inducible GPC3-CAR and a CD147-specific binding agent disclosed herein linked to an inducer of the inducible GPC3-CAR. In some embodiments, the methods include administering to the subject a composition including a modified T cell, NK cell, NKT cell, DNT cell, neutrophil, or macrophage expressing an inducible GPC3-CAR and a CD147-specific binding agent linked to the inducer (for example, transduced with one or more vectors encoding the GPC3-CAR (*e.g.*, SEQ ID NO: 16) and the CD147-specific binding agent) and a pharmaceutically acceptable carrier. In other examples, the methods include administering to the subject one or more expression vectors encoding the inducible GPC3-CAR and the CD147-specific binding agent linked to the inducer and a pharmaceutically acceptable carrier.

The modified cells (such as T cells, NK cells, NKT cells, DNT cells, neutrophils, or macrophages) expressing a CD147-CAR or CD147-specific binding agent described herein can be incorporated into pharmaceutical compositions. Such compositions typically include a population of

cells (such as CD147-CAR-NK cells or CD147-CAR-T cells) and a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration (see, *e.g., Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition, 2005). Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, balanced salt solutions, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Supplementary active compounds can also be incorporated into the compositions. Actual methods for preparing administrable compositions include those provided in *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005).

In some examples, the composition includes about 10^4 to 10^{12} of the modified NK cells or T cells (for example, about 10^4-10^8 cells, about 10^6-10^8 cells, or about 10^6-10^{12} cells). For example, the composition may be prepared such that about 10^4 to 10^{10} modified NK cells or modified T cells cells/kg (such as about 10^4, 10^5, 10^6, 10^7, or 10^8 cells/kg) are administered to a subject. In specific examples, the composition includes at least 10^4, 10^5, 10^6, or 10^7 CD147-CAR-NK cells or CD147-CAR-T cells. The population of modified NK cells or modified T cells is typically administered parenterally, for example intravenously; however, injection or infusion to a tumor or close to a tumor (local administration) or administration to the peritoneal cavity can also be used. Appropriate routes of administration can be determined based on factors such as the subject, the condition being treated, and other factors.

Multiple doses of the population of modified NK cells or modified T cells can be administered to a subject. For example, CD147-CAR-NK cells or CD147-CAR-T cells can be administered daily, every other day, twice per week, weekly, every other week, every three weeks, monthly, or less frequently. A skilled clinician can select an administration schedule based on the subject, the condition being treated, the previous treatment history, and other factors.

In additional examples, the subject is also administered at least one, at least one, at least two, at least three, or at least four cytokine(s) (such as IL-2, IL-15, IL-21, and/or IL-12) to support survival and/or growth of the modified NK cells or modified T cells. In specific, non-limiting examples, at least one cytokine includes IL-2 and IL-15. The cytokine(s) are administered before, after, or substantially simultaneously with the modified NK cells or modified T cells. In specific examples, at least one cytokine (*e.g.*, IL-2) is administered simultaneously, for example, with CD147-CAR-NK cells.

In some examples, the subject being treated has a solid tumor, for example, a solid tumor expressing CD147. Examples of solid tumors, include sarcomas (such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, soft tissue sarcoma, and other sarcomas), synovioma, mesothelioma, Ewing sarcoma, leiomyosarcoma, rhabdomyosarcoma, colon cancer, colorectal cancer, peritoneal cancer, esophageal cancer (such as esophageal squamous cell carcinoma), pancreatic cancer, breast cancer (including basal breast carcinoma, ductal carcinoma and lobular breast carcinoma), endometrial cancer, lung cancer (such as non-small cell lung cancer), ovarian cancer, prostate cancer,

liver cancer (including hepatocellular carcinoma), gastric cancer, squamous cell carcinoma (including head and neck squamous cell carcinoma), basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, medullary carcinoma, bronchogenic carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms tumor, cervical cancer, fallopian tube cancer, testicular tumor, seminoma, bladder cancer (such as renal cell cancer), melanoma, and CNS tumors (such as a glioma, glioblastoma, astrocytoma, medulloblastoma, craniopharyrgioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma). Solid tumors also include tumor metastases (for example, metastases to the lung, liver, brain, or bone). In some examples, the subject has hepatocellular carcinoma, neuroblastoma, breast cancer, gastric cancer, endometrial cancer, bladder cancer (such as renal cell carcinoma), lung cancer (such as non-small cell lung cancer), cervical cancer, medulloblastoma, esophageal cancer (such as esophageal squamous cell carcinoma), prostate cancer, seminoma, glioblastoma, osteosarcoma, astrocytoma, or soft tissue sarcoma. In particular examples, the subject has hepatocellular carcinoma or neuroblastoma.

In other examples, the subject has a hematological malignancy, for example, a hematological malignancy expressing CD147. Examples of hematological malignancies include leukemias, including acute leukemias (such as 11q23-positive acute leukemia, acute lymphocytic leukemia (ALL), T-cell ALL, acute myelocytic leukemia, acute myelogenous leukemia (AML), and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), lymphoblastic leukemia, polycythemia vera, lymphoma, diffuse large B cell lymphoma, Burkitt lymphoma, T cell lymphoma, follicular lymphoma, mantle cell lymphoma, Hodgkin disease, non-Hodgkin lymphoma, multiple myeloma, Waldenstrom macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia, and myelodysplasia. In particular examples, the subject has acute lymphocytic leukemia (ALL), T-cell ALL, acute myelocytic leukemia, or acute myelogenous leukemia (AML).

In some examples, the subject is also treated with one or more of surgery, radiation therapy and chemotherapeutic agents. Exemplary chemotherapeutic agents include (but are not limited to) alkylating agents, such as nitrogen mustards (such as mechlorethamine, cyclophosphamide, melphalan, uracil mustard or chlorambucil), alkyl sulfonates (such as busulfan), nitrosoureas (such as carmustine, lomustine, semustine, streptozocin, or dacarbazine); antimetabolites such as folic acid analogs (such as methotrexate), pyrimidine analogs (such as 5-FU or cytarabine), and purine analogs, such as mercaptopurine or thioguanine; or natural products, for example vinca alkaloids (such as vinblastine, vincristine, or vindesine), epipodophyllotoxins (such as etoposide or teniposide), antibiotics (such as dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, or mitocycin C), and enzymes (such as L-asparaginase). Additional agents include platinum coordination complexes (such as cis-diamine-dichloroplatinum II, also known as cisplatin), substituted ureas (such as hydroxyurea), methyl hydrazine

derivatives (such as procarbazine), and adrenocrotical suppressants (such as mitotane and aminoglutethimide); hormones and antagonists, such as adrenocorticosteroids (such as prednisone), progestins (such as hydroxyprogesterone caproate, medroxyprogesterone acetate, and magestrol acetate), estrogens (such as diethylstilbestrol and ethinyl estradiol), antiestrogens (such as tamoxifen), and androgens (such as testosterone proprionate and fluoxymesterone). Examples of the most commonly used chemotherapy drugs include adriamycin, melphalan (Alkeran®) Ara-C (cytarabine), carmustine, busulfan, lomustine, carboplatinum, cisplatinum, cyclophosphamide (Cytoxan®), daunorubicin, dacarbazine, 5-fluorouracil, fludarabine, hydroxyurea, idarubicin, ifosfamide, methotrexate, mithramycin, mitomycin, mitoxantrone, nitrogen mustard, paclitaxel (or other taxanes, such as docetaxel), vinblastine, vincristine, VP-16, while newer drugs include gemcitabine (Gemzar®), trastuzumab (Herceptin®), irinotecan (CPT-11), leustatin, navelbine, rituximab (Rituxan®) imatinib (STI-571), Topotecan (Hycamtin®), capecitabine, ibritumomab (Zevalin®), and calcitriol. A skilled clinician can select appropriate additional therapies (from those listed here or other current therapies) for the subject, depending on factors such as the subject, the cancer being treated, treatment history, and other factors.

EXAMPLES

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

Example 1

Materials and Methods

Antibodies and Reagents: Purified anti-CD247 (also known as T-cell surface Glycoprotein CD3 Zeta Chain, CD3) antibody (clone 6B10.2, BioLegend), purified anti-human CD147, FITC-conjugated anti-human CD147 (clone HIM6, BioLegend), PE- or APC-conjugated anti-human CD3 antibody (clone OKT3, BioLegend), FITC or BV 510-conjugated anti-human CD56 antibody (clone HCD56, BioLegend), PE-conjugated anti-human CD69 antibody (clone FN50, BioLegend), APC/Fire 750-conjugated anti-human CD226 antibody (also known as DNAM-1, clone 11A8, BioLegend), APC/Fire 750-conjugated anti-human KLRG1 (MAFA) antibody (clone SA231A2, BioLegend), BV421-conjugated anti-human CD335 (NKp46) antibody (clone 9E2, BioLegend), PE/Cy7-conjugated anti-human CD158b (KIR2DL2/L3, BioLegend) antibody (clone DX27, BioLegend), PE/Cy7-conjugated anti-human CD244 (2B4) antibody (clone C1.7, BioLegend), PE-conjugated anti-human CD152 (CTLA-4) antibody (clone BNI3), APC-conjugated anti-human CD366 (Tim-3) antibody (clone F38-2E2), PerCP/Cy5.5 anti-human TIGIT (VSTM3) antibody (clone A15153G), FITC-conjugated anti-human CD223 (LAG-3) antibody (clone 11C3C65, BioLegend), and PerCP/Cy5.5-conjugated anti-human CD94 (clone DX22, BioLegend) were purchased from BioLegend (San Diego, CA, USA).

APC-conjugated anti-human CD16 antibody (clone B73.1, BD Biosciences), FITC-conjugated anti-human CD3 antibody (clone UCHT1, BD Biosciences), BV480-conjugated anti-human CD85j antibody (LIR-1) antibody (clone GHI/75, BD Biosciences), BV711-conjugated anti-human CD314 (NKG2D) antibody (clone 1D11, BD Biosciences), and PE- or FITC-conjugated anti-human CD107a antibody (clone H4A3, BD Biosciences) were purchased from BD Biosciences (San Jose, CA, USA).

FITC-conjugated anti-human KIR/CD158 antibody (clone 180704, R&D Systems), PE-conjugated anti-human KIR2DL1/KIR2DS5 antibody (clone 143211, R&D Systems), APC-conjugated anti-human KIR3DL1 antibody (clone DX9, R&D Systems), AF405-conjugated anti-human KIR3DL2/CD158k antibody (clone 539304, R&D Systems), APC-conjugated anti-human NKG2A/CD159a antibody (clone 131411, R&D Systems), and PE-conjugated anti-human NKG2C/CD159c antibody (clone 134591, R&D Systems) were purchased from R&D Systems. AF647 Goat anti-human IgG F(ab')^2 fragment antibody was purchased from Jackson ImmunoResearch (West Grove, PA, USA).

Bioinformatic analysis from public cancer patient database: Patient survival data and RSEM (RNA-Seq by Expectation Maximization) normalized expression datasets about CD147 were generated from The Cancer Genome Atlas (TCGA) and were downloaded from OncoLnc (oncolnc.org). Data were plotted for Kaplan-Meier curves using GraphPad Prism 5.0 (GraphPad). RSEM normalized expression datasets derived from TCGA come from FireHose Broad GDAC, which was developed by The Broad Institute (gdac.broadinstitute.org). Figures were generated by GraphPad Prism 5.

Cell lines: 293T, K562, Daudi cell, SK-Hep1, and HepG2 cell lines were purchased from American Type Culture Collection (ATCC). To establish the Daudi-FFluc cell, CD147-positive HepG2 and SK-Hep1 cells were transduced with the lentiviral vector encoding FFLuc-GFP, as previously described. The protocol for collection of peripheral blood from healthy donors was approved by the institutional review board (IRB) and ethics review committees at the Rutgers-New Jersey Medical School (Newark, NJ).

NK-92MI cell culture and generation of CAR- modified NK-92MI cells: NK-92MI cell line was purchased from ATCC® (CRL-2408™, USA). NK-92MI, an interleukin-2 (IL-2) independent NK cell line, is derived from NK-92 (ATCC® CRL-2407™) cell line (Gong *et al., Leukemia* 8:652-658, 1994) stably expressed with human IL-2 cDNA (Tam *et al., Hum Gene Ther* 10:1359-1373, 1999; Tam *et al., J. Hematother.* 8:281-290, 1999). NK-92MI cell lines were maintained in the specific NK-92MI culture medium (alpha minimum essential medium, alpha-MEM) without ribonucleosides and deoxyribonucleosides but with 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. To make the complete growth medium, the following components were added to the base medium: 0.2 mM inositol, 0.1 mM 2-mercaptoethanol, 0.02 mM folic acid, horse serum to a final concentration of 12.5%, and fetal bovine serum to a final concentration of 12.5%. NK-92MI cells were transduced with retroviral supernatants on day 3 in plates coated with recombinant fibronectin fragment (FN CH-296; Retronectin; Takara, Japan). After transduction, NK cells were expanded using IL-2. To check the percentage of CD147-CAR expression on NK-92MI cells, these cells were stained for CD3 and CD56 to stain NK cells,

followed by Flow Cytometry analysis.

Generation of CD147-knockout cell line: To generate the CD147 knock-out hepatocellular cell line, a lentiviral delivery system was used, guide RNA targeting CD147 sequence: #1 (5-TTGACATCGTTGGCCACCGC-3; SEQ ID NO: 10), #3 (5-GTGGACGCAGATGACCGCTC-3; SEQ ID NO: 11). Lentivirus was produced in HEK 293T by transfecting lenti-CRISPR v2 with packaging plasmids pSPAX2 and pMD2G. After 3 days, supernatants were filtered (0.45 μm) and incubated with hepatocellular cell cells and 8 μg/mL polybrene (Sigma). After 48 hours incubation, transduced cells were changed to fresh medium and selected with 8.0 μg/ml puromycin for 5 days. Western blots and flow cytometry analysis were performed to confirm the efficacy of the knockout cell lines.

Plasmid construction and retrovirus production: A codon-optimized DNA fragment was synthesized by GENEWIZ encoding the CD147-specific scFv from the 5F6 clone and sub-cloned in-frame into the SFG retroviral vector retroviral backbone in-frame with the hinge component of human IgG1, CD28 trans-membrane domain, intracellular domain CD28 plus 4-1BB, and the ζ chain of the human TCR/CD3 complex. To produce CD147-CAR retrovirus, 293T cells were transfected with a combination of plasmid containing CD147-specific scFv, RDF, and PegPam3, as previously described (Loskog *et al., Leukemia* 20:1819-1828, 2006). The construct of CD19-CD28-CAR and CD19-4-1BB-CAR has been previously described (Xiong *et al., Mol. Ther.* 26:963-975, 2018).

Transduction of NK-92MI cells with CD147-CAR: NK cells were harvested on day 7 of expansion and transduced with CD147-CAR retrovirus in plates coated with Retronectin (FN CH-296, Takara, Japan). Two days later, cells were transferred to G-Rex 6 multi-well cell culture plates and maintained in 35 ml of complete RPMI-1640 media with 200 U/ml IL-2 (PeproTech). The medium was changed every 3-4 days and 2×10^7 cells were kept in each well for continued culture at each time. Total cell numbers were counted using Trypan Blue exclusion. To check the percentage of NK cells and the expression of CAR, cells were stained for CD3, CD56, and IgG $F(ab')_2$, and analyzed by Flow Cytometry.

Flow Cytometry Analysis and Sorting: CAR-NK cells were stained with fluorescence-conjugated antibodies in FACS staining buffer with 1% fetal bovine serum (FBS) on ice for 30 minutes, washed with PBS, and analyzed on a FACS LSRII or an LSR Fortessa flow cytometer (BD). PMT voltages were adjusted and compensation values were calculated before data collection. Data were acquired using FACS Diva software (BD) and analyzed using FlowJo software (Tree Star).

For the flow cytometry single live cell sorting, all of the sample cells were stained with fluorescence-conjugated antibodies with (RPMI1640 with 1% FBS) on ice for 30 minutes, washed with PBS twice, re-suspended in completed culture medium, and sorted by SORP BD FACS Aria III. After sorting, collection samples were washed with pre-warmed medium once, and cultured for use.

CAR-NK Degranulation assay (CD107a): CAR-NK cells (1×10^5) were incubated with target cells in U-bottomed 96-well plates in complete NK-92MI culture media at 37°C for 4 hours or overnight. Afterward, cells were harvested, washed, stained for CD3, CD56, and CD107a with GolgiStop (BD) for 30 minutes on ice, and analyzed by flow cytometry.

Cytokine release assays: The IFN gamma and TNF-alpha cytokines secreted by the CAR-NK were measured by a commercial ELISA kit (Invitrogen - Thermo Fisher Scientific) as per the manufacturer's protocol.

^{51}Cr release assay: To evaluate the cytotoxic activity of CAR-NK cell, a standard 4-hour ^{51}Cr release assay was used. Briefly, target cells were labeled with ^{51}Cr at 37°C for 2 hours and then resuspended at 2×10^5/mL in NK-92MI culture medium with 10% FBS without IL2. Then, 2×10^4 target cells were incubated with serial-diluted CAR-NK cells at 37°C for 4 hours. After centrifugation, the supernatants were collected and the released ^{51}Cr was measured with a gamma counter (Wallac, Turku, Finland). The cytotoxicity (as a percentage) was calculated as follows: [(sample − spontaneous release) / (maximum release − spontaneous release)] × 100.

FFLuc reporter system assay: To quantify the cytotoxicity of CAR-modified immune cells, a FFLuc reporter system assay was developed. Briefly, at day 1, target cells were pre-seeded at 2 x 10^4 or 3 x 10^4 target cells/well (FFluc-GFP stably transduced cell) onto an optical 96-well plate (Greiner Bio-One™ No.: 655098) in 100 μl/well of the target cell's full nutrition medium and incubation at 37°C with 5% CO_2 overnight. The next day, serial dilution of the effector cell was prepared according to the ratio of effector/target and the indicated effector cells were added into each well (100 μl/well). The reaction was incubated at 37°C with 5% CO_2 for 4 hours and then the supernatant was gently discarded. 100 μl working D-Luciferin was added to each well and incubated at 37°C with 5% CO_2 for 5 mins, with the lights turned off. A microplate reader (PerkinElmer, USA) was used to quantify the data. The data were quantified by converting the obtained values to percentage of specific lysis by the following equation: Specific Lysis Percentage (%) = [1-(S-E)/(T-M)]*100, where S is the value of luminescence of the sample well, E is the value of luminescence of the "effector cell only" well compared to the sample well, T is the mean value of luminescence of "Target cell only" wells, and M is the mean value of luminescence of "blank medium only" wells.

Animal Studies: All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC). NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice from The Jackson Laboratory (Bar Harbor, ME) were used for all *in vivo* experiments. To establish a hepatocellular carcinoma cell line xenograft model, both male and female NSG mice (8-week-old) were injected subcutaneously with 4×10^6 SK-Hep1 cells in 100 μL of PBS Corning® Matrigel® Matrix in the right flank. When the tumor burden reached ~40-50 mm^2, mice were randomly allocated into three groups. Beginning treatment on day 1, the mice were injected (i.v.) with 5×10^6 CD147-CAR-NK-92MI cells in 100 μL of PBS. Control groups were infused with parental NK-92MI cells or vehicle (PBS). On the next day (day 2), all the animals were injected (i.v.) with IL-2 (20,000 units/mouse). Animal weight and tumor burden were collected twice a week. The tumor size was measured by a caliper and the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were recorded. Tumor sizes based on caliper measurements were calculated by the modified ellipsoidal formula. The tumor size was calculated as follows: Tumor size (mm^2) = 1/2(length × width^2). When the tumor burden was above 2000 mm^2 or the animal's weight

reduced > 20%, mice were euthanized according to IACUC guidelines. The animal survival data were recorded simultaneously.

For the patient-derived xenograft (PDX) model, patient hepatocellular carcinoma animal models were developed by The Jackson Laboratory. Briefly, fresh PDX specimens were implanted subcutaneously into the flanks of 6-8 week old NOD SCID gamma (NSG) mice. After the tumor burden reached ~40-50 mm^2, mice were randomly allocated into three groups for further analysis. The main treatment procedure used was as described above. Xenografts specimens were fixed with 10% formalin, embedded in paraffin for cutting, and processed for IHC staining or were directly frozen into liquid nitrogen for further analysis.

Statistical Analysis: Tumor size statistical analysis was performed by two-way ANOVA with Bonferroni post-tests. The overall survival statistics were calculated using the log-rank test. Other statistical significance was determined using a two-tailed unpaired Student's t test and a two-tailed paired Student's t test. All statistical calculation graphs were generated by GraphPad Prism 5.0. $P < 0.05$ (*), $P < 0.01$(**), and $P < 0.001$(***) were considered statistically significant.

Example 2

CD147 is expressed in hepatocellular carcinoma cell lines and liver cancer specimens from patients

To determine whether CD147 is an effective, valid target for hepatocellular carcinoma and other types of cancer, the correlation between patient survival and expression level of CD147 from TCGA (The Cancer Genome Atlas, cancergenome.nih.gov) datasets was analyzed. Comparison of survival percentage from two different patient subsets ($CD147^{high}$ and $CD147^{low}$) showed that there was a strong negative correlation between CD147 and survival percentage (FIG. 1A). Specifically, $CD147^{high}$ in multiple tumor tissues demonstrated low survival percentage (FIG. 1A). In addition, a comparison of CD147 expression between normal tissue (NT) and tumor sample (TP) in multiple cancer types showed significant upregulation of CD147 expression among different types of tumor tissue (FIG. 1B).

To verify the data from the bioinformatics analysis, the expression of CD147 among different tumor cell lines and other tissue was analyzed by Western-blot, which includes wildtype NK-92 (a human NK cell line), T2 (a mutant T×B cell hybrid), 721.221 (an HLA-A, -B, -C null human cell line), MDA-MB-231 (a human breast carcinoma cell line), K562 (a human myelogenous leukemia cell line), HepG2 (a human hepatocellular carcinoma cell line), SK-Hep1 (a human liver adenocarcinoma cell line), Raji (a human B lymphocyte Burkitt's lymphoma cell line), Daudi (a human B lymphoblast cell line), NK-92-MI (an interleukin 2-independent natural killer cell line), and human peripheral blood monocytes (PBMCs). CD147 molecules were highly upregulated in HepG2 and SK-Hep1 cell lines (two hepatocellular carcinoma cell lines), compared to PBMCs (FIG. 1C). The expression of CD147 on PBMCs was relatively low, compared to tumor cell lines (FIG. 1C). Similar results were obtained by flow cytometry analysis (FIG. 1D). Furthermore, the results of immunohistochemistry (IHC) assays confirmed that CD147 was significantly upregulated in HCC tissue isolated from a PDX mouse model (FIG. 1E).

Example 3

Generation and Characteristics of CD147-CAR-NK Cells

A CD147-CAR using the SFG vector (Loskog *et al., Leukemia* 20:1819-1828, 2006; Xiong *et al., Mol. Ther.* 26:963-975, 2018) was constructed. The CD147-CAR contained a modified single-chain variable fragment (scFv) of anti-CD147 antibody (derived from clone 5F6, as described in Example 1), an IgG-CH2CH3 spacer, a trans-membrane domain of CD28, intracellular domain of CD28-4-1BB, and intracellular signaling domains of the TCR-zeta chain (FIG. 2A).

First, this CAR construct was tested in the NK-92MI cell line. After transduction, NK-92MI expressed the CD147-CAR molecules (FIG. 2B). After sorting by flow cytometry, the percentage of CD147-postive NK-92MI cells was above 96% (FIG. 2B). The expression of CD147-CAR molecules in NK-92MI cells was further verified by Western blot. Compared to the parental NK-92MI cell line, the CD147-CAR-NK-92MI expressed the chimeric scFv-CD147-CAR. The approximate molecular weight was about 80-85 kD (FIG. 2C). The CD147-CAR-NK-92MI cell line was further characterized by flow cytometry. Comparable activating receptor (*e.g.*, CD56, NKG2D, NKP46, NKG2A, CD16, CD94/NKG2C, CD226 (also known as DNAM-1), and CD244 (also known as 2B4)) and inhibitory receptor (*e.g.,* KLRG1, LAG-3, CTLA-4, TIM-3, PD-1, and TIGIT) expression were observed (FIG. 2D). Given the low level of CD147 expression on NK92-MI cells, the expression of CD147 on CD147-CAR-NK-92MI cell line was also analyzed by flow cytometry. Expression of CD147-CAR on NK-92MI was stable for more than 30 days post-transduction. However, CD147-CAR expression was associated with loss of CD147 on NK-92MI cell line, indicating the limiting fratricide among CD147-CAR-NK-92MI cells (FIGS. 2E-2G). Notably, the loss of CD147 molecule expression on CD147-CAR-NK-92MI cells did not affect their functionalities and expression *in vitro*.

Example 4

CD147-CAR NK cells specifically kill hepatocellular carcinoma (HCC) in vitro

After successful establishment of CD147-CAR-NK cells, the capacity of CD147-CAR-NK-92MI cells to eradicate $CD147^{+}$ HCC cell lines (including SK-Hep1 and HepG2 cells) was tested. Compared with control CD19-CD28-CAR-NK-92MI and CD19-4-1BB-CAR-NK-92MI cells, CD147-CAR-NK-92MI cells demonstrated significant cytotoxicity against two HCC cell lines, SK-Hep1 and HepG2 (FIGS. 3A and 3B), as well as Huh5 and HCO2 cell lines (FIG. 4A-4C). In addition, the production of both TNF-alpha and IFN-gamma by CD147-CAR-NK-92MI cells was significantly higher than that of CD19-CD28-CAR-NK-92MI and CD19-4-1BB-CAR-NK-92MI cells stimulated by SK-Hep1 and HepG2 cells (FIGS. 3C and 3D). Interestingly, activation of CD147-CAR-NK-92MI cells by their susceptible target cells can be blocked by the anti-CD147 antibody (clone HIM6), but not the control IgG1 (FIGS. 5A-5C). The specificity of this anti-CD147 antibody was further verified by testing its effects on cytotoxicity of CD19-4-1BB-CAR-NK-92MI cells. This anti-CD147 antibody could not block

the cytotoxicity of CD19-4-1BB-CAR-NK-92MI cells against CD19-positive Daudi cell line (FIG. 6), indicating the selectivity of the CD19-4-1BB-CAR-NK-92MI cells and of the anti-CD147 antibody.

To further confirm the specificity of CD147-CAR-NK-92MI cells, the CD147-knockout ($CD147^{-/-}$) SK-Hep1 cell line ($CD147^{-/-}$-SK-Hep1) and CD147-knockout ($CD147^{-/-}$) HepG2 cell line ($CD147^{-/-}$-HepG2,) were generated. The $CD147^{-/-}$-HepG2 and $CD147^{-/-}$-SK-Hep1 cells were not recognized by CD147-CAR-NK-92MI cells (FIGS. 5D-5G), which was quantified by CD107a surface expression when co-cultured with CD147-KO cell lines.

Both CD107a assay and cytokine production assay can be used to evaluate the activation of CD147-CAR-NK-92MI cells by the susceptible target cells. To directly test whether CD147-CAR-NK-92MI cells can kill CD147-positive HCC cells, the 4-hour standard Chromium-51 (^{51}Cr) release assay (a gold standard assay for evaluating the cytotoxicity of CTLs and NK cells in the field of immunology) was used. The CD147-CAR-NK-92MI cells killed SK-Hep1 and Daudi cells. Similar killing activities by CD147-CAR-NK-92MI cells against additional HCC cell lines, such as the Huh7 cell line (Kasai *et al., Hum Cell* 31:261-267, 2018) and HCO2 cell line (Trinh *et al., PLoS One* 10:e0136673, 2015).

To further verify the killing activity of CD147-CAR-NK-92MI cells, a novel, easy-to-use, and non-radioactive approach for the assessment of CD147-CAR-NK-92MI cell cytotoxicity using a luciferase bioluminescent signal was developed. First, the FFLuc-EGFP-SK-Hep1 and FFLuc-EGFP-HepG2 cell lines were generated. To evaluate the direct killing of target cells, CD147-CAR-NK-92MI cells were co-cultured with FFLuc-EGFP-SK-Hep1 and FFLuc-EGFP-HepG2 cell lines, respectively. After a 4-hour incubation of CD147-CAR-NK-92MI cells in a 96-well optical-bottom microplate, which was pre-seeded with target cell stably expressing the EGFP-firefly luciferase fusion gene (EGFP-FFluc), the chemical bioluminescent signal of EGFP-FFluc was quantified by a fluorescent microplate reader. The FFLuc signal was converted into the percentage of specific lysis, as described in the Example 1, similar to the ^{51}Cr release assay (FIGS. 8H and 8I).

At first, CD147-CAR modified primary T and NK cells isolated from human peripheral blood mononuclear cells (PBMCs) can eradicate multiple HCC cell lines (including SK-Hep1, Huh7, and HepG2, etc.), but not kappa-CAR modified T cells (FIGS. 8A-8C). We also demonstrated that CD147-CAR modified human primary NK cells effectively killed HCC cell lines, *in vitro*, by ^{51}Cr release assay (FIGS. 8D-8F). To further demonstrate primary NK naturally killing ability through the NKGD/NKG2DL interaction in addition to CD147-CAR-primary NK cytotoxicity, we found anti-NKG2D further blocked the killing of $CD147^{-/-}$-SK-Hep1 cells by CD147-CAR-NK (FIG. 8G).

The dose-dependent specific lysis was comparable with ^{51}Cr release assays. The cytotoxicity activity measured by this approach was further quantified under a common inverted fluorescence microscope to evaluate the morphology and dynamics of EGFP signal in target cells (FIGS. 7A and 7B). Therefore, two complementary approaches demonstrated that CD147-CAR NK cells specifically kill hepatocellular carcinoma (HCC) *in vitro*.

CD147-CAR-NK-92MI cells could not kill the $CD147^{-/-}$-SK-Hep1 and $CD147^{-/-}$-HepG2, compared to parental SK-Hep1 and HepG2 cells (FIGS. 8K and 8L). The specificity of CD147-CAR-

NK-92MI cell cytotoxicity was further verified by adding anti-CD147 antibodies in the effector and target cell co-culture system (FIGS. 8M and 8N). To further validate CD147 as an effective and valid target for HCC, the cytotoxicity of CD147-CAR-T cells against two different HCC cell lines – HepG2 (FIG. 10A) and SK-Hep1 (FIG. 10B) was tested. When CD147 molecules were deleted in SK-Hep1 cell line (CD147-knockout SK-Hep1 cell line), the specific lysis of CD147-CAR-T cells had significantly decreased (FIG. 10C), which further validated the specificity cytotoxicity of CD147-CAR-T cells against CD147 positive HCC cell lines.

Example 5

CD147-CAR-NK Cells Control Progression of HCC *In Vivo*

To evaluate whether CD147-CAR can kill HCC, *in vivo*, two different xenograft models were used. First, CD147-CAR-modified primary T and NK cells derived from PBMCs were evaluated in a SK-Hep1 xenograft mouse model. CD147-CAR-modified primary T cells significantly suppressed tumor size and prolonged survival (FIGS. 11A-11D). To further evaluate the efficacy of CD147-CAR modified primary NK cells, we included a non-transduced (NT) primary NK group as an additional control (FIGS. 12A-12D). Mice receiving parental NT-NK control group and PBS vehicle control group developed rapid disease progression. In contrast, mice receiving CD147-CAR-primary T and NK cells were significantly protected from rapid progression and their median survival was prolonged ($P < 0.05$), with comparable body weights among the different groups (FIGS. 11C and 12C), indicating the tolerable toxicity of CD147-CAR-modified primary T and NK cells, *in vivo*.

Furthermore, to further develop additional 'off-the-shelf' cell therapy strategies, we evaluated the efficacy of CD147-CAR modified NK-92MI cells. Due to the malignant nature of NK-92MI, CAR-modified NK-92MI cells need to be irradiated before administered to patients46,61. The cytotoxicity of non-irradiated and irradiated CD147-CAR-NK-92MI cells were compared by standard 4-hour ^{51}Cr release assays (FIG. 13). Comparable cytotoxicity between non-irradiated and irradiated CD147-CAR-NK-92MI cells was observed, *in vitro* (FIG. 13).

The efficacies between non-irradiated and irradiated CD147-CAR-NK-92MI cells in the xenograft NSG mouse model were further compared (FIGS. 14A-14D). Comparable efficacies, *in vivo*, measured by median survival, between non-irradiated and irradiated CD147-CAR-NK-92MI-infused mice were observed (FIGS. 14A-14D).

To further evaluate the efficacy of CD147-CAR-NK-92MI cells (injected on day 1, day 3, and day 5 after tumor implantation) to control tumor growth, disease progression was measured by tumor size (FIG. 15A). Mice receiving parental NK-92MI and PBS vehicle control groups developed rapid disease progression (FIG. 15B). In contrast, mice receiving CD147-CAR-NK-92MI cells were significantly protected from rapid progression and their median survival was prolonged ($P < 0.01$), with comparable body weights among the different groups (FIG. 15C and 15D), indicating the tolerable toxicity of CD147-CAR-NK-92MI cells, *in vivo*.

Although cancer cell lines may have significant limitations in their ability to precisely model biology and therapeutic effects, patient-derived xenografts (PDXs) models are biologically stable and can mimic human clinic conditions regarding mutational status, gene expression patterns, and tumor heterogeneity. Thus, a second xenograft mouse model was employed, using metastatic liver cancer tissue from a patient. The effect of CD147-CAR-NK-92MI cells administered on day 0, day 4, day 8, day 11, day 15, day 22, day 25, and day 35 after engraftment was tested. The median survival of mice treated with CD147-CAR-NK-92MI cells was 63 days, which was significantly higher than that of control mice (median survival about 42 days). Reduced tumor burden and disease progression were observed in the mice treated with CD147-CAR-NK-92MI cells (FIGS. 16A-16D), indicating the effectiveness of CD147-CAR-NK-92MI cells in suppressing liver cancer progression in a PDX mouse model.

Example 6

CD147-CAR-T Cells Specifically Kill CD147-Positive Tumor Cells

The ability of CD147-CAR-T cells to kill HCC cells was tested against 8 HCC cell lines (Huh7, Huh7.5, HepG2, SK-Hep1, Hep3B, Hu1545, HCO2, and LH86). Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors. To transduce the PBMCs, cells were activated with 1 μg/ml anti-CD3 (clone, OKT3, Ortho Biotech, Bridgewater, NJ, USA) and 1 μg/ml anti-CD28 with 100 U/ml recombinant human IL- 2 (Proleukin; Chiron, Emeryville, CA, USA) in 10% FBS RPMI-1640 media. To produce CD147-CAR-T cells, activated T cells were transduced with retroviral supernatants on day 3 in plates coated with recombinant fibronectin fragment (FN CH- 296; Retronectin; Takara Shuzo, Otsu, Japan). After transduction, T cells were expanded using IL- 2 and then used for assays.

FFLuc reporter system assay as described in Example 1 was used to test for specific killing of FFLuc-EGFP-HepG2 by CD147-CAR-T cells (FIG. 17A). Effector cells (CD147-CAR-T cells) were co-cultured with target cells at $1x10^4$ per well FFLuc-GFP-HepG2 in a 96-well optical-bottom microplate for 6 hours. The control group used the wild type kappa-CAR-T cells incubating with CD147-positive FFLuc-EGFP-HepG2. Decreased cytotoxicity of CD147-CAR-T cells using knockout-CD147 FFLuc-GFP-HepG1 was shown by FFLuc report system assay (FIG. 17B). Effector cells (CD147-CAR-T and Kapp-CAR-T cells) were co-cultured with target cells CD147-positive FFLuc-EGFP-HepG2 ($1x10^4$) in a 96-well optical-bottom microplate for 6 hours. Cytotoxicity of CD147-CAR-T cells was measured by the luminescent signal read by microplate reader. Cytotoxicity of CD147-CAR-T cells was measured by a standard 4-hr ^{51}Cr release assay (FIG. 17C). Kappa-CAR-T cells were used as a negative control group.

Example 7

Effect of CD147-CAR-T Cells on Neuroblastoma Cells

CD107a degranulation on CD147-CAR-T cells was observed after 10 hours with medium (control) or SK-N-SH tumor cells (FIG. 18A), as well as DaoY and D283 cells. The ratio of effector and target was 1:1.2. Cells were gated for CD56 positive subsets for quantifying surface CD107a expression.

Quantitative data for percentage of surface CD107a expression on CD147-CAR-NK-92MI cells upon different stimulations, as indicated (FIG. 18B). CD147-CAR-NK-92MI cells were also cytotoxic to DaoY cells after 3 hours (FIG. 19).

5

Example 8

HCC Patient-Derived Primary CD147-CAR-NK Cells Specifically Kill CD147-Positive Tumor Cells *In Vitro*

Due to CD147's broad expression pattern across multiple solid tumor types, CD147 is an attractive target for CD147-CAR-based cancer immunotherapy. Here, we examined whether CD147 is
10 upregulated in human HCC tissue samples. Different stages of HCC tumor tissue stained strongly positive for CD147, compared to healthy liver tissue (FIG. 21A).

To evaluate whether CD147-CAR modified primary NK cells directly isolated from HCC liver can kill HCC, *in vitro*, NK cells were isolated from different zones of liver tissue (FIG. 21B), which includes a tumor zone, tumor adjacent zone, and a non-tumor zone in a human liver with HCC. Then,
15 these NK cells were expanded (FIG. 21C). CD147-CAR were generated using these expanded NK cells from human HCC liver tissue. The transduction efficiency of activated NK cells was generally greater than 70% (FIG. 21D). CD147-CAR-NK cells specifically recognized tumor cells expressing CD147. The anti-tumor activity of CD147-CAR-NK was evaluated against HCC cell lines (FIG. 21E). Together, this demonstrates that CD147-CAR-redirected primary human liver NK cells kill the CD147-positive
20 target cells specifically and selectively.

Example 9

SynNotch GPC3-Inducible CD147-CAR T Cells Selectively Target GPC3+CD147+ HCC Cells, but not GPC3+CD147- or GCP3-CD147+ HCC Cells

25 To generate an anti-GPC3 synNotch induced receptor vector, anti-GPC3 (mouse GC33 clone) scFv that can specifically bind with human GPC3 antigen was synthesized by GENEWIZ. The sequence encoding a signal peptide and a myc-tag at the N-terminal were fused with the synNotch-Gal4VP64 induced element derived from (Addgene plasmid #79125) by overlap PCR. The fragments were inserted into the SFG gamma retrovirus vector which were digested by restriction endonucleases NcoI and XhoI.
30 For construction of the anti-CD147-CAR-mCherry vector, the entire CD147-CAR element was inserted into pHR_Gal4UAS_pGK_mCherry (Addgene plasmids #79124) which was digested by restriction endonucleases MluI and NdeI. The expression of the mCherry gene was under control of the pGK promoter. In this strategy, eGFP and mCherry double positive cells were gated as synNotch CAR modified cells for further analysis and functional evaluation.
35 To generate the anti-CD147 synNotch induced receptor vector, anti-CD147 scFv was fused with the synNotch-Gal4VP64 induced element derived from pHR_PGK_antiCD19_synNotch_Gal4VP64 (Addgene plasmid #79125) by overlap PCR. A myc-tag was added to the N-terminal. The fragments were inserted into the SFG gamma retrovirus vector after the signal peptide, which were digested by

restriction endonucleases SalI and MluI.

For construction of the anti-GPC3-CAR-mCherry vector, the entire GPC3-CAR element, which contains anti-GPC3 (mouse GC33 clone) scFv, was inserted into pHR_Gal4UAS_pGK_mCherry (Addgene plasmids #79124) which was digested by restriction endonucleases MluI and NdeI.
5 Expression of the mCherry gene was under control of the pGK promoter. In this strategy, myc-tagged and mCherry double positive cells were gated as synNotch CAR modified cells for further analysis and functional evaluation.

Table 2. Primers for plasmid construction

Construct name	**Primer name**	**Primer sequence**	**SEQ ID NO:**
pSFG-Myc-αCD147-synNotch-Gal4VP64	SFG-Myc-CD147.FOR	5'-TGCGT CGACG AGCAG AAACT CATCT CTGAA GAGGA TCTGG AGATG AAGCT GGAAG AGAGC GGCGG-3'	18
	Fusion-Notch.FOR	5'-GGCAC CAAGC TGGAG ATCAA GATCC TGGAC TACAG CTTCA CAGG-3'	19
	Fusion-CD147.REV	5'-CCTGT GAAGC TGTAG TCCAG GATCT TGATC TCCAG CTTGG TGCC-3'	20
	SFG-Myc-CD147.REV	5'-CTAAC GCGTT CATGA TCCGA GCATG TCCAG GTCAA AG-3'	21
pHR-Gal4UAS-GPC3-CAR-PGK-mCherry	FP-FOR-GPC3	5'- TCGACATTCGTTGGATCCGCCAGCATGGAGTTTGGTTTAAGC -3'	22
	FP-GPC-bbz-overlap	5'-CGGCT CCGGA ACCAA GCTGG AGATT AAGGA GCCCA AATCT CCTGA CAAAA CTCAC-3'	23
	RP-GPC-bbz-overlap	5'-GTGAG TTTTG TCAGG AGATT TGGGC TCCTT AATCT CCAGC TTGGT TCCGG AGCCG-3'	24
	RP-REV-bbz	5'-TAGAA TTCGT TAACC TCGAG TTAGC GAGGG GGCAG GGCCT GC-3'	25
pSFG-Myc-αGPC3-synNotch-Gal4VP64-IRES-GFP	FP -NcoI	5'-TGCCA CCATG GCAAT GGAGT TTGGT TTAAG CTGGC TGTTT TTAGT GGCCA TTTTA AAGGG CGTG-3'	26
	RP-MluI	5'-CAGGA TACGC GTCTT AATCT CCAGC TTGGT TCCGG-3'	27
	FP-Notch-MluI	5'-TTAAG ACGCG TATCC TGGAC TACAG CTTCA CAGGT G-3'	28
	RP-Notch-XhoI	5'-TCCCG CTCGA GTCAT GATCC GAGCA TGTCC AGG-3'	29
pHR-Gal4UAS-CD147-CAR-PGK-mCherry	FP-BamHI	5'-TCGTT GGATC CACGC GTCGT ACGTT AATTA ACCCG GGCAT ATGTT GACTT GCGGC CGCAA C-3'	30
	RP-BIpI	5'-CCATT GCTCA GCGGT GCTG-3'	31
	FP-MluI-147 insert	5'-GATCC ACGCG TATGG AGTTT GGGCT GAGCT GGC-3'	32
	RP-NdeI-147 insert	5'-GTCAA CATAT GTTAG CGAGG GGGCA GGGCC TGCAT G-3'	33
pSFG-	FP-CD147	5'-CTAGA CTGCC ATGGA GTTTG GGCTG AGCTG-3'	34

Construct name	Primer name	Primer sequence	SEQ ID NO:
CD147-CAR-28bbz	Insert		
	RP-CD147 Insert	5'-GACGG TGACG TACGT CTTGA TCTCC AGCTT GGTG-3'	35

To mitigate off-tumor toxicity to normal tissue, the effect of density of CD147 expression in different types of cells (with a focus on hematopoietic cells) on anti-tumor activity of CD147-CAR was tested. The CD147 expression among HepG2, Raji, Daudi, and PBMCs was assessed. Different
5 expression levels of CD147 were observed (FIG. 22A). Notably, those cells (*e.g.*, PBMCs) expressing low levels of CD147 did not trigger cytotoxicity activity of CD147-CAR-NK-92MI cells, even when CD147-CAR cells were cultured with target cells at the high effector and target ratio (E:T ratio) of 10 to 1 (FIG. 22B). These findings suggest that the optimized scFv sequence of anti-CD147 only allows the specific scFv domain to bind cells with high-expressing CD147 molecules, which can mitigate off-tumor
10 toxicity towards normal tissues that express low levels of CD147 molecules.

To further mitigate off-tumor toxicity of CD147-CAR, a synNotch receptor that can release transcription factors, which in turn drives expression of a CAR against a different tumor antigen, was used. This 'logic-gated' synNotch CAR can only kill dual antigen positive tumor cells, but not single tumor antigen positive tumor cells. A combination approach was designed, consisting of GPC-3 and
15 CD147 to mitigate off-tumor toxicity. Briefly, an SFG retroviral vector encoding an anti-GPC3-specific synNotch receptor linking a Gal4-VP64 intracellular transcription activation domain was constructed. A constitutively expressed enhanced GFP (eGFP) was placed downstream of the GPC3-synNotch to identify transduced cells (FIG. 23A).

A lentiviral vector was constructed in which the anti-CD147-CAR was placed under control of
20 the upstream activating sequence (UAS) promoter that can be activated by Gal4-VP64 transcription factors released after engagement of the synNotch receptor. A constitutively expressed monomeric red fluorescent protein Cherry (mCherry) was placed downstream of the inducible CD147-CAR to identify transduced cells (FIG. 23A).

Human PBMCs were co-transduced with both lentiviral and retroviral vectors (FIG. 23C). The
25 double positive cells were verified by eGFP (a marker for anti-GPC3-synNotch) and mCherry (a marker for CD147-CAR) using flow cytometry analysis (FIG. 23D). Four subsets of transduced T cells (including mCherry positive only, GFP positive only, GFP and mCherry double positive, and GFP and mCherry double negative subsets) were analyzed (FIG. 23E). These transduced T cells were primed by a $GPC3^{high}CD147^{low}$ HepG2 cell line to induce CD147-CAR expression on the surface (FIG. 23F). No
30 CD147-CAR expression was observed in the absence of synNotch engagement. No leakiness of CAR expression was observed in transduced primary T cells. However, about 10% of CAR expression leakiness was observed in transduced NK-92MI cells (data not shown). This observation was further verified by a gamma secretase inhibitor (MK-0752, Notch signaling inhibitor) treatment assay (FIG. 24).

The CD147-CAR expression on GPC3-synNotch-GFP and CD147-CAR-mCherry double positive T cells was dramatically inhibited upon MK-0752 treatment (FIG. 24).

Following GPC3-synNotch-GFP and CD147-CAR-mCherry co-transduction T cells and priming by the GPC3^{high}CD147^{low} HepG2 cell line, the activity of transduced T cells were triggered by different subsets of HCC cell lines for 2 hours to assess killing efficacy. The different subsets of HCC cell lines were: CD147+GPC3^{high} HepG2 cell line, CD147^{ko}GPC3^{high} HepG2 cell line, CD147+GPC3^{low} HepG2 cell line, CD147^{ko}GPC3^{low} HepG2 cell line (FIG. 23G). Phorbol-12-myristate-13-acetate (PMA)/ionomycin (IONO) was used as a positive control.

GPC3-synNotch-GFP and CD147-CAR-mCherry double positive T cells that were primed with CD147^{ko}GPC3^{high} HepG2 cells could be specifically activated by the CD147+GPC3^{high} HepG2 cells (FIG. 23H), which was quantified by CD107a surface expression when cocultured with different target cell lines. Similar results were obtained when a myc-tagged CD147 specific-synNotch-GFP and inducible GPC3-CAR-mCherry were co-transduced into T cells (FIGS. 25A-25H).

Together, the data suggest that only synNotch GPC3-inducible CD147-CAR T cells can specifically be activated by GPC3+CD147+ HepG2 cells, but not GPC3+CD147- or GPC3-CD147+ HepG2 cells. These activated CD147-CAR T cells can kill CD147+GPC3+ HepG2 cells, but not CD147- GPC3+ HepG2 cells (FIGS. 26A-26D).

In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A chimeric antigen receptor comprising:

(a) an antigen binding domain comprising that specifically binds CD147, comprising an amino acid sequence comprising the variable heavy chain (VH) domain complementarity determining region 1 (CDR1), CDR2 and CDR3 amino acid sequences of amino acid positions 26-32, 52-59, and 101-104 of SEQ ID NO: 2, respectively, and the variable light chain (VL) domain CDR1, CDR2 and CDR3 amino acid sequences of amino acid positions 155-165, 181-187, and 220-228 of SEQ ID NO: 2, respectively;

(b) a hinge domain;

(c) a transmembrane domain; and

(d) an intracellular domain comprising one or more co-stimulatory molecule intracellular domains and an intracellular signaling domain.

2. The chimeric antigen receptor of claim 1, wherein the antigen binding domain has at least 90% sequence identity to the amino acid sequence SEQ ID NO: 2.

3. The chimeric antigen receptor of claim 2, wherein the antigen binding domain comprises the amino acid sequence of SEQ ID NO: 2.

4. The chimeric antigen receptor of any one of claims 1 to 3, wherein the antigen binding domain binds to cells expressing high levels of surface CD147

5. The chimeric antigen receptor of any one of claims 1 to 4, wherein the one or more co-stimulatory molecule intracellular domains comprise intracellular domains of CD28 and 4-1BB.

6. The chimeric antigen receptor of any one of claims 1 to 5, wherein the intracellular signaling domain comprises a signaling domain of CD3ζ.

7. The chimeric antigen receptor of any one of claims 1 to 6, wherein the hinge domain comprises an IgG1 hinge domain.

8. The chimeric antigen receptor of any one of claims 1 to 7, wherein the transmembrane domain is a CD28 transmembrane domain or a CD8a transmembrane domain.

9. The chimeric antigen receptor of any one of claims 1 to 8, comprising an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 5.

10. The chimeric antigen receptor of claim 9, comprising the amino acid sequence of SEQ ID NO: 5.

11. The chimeric antigen receptor of any one of claims 1 to 10, further comprising one or more additional antigen binding domains.

5 12. The chimeric antigen receptor of claim 11, wherein the one or more additional antigen binding domains specifically binds to glypican 3, alpha-fetoprotein, or Mucin-1.

13. The chimeric antigen receptor of any one of 1 to 12, further comprising an inducible suicide molecule.

10

14. The chimeric antigen receptor of claim 13, wherein the suicide molecule comprises caspase 9.

15. The chimeric antigen receptor of claim 13 or claim 14, wherein expression of the suicide molecule is inducible by tetracycline, doxycycline, or rapamycin.

15

16. The chimeric antigen receptor of claim 13 or claim 14, comprising an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 7.

17. The chimeric antigen receptor of claim 16, comprising the amino acid sequence of SEQ ID NO:
20 7.

18. The chimeric antigen receptor of any one of claims 1 to 17, further comprising a cytokine receptor intracellular domain.

25 19. The chimeric antigen receptor of claim 18, wherein the cytokine receptor intracellular domain is an interleukin-15 receptor intracellular domain, an interleukin-12 receptor intracellular domain or an interleukin 18 receptor intracellular domain.

20. A nucleic acid molecule encoding the chimeric antigen receptor of any one of claims 1 to 19.

30

21. The nucleic acid molecule of claim 20, comprising the nucleic acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 14.

22. A vector comprising the nucleic acid molecule of claim 20 or claim 21.

35

23. The vector of claim 22, further comprising a nucleic acid molecule encoding a second chimeric antigen receptor targeting hepatitis B virus or hepatitis C virus.

24. The vector of claim 22, further comprising an inducible promoter or enhancer nucleic acid molecule operably linked to the chimeric antigen receptor nucleic acid molecule.

25. The vector of claim 24, wherein the enhancer nucleic acid molecule is a Gal4 upstream activation
5 sequence.

26. The vector of claim 25, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 15.

10 27. The vector of any one of claims 22 to 26, wherein the vector is a viral vector.

28. The vector of claim 27, wherein the viral vector is a lentiviral vector or a retrovirus vector.

29. A single-chain variable fragment (scFv) that specifically binds CD147, comprising an amino
15 acid sequence comprising the variable heavy chain (VH) domain complementarity determining region 1 (CDR1), CDR2 and CDR3 amino acid sequences of amino acid positions 26-32, 52-59, and 101-104 of SEQ ID NO: 2, respectively, and the variable light chain (VL) domain CDR1, CDR2 and CDR3 amino acid sequences of amino acid positions 155-165, 181-187, and 220-228 of SEQ ID NO: 2, respectively.

20 30. The scFv of claim 29, wherein the amino acid sequence has at least 90% sequence identity to the amino acid sequence SEQ ID NO: 2.

31. The scFv of claim 30, wherein the amino acid sequence comprises the amino acid sequence of SEQ ID NO: 2.

25

32. The scFv of any one of claims 29 to 31, wherein the scFv binds to cells expressing high levels of surface CD147.

33. A nucleic acid molecule encoding the scFv of any one of claims 29 to 32.

30

34. The nucleic acid molecule of claim 33, comprising the nucleic acid sequence of SEQ ID NO: 1.

35. A vector comprising the nucleic acid sequence of claim 33 or claim 34.

35 36. A T cell, natural killer (NK) cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage expressing the chimeric antigen receptor of any one of claims 1 to 19.

37. A T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage comprising the nucleic acid of claim 20, claim 21, claim 33, or claim 34 or the vector of any one of claims 22 to 28 or claim 35.

5 38. The T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage of claim 37, further comprising a nucleic acid molecule encoding an activator of the inducible promoter or enhancer.

39. The T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or
10 macrophage of claim 38, wherein the nucleic acid encoding the activator further encodes an anti-GPC3 specific binding agent.

40. A T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage comprising the vector of claim 24 or claim 35, further comprising a nucleic acid molecule
15 encoding an activator of an inducible promoter or enhancer and a vector comprising an anti-GPC3 chimeric antigen receptor operably linked to the inducible promoter or enhancer.

41. The T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage of claim 40, wherein the vector comprising an anti-GPC chimeric antigen receptor
20 comprises the nucleic acid sequence of SEQ ID NO: 16.

42. The T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage of any one of claims 36 to 41, wherein the T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage is obtained from peripheral blood, cord blood,
25 lymph node, bone marrow, tumor tissue, or cell line.

43. The NK cell of any one of claims 36 to 42, wherein the NK cell is an NK-92 cell or NK-92MI cell.

30 44. A method of producing CD147-CAR-T cells or CD147-CAR-NK cells, comprising transducing or transfecting T cells or NK cells with the vector of any one of claims 22 to 28.

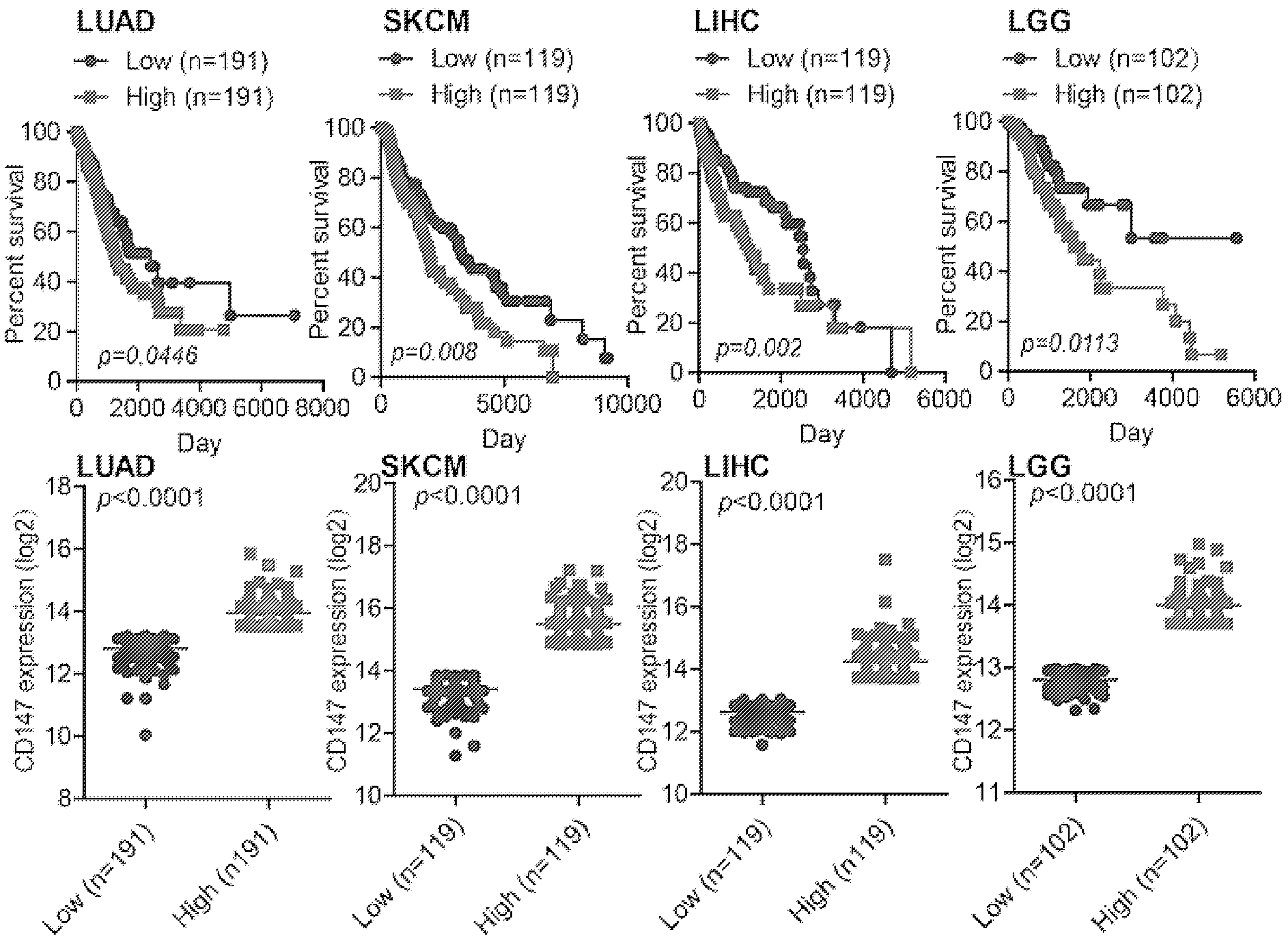
45. A method of treating a subject with cancer, comprising administering an effective amount of the T cell, NK cell, natural killer T (NKT) cell, double negative T (DNT) cell, neutrophil, or macrophage of
35 any one of claims 36 to 43 to the subject.

46. The method of claim 45, wherein the subject has a cancer that expresses CD147.

47. The method of claim 45 or claim 46, wherein the subject has hepatocellular carcinoma, neuroblastoma, breast cancer, pancreatic cancer, leukemia, lymphoma, multiple myeloma, colorectal cancer, lung cancer, melanoma, renal cell carcinoma, sarcoma, or nasopharyngeal carcinoma.

5 48. The method of any one of claims 45 to 47, further comprising treating the subject with at least one of surgery, radiation, chemotherapy, an additional immunotherapy, or a combination of two or more thereof.

FIG. 1A



SUBSTITUTE SHEET (RULE 26)

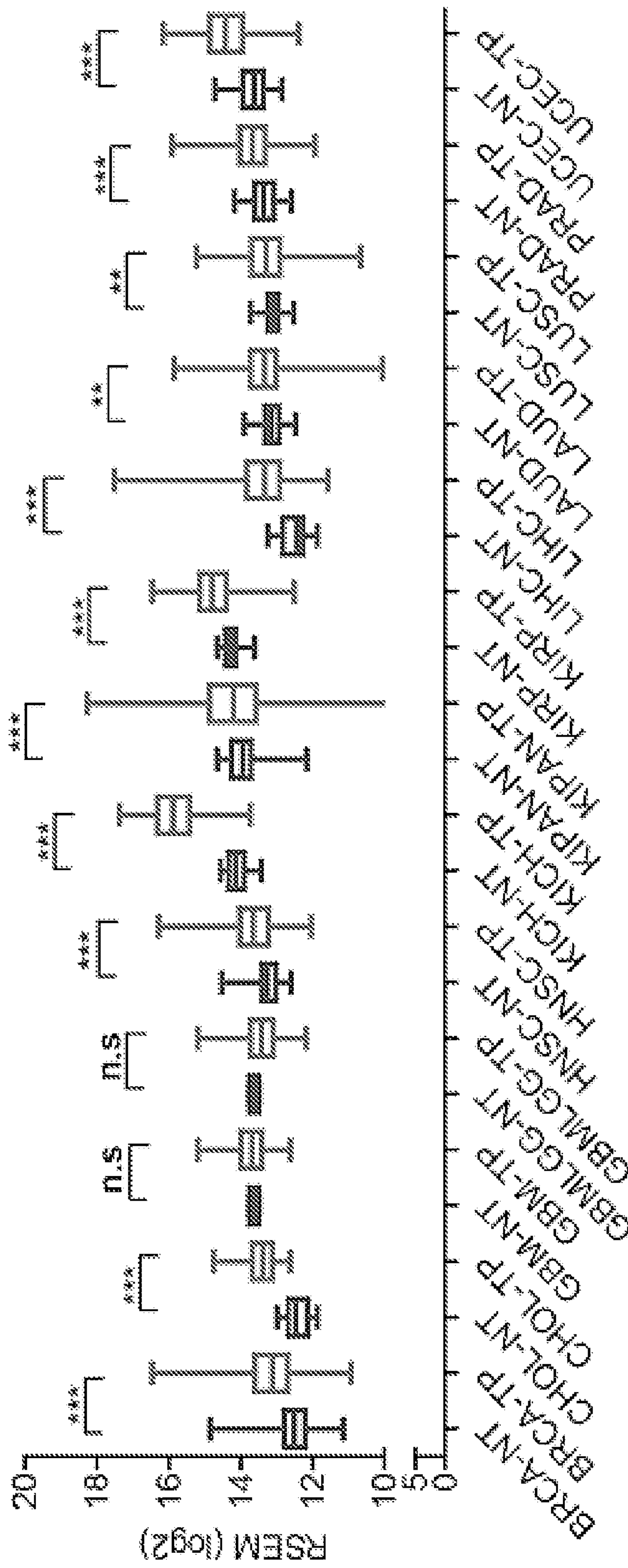


FIG. 1B

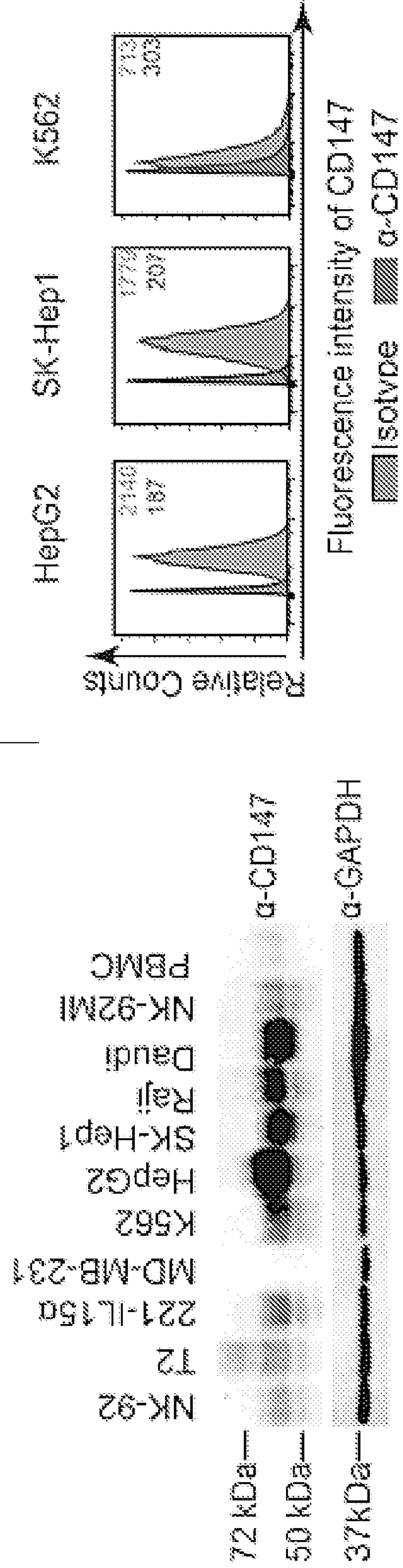


FIG. 1C

FIG. 1D

SUBSTITUTE SHEET (RULE 26)

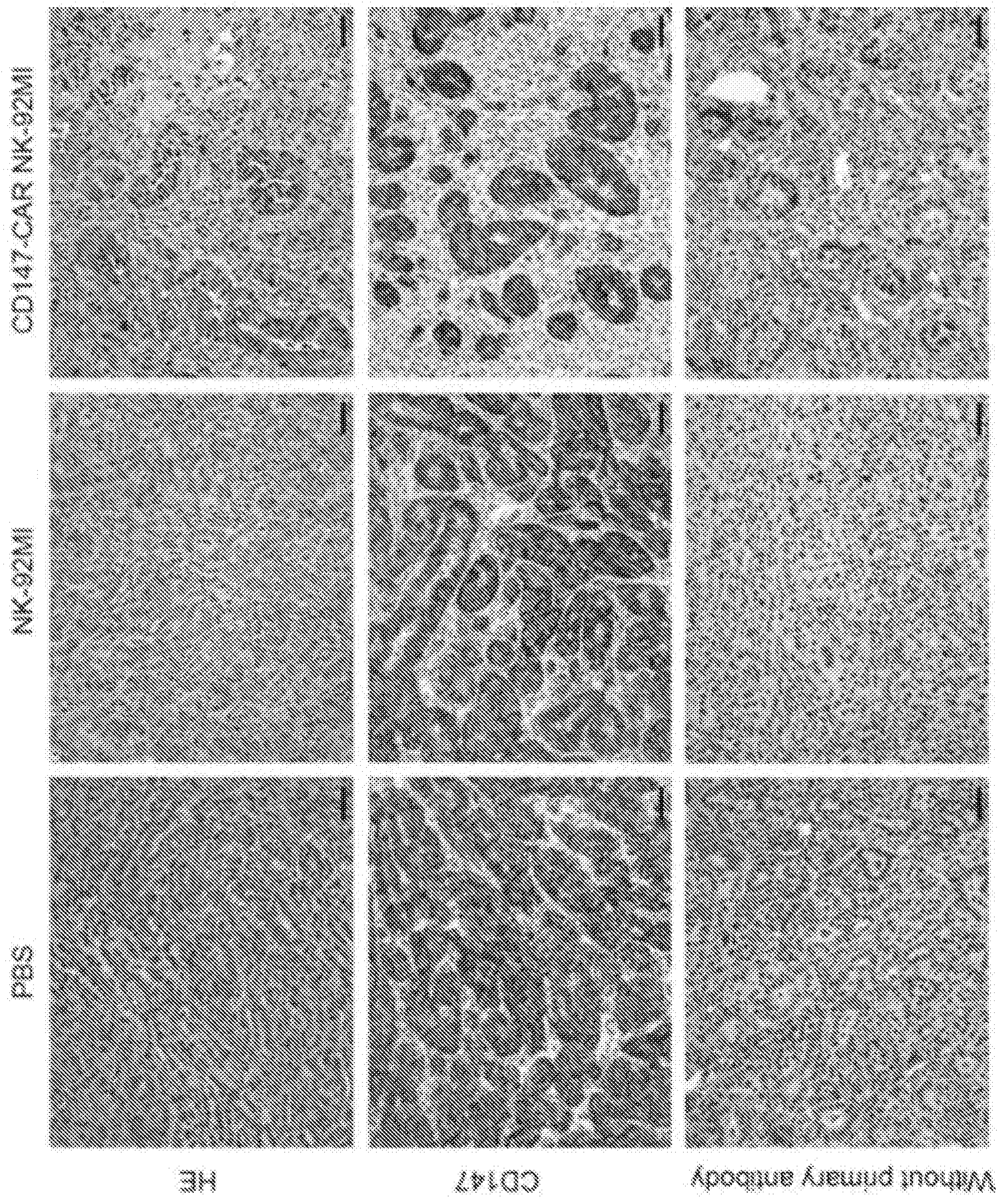


FIG. 1E

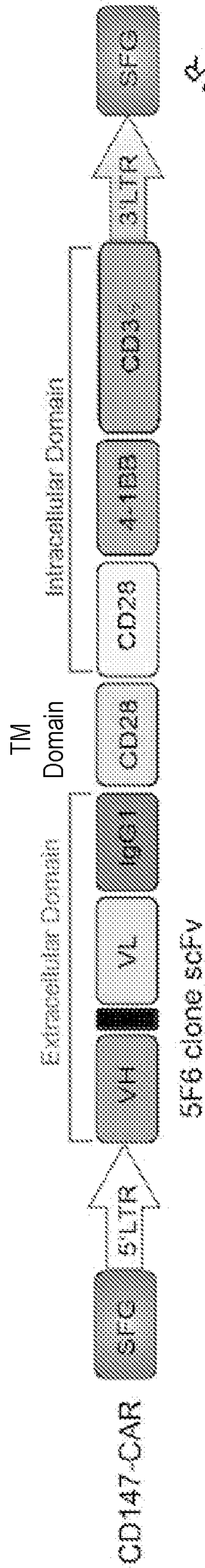


FIG. 2A

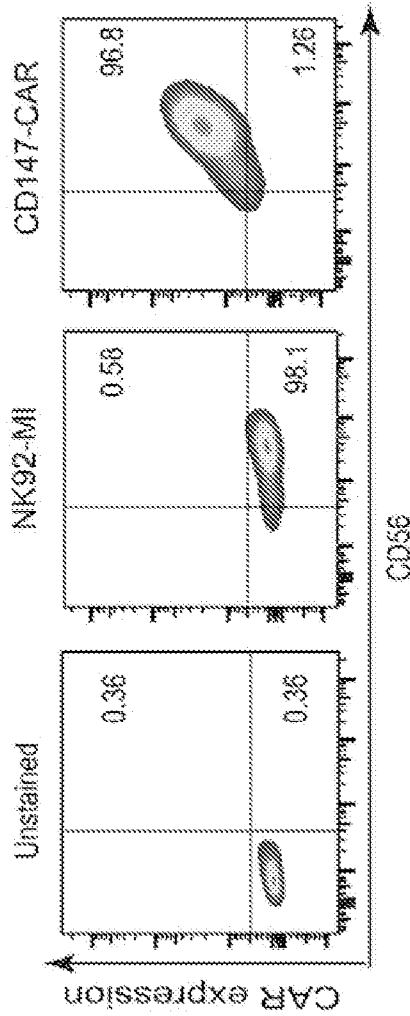


FIG. 2B

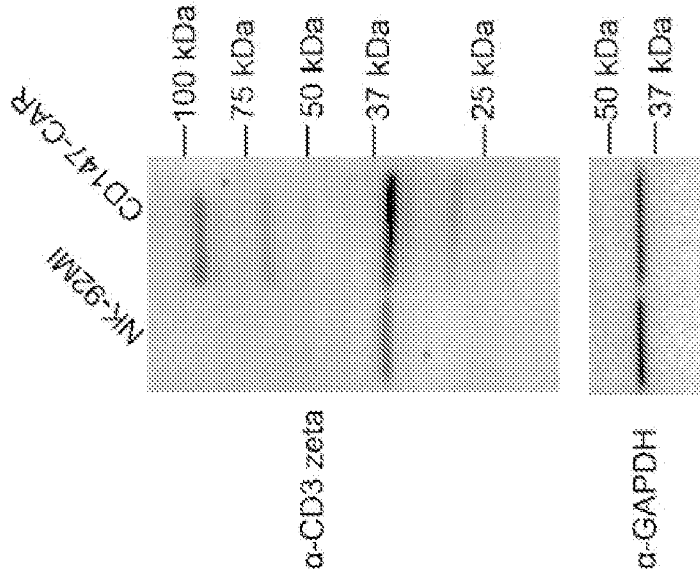


FIG. 2C

SUBSTITUTE SHEET (RULE 26)

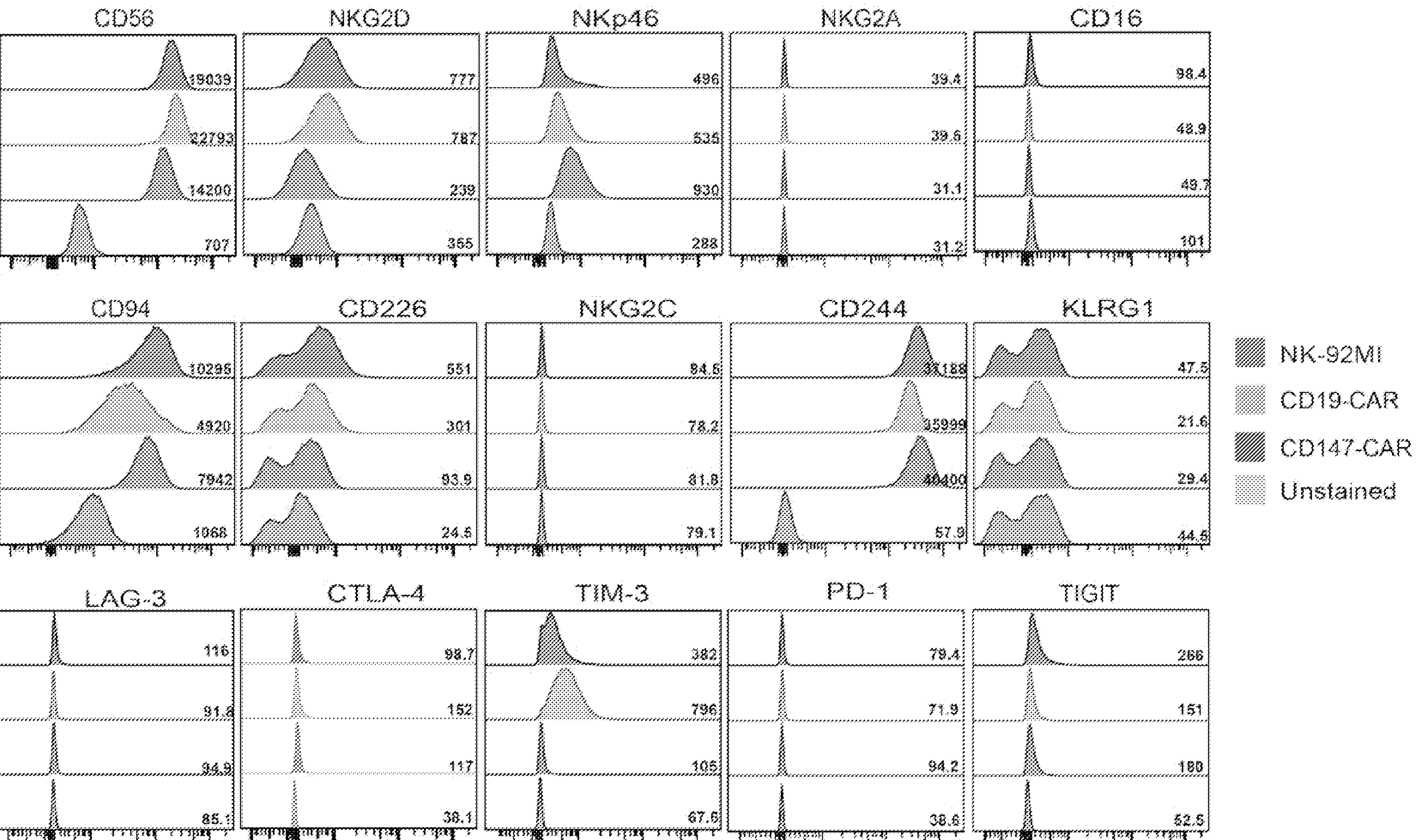


FIG. 2D
CD56
NKG2D
NKp46
NKG2A
CD16
CD94
CD226
NKG2C
CD244
KLRG1
LAG-3
CTLA-4
TIM-3
PD-1
TIGIT
NK-92MI
CD19-CAR
CD147-CAR
Unstained

FIG. 2E

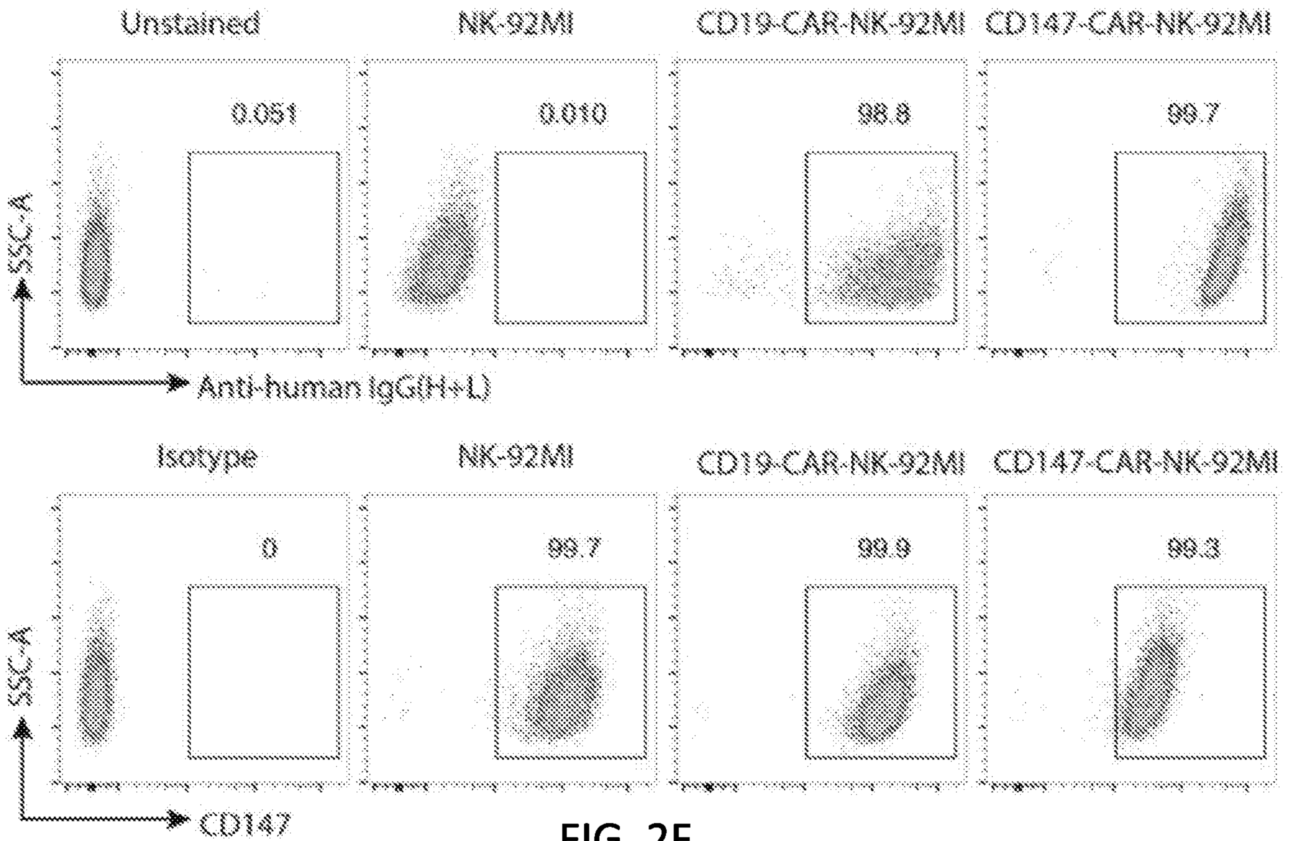


FIG. 2F

FIG. 2G

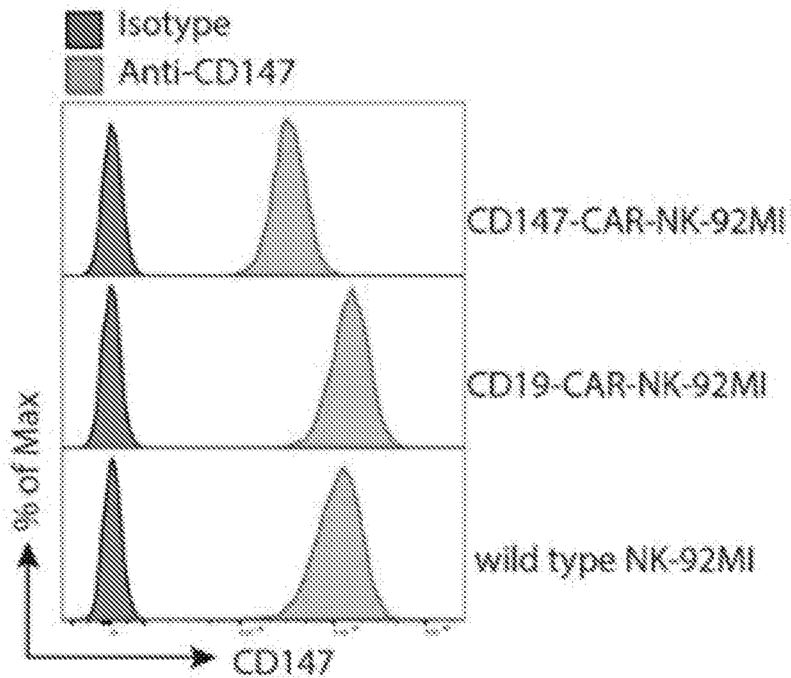


FIG. 3B

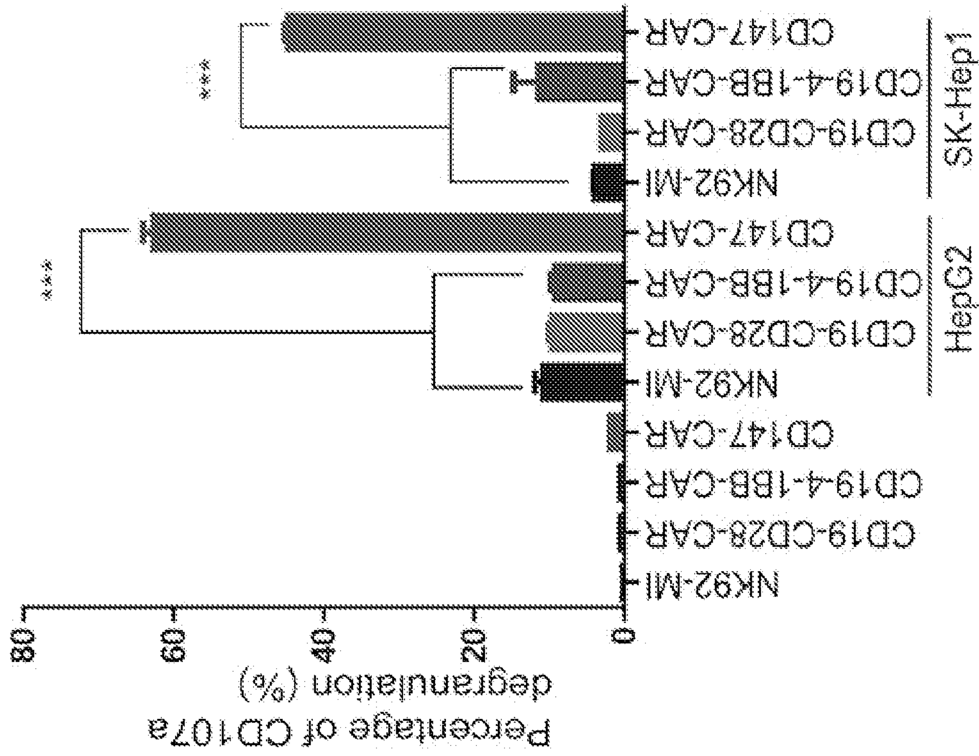


FIG. 3A

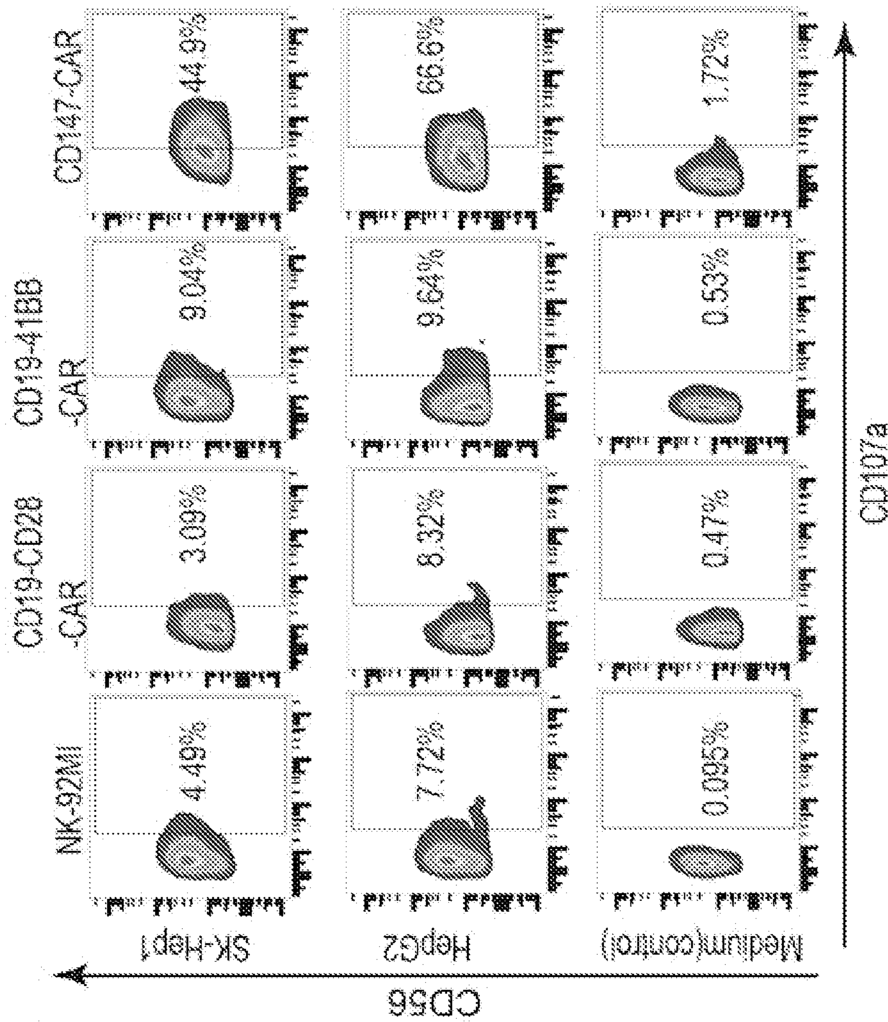


FIG. 3D

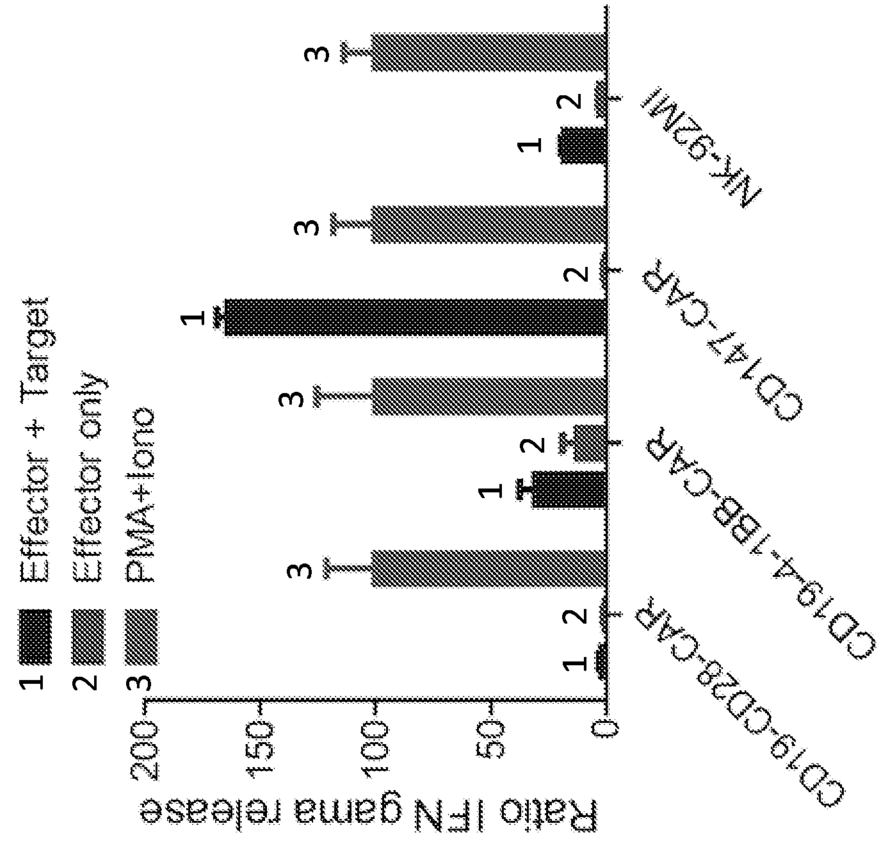


FIG. 3C

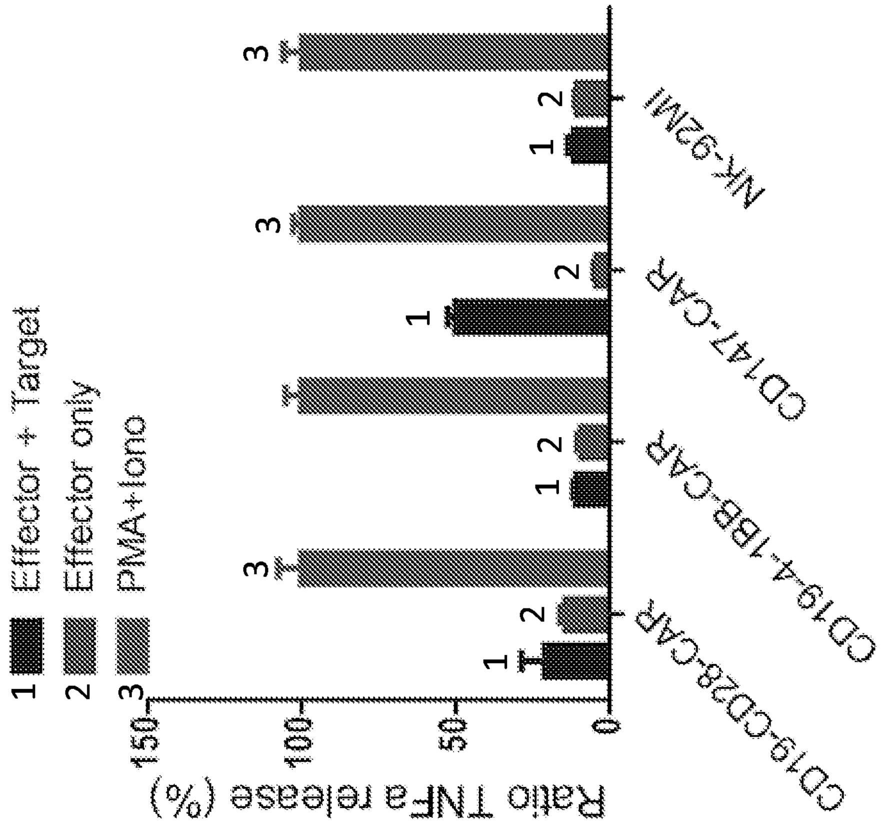


FIG. 4A

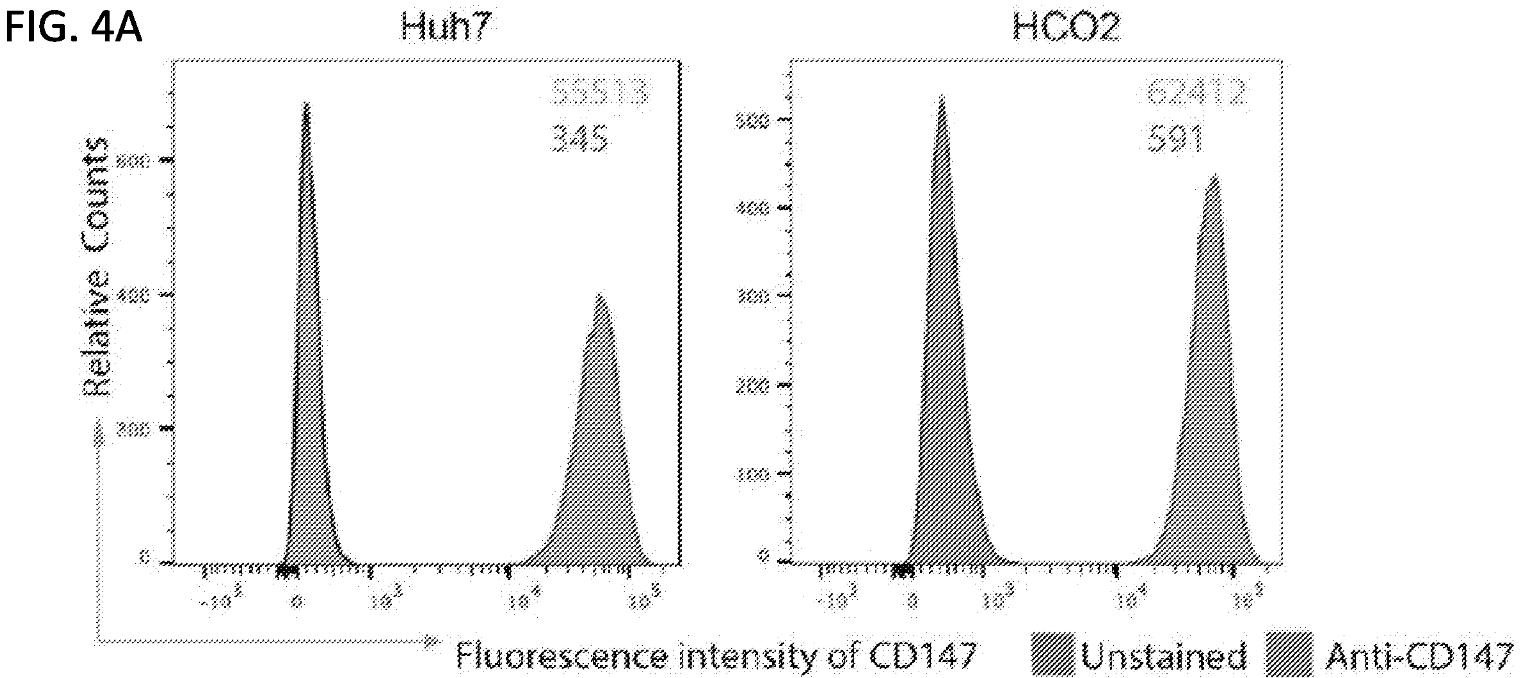


FIG. 4B

FIG. 4C

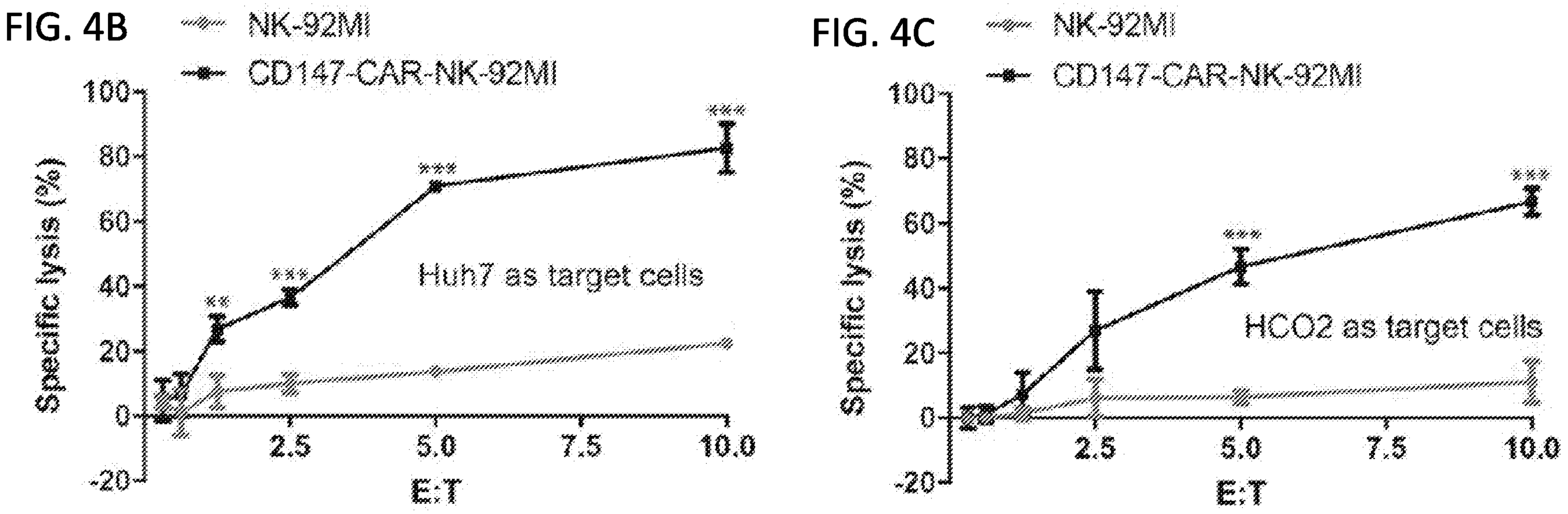
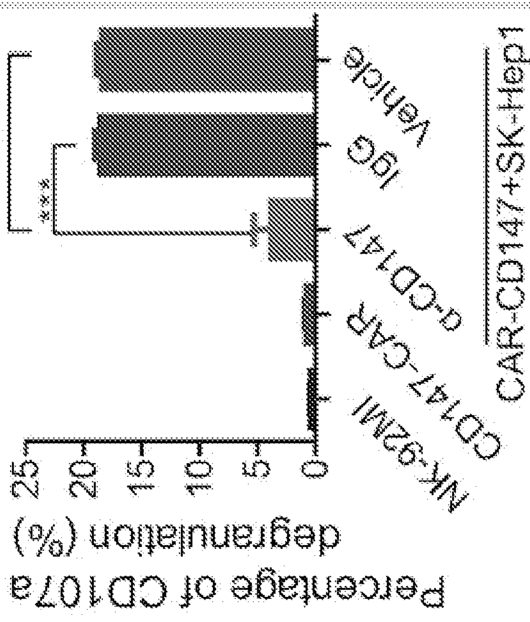


FIG. 5B



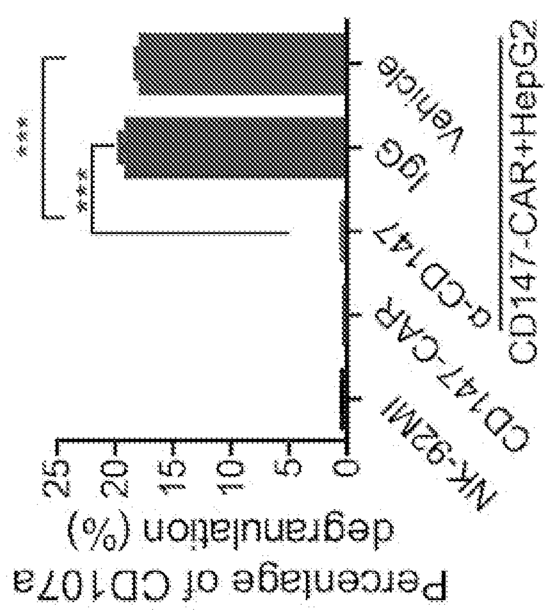
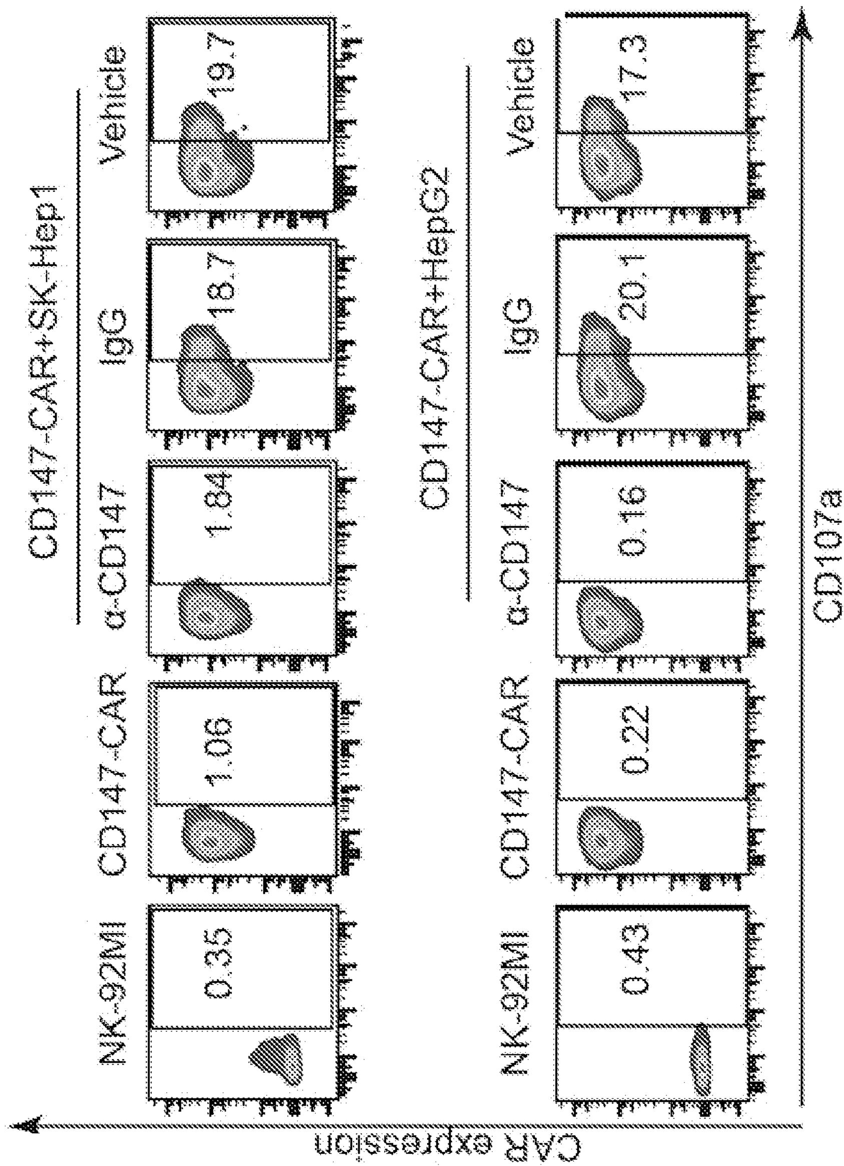


FIG. 5C

FIG. 5A



SUBSTITUTE SHEET (RULE 26)

FIG. 5D

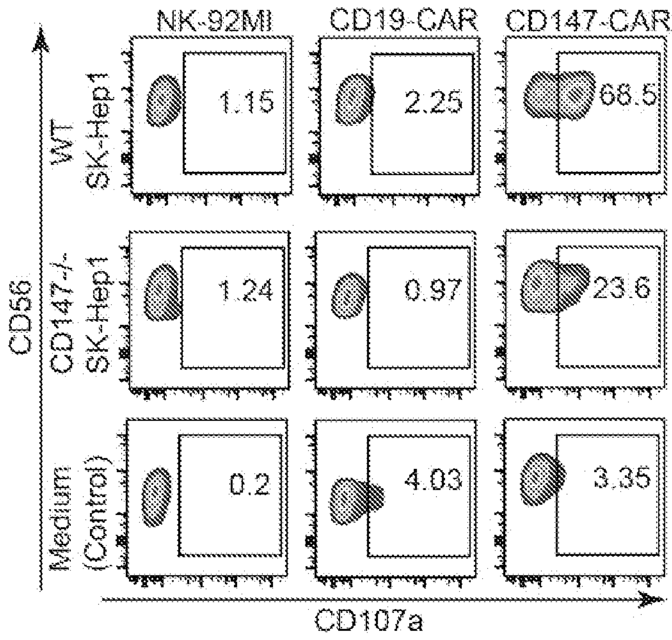
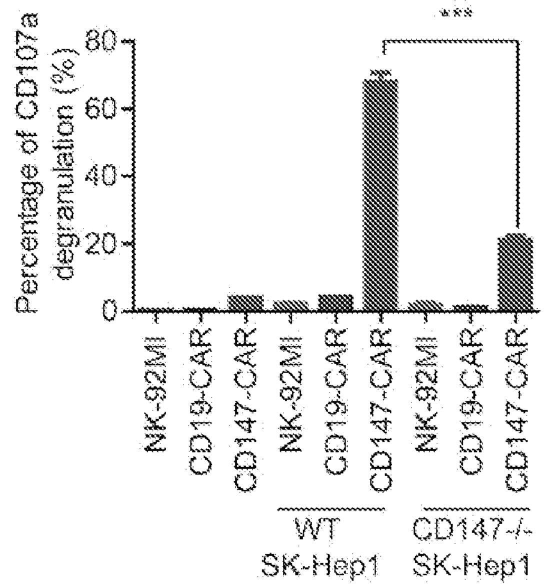


FIG. 5E



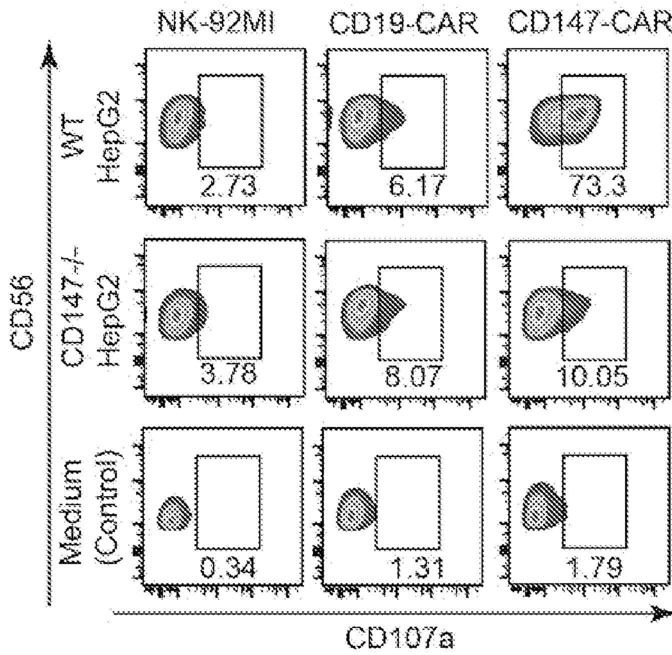


FIG. 5F

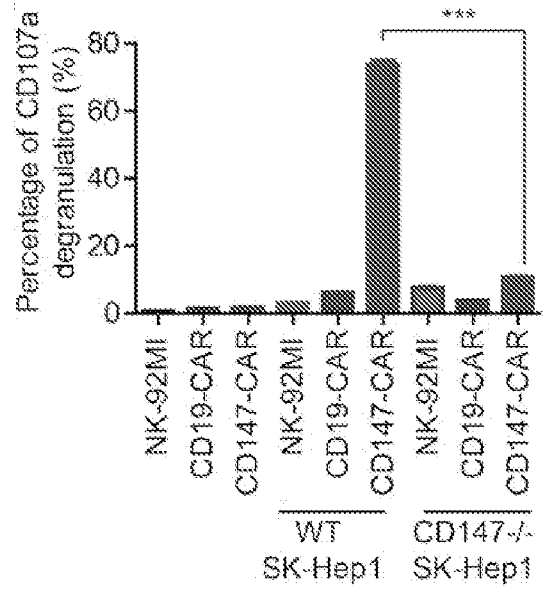
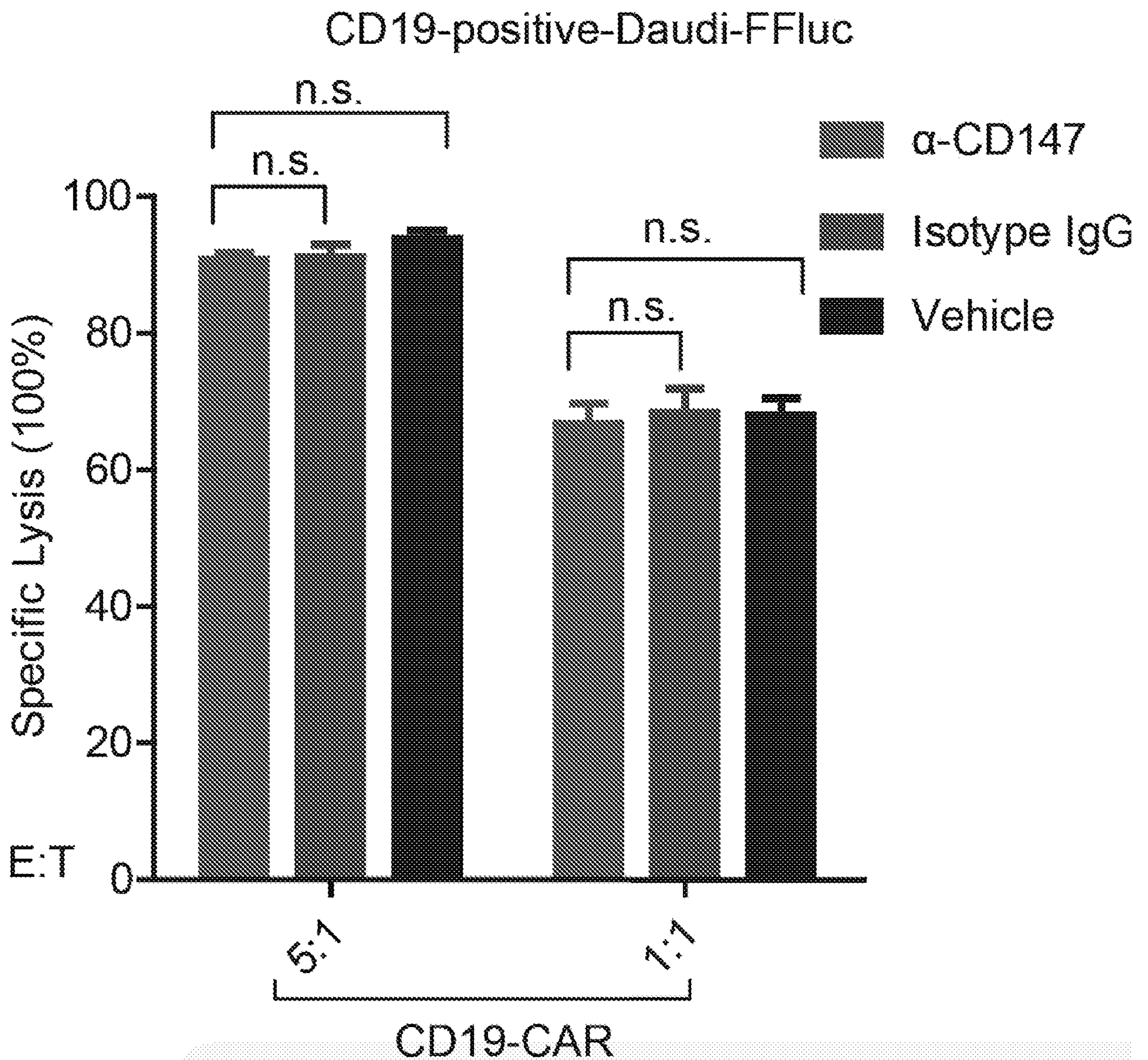


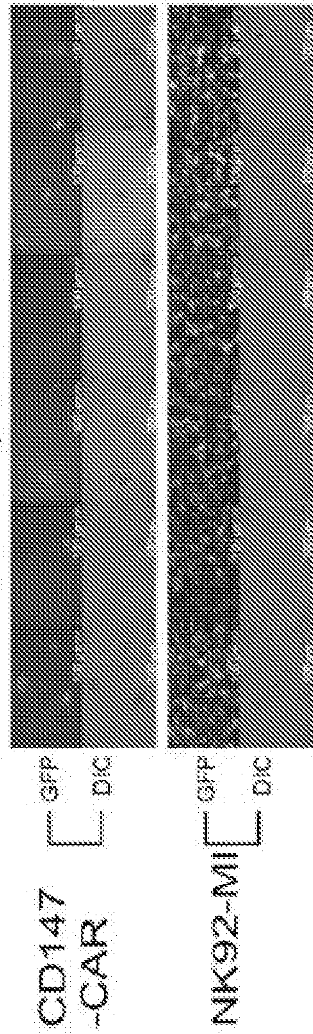
FIG. 5G

FIG. 6



SUBSTITUTE SHEET (RULE 26)

FIG. 7A



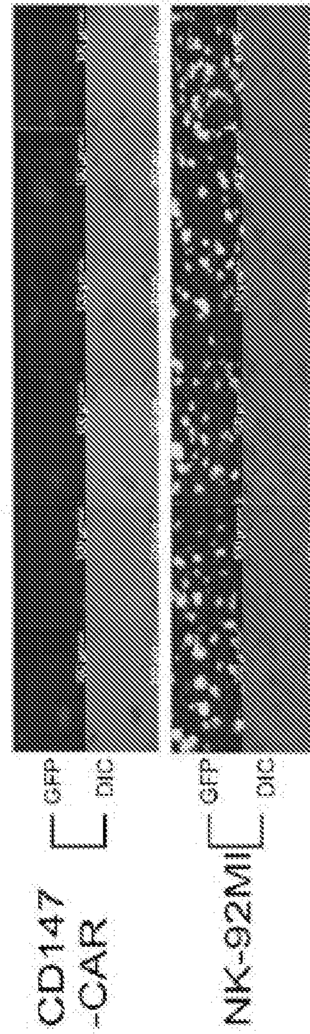
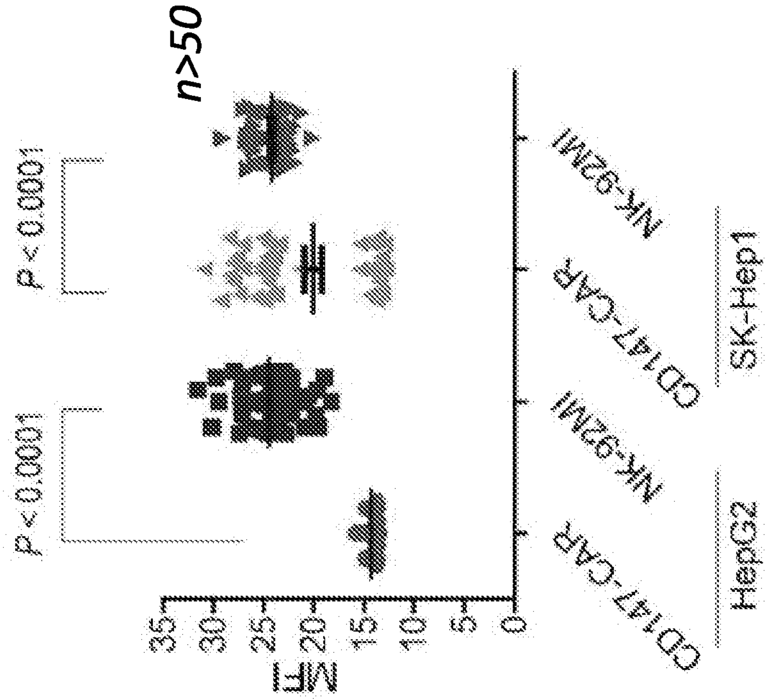


FIG. 7B



SUBSTITUTE SHEET (RULE 26)

FIG. 8A

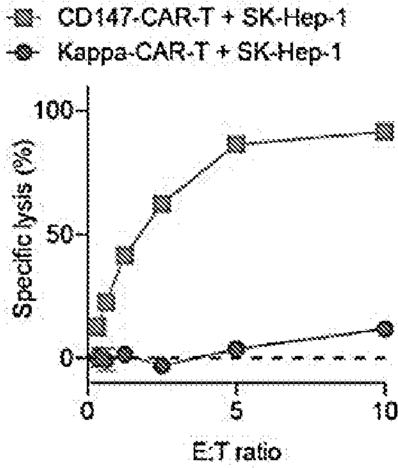


FIG. 8B

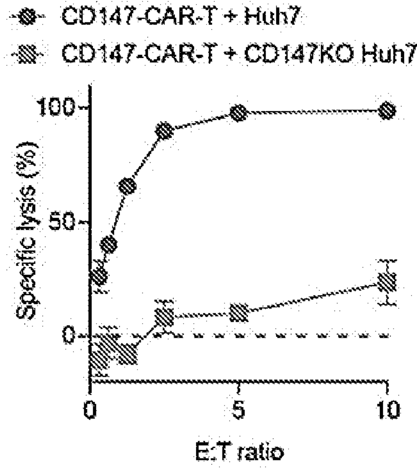
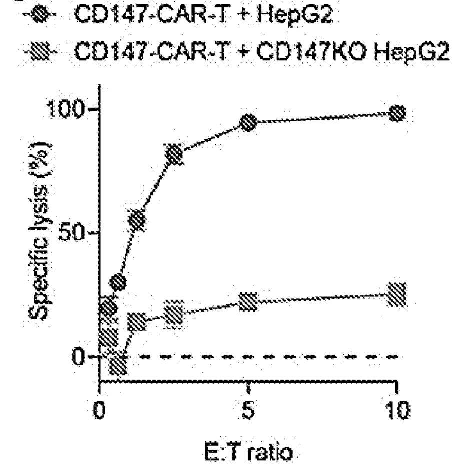


FIG. 8C



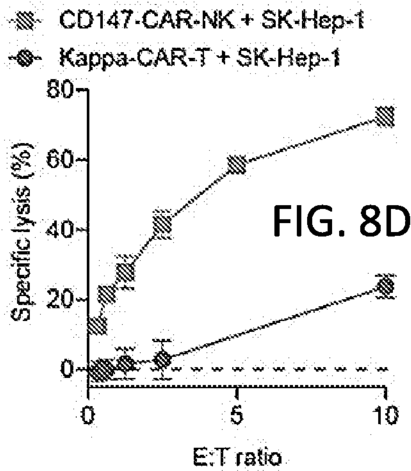


FIG. 8D

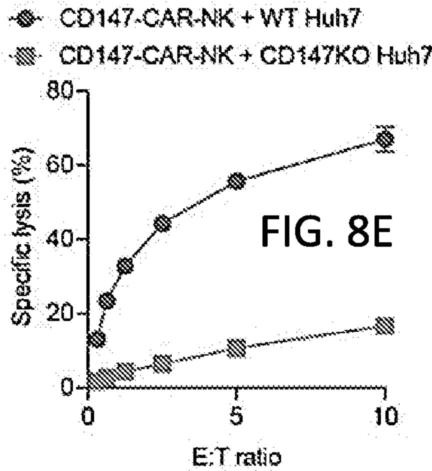


FIG. 8E

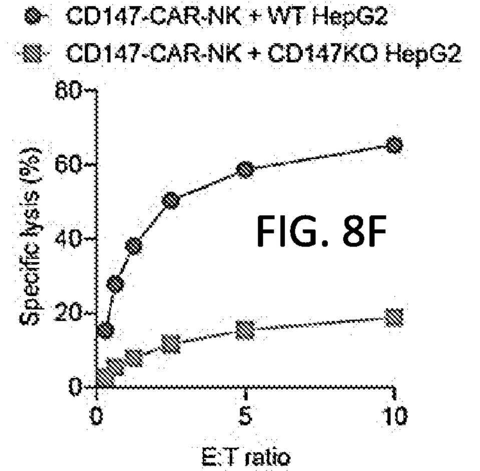
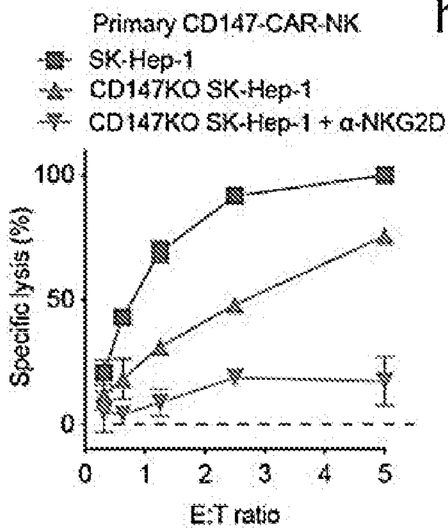


FIG. 8F



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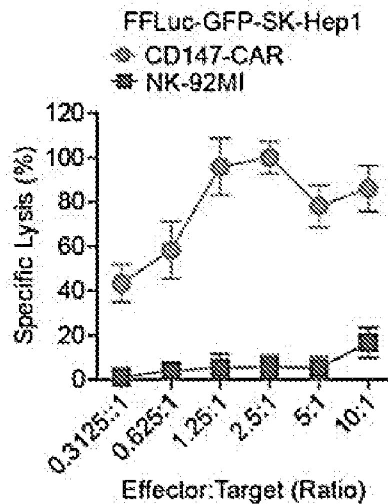


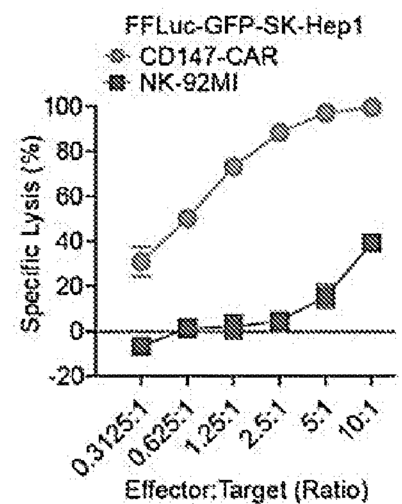


FIG. 8G

FIG. 8H

FIG. 8I

FIG. 8J

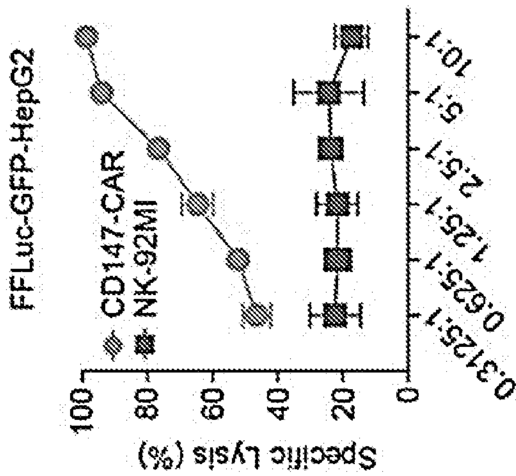


FIG. 8K

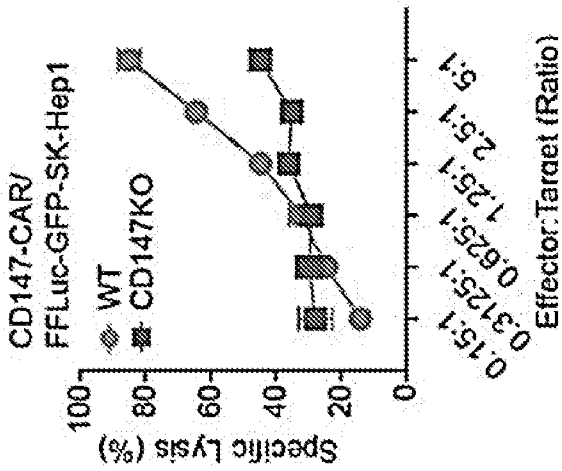


FIG. 8L

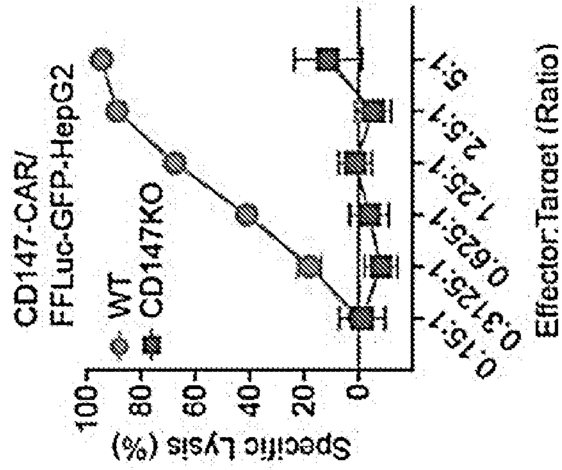


FIG. 8M

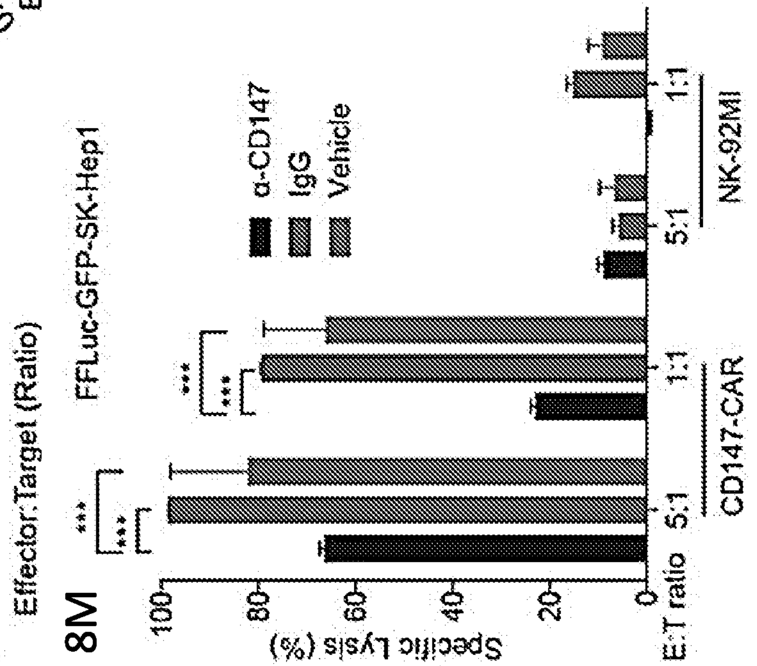


FIG. 8N

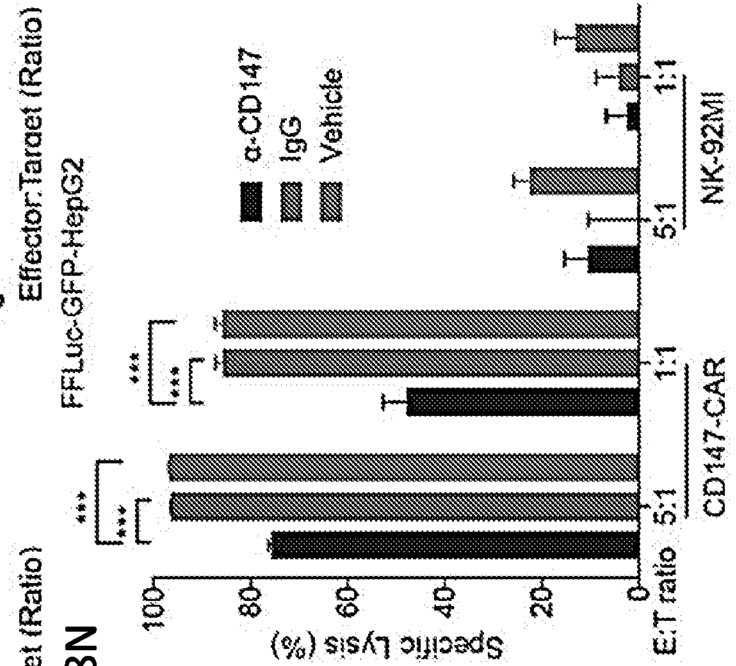
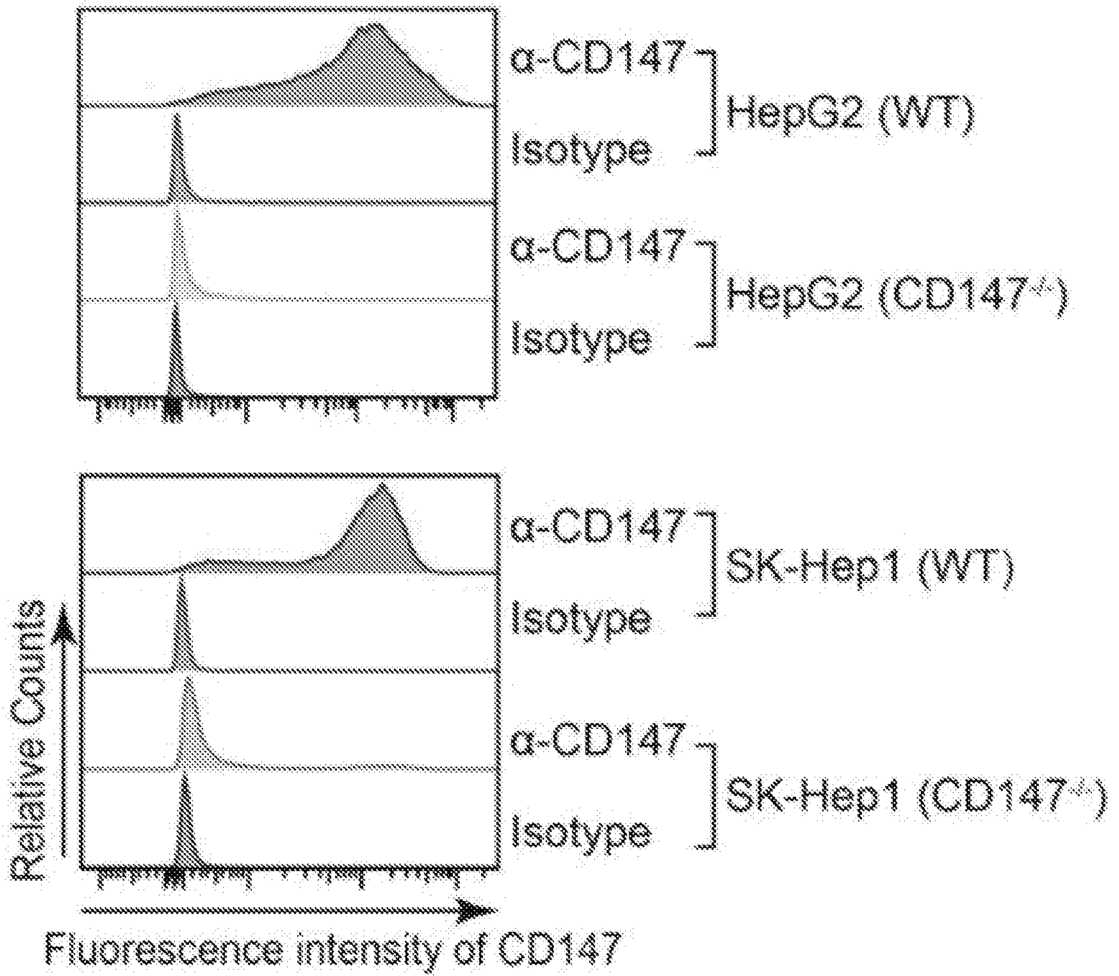


FIG. 9A



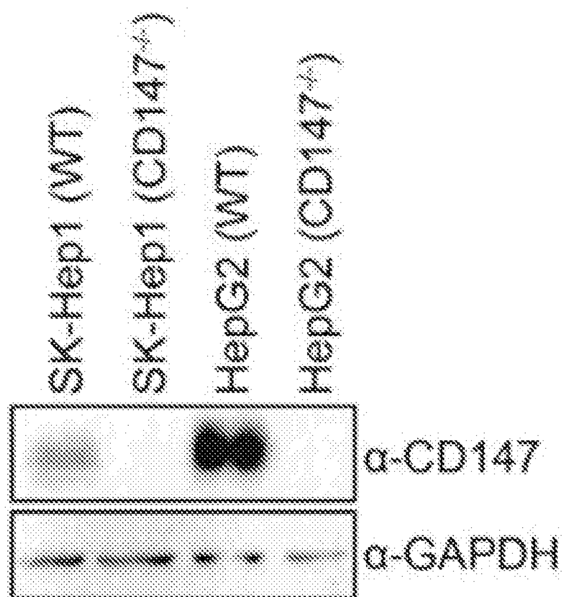


FIG. 9B

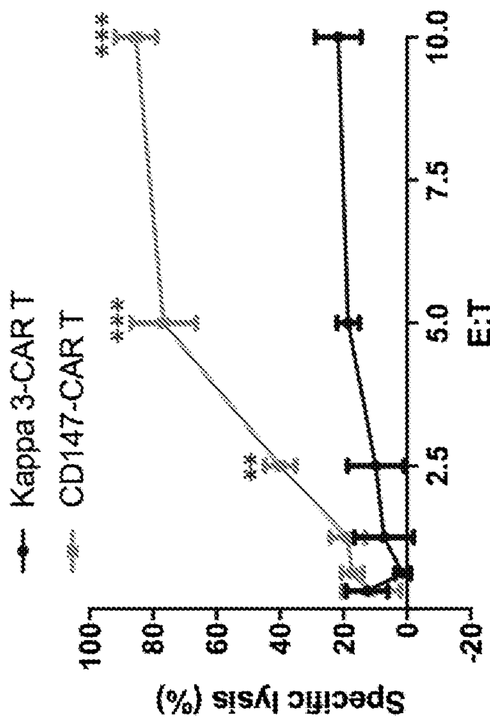


FIG. 10A

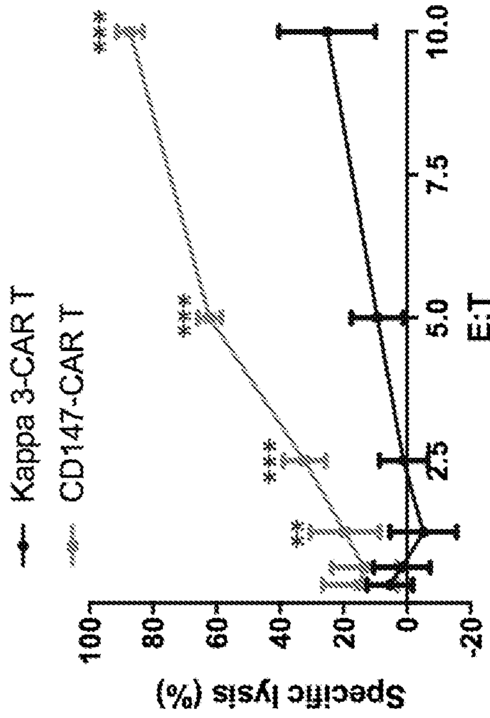


FIG. 10B

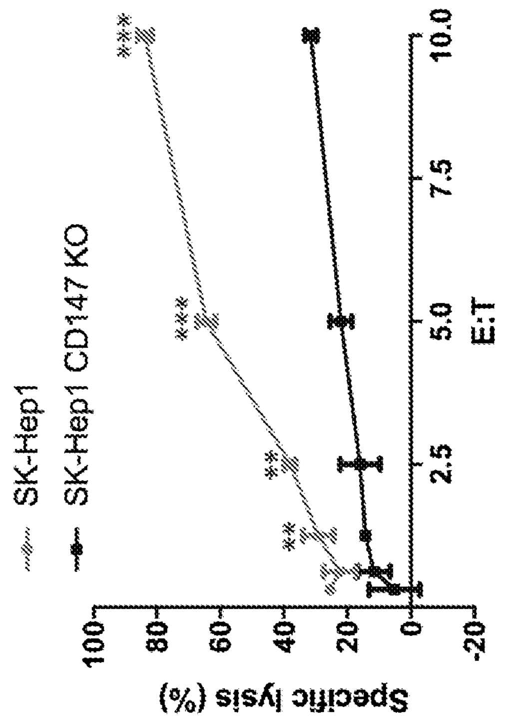


FIG. 10C

FIG. 11A

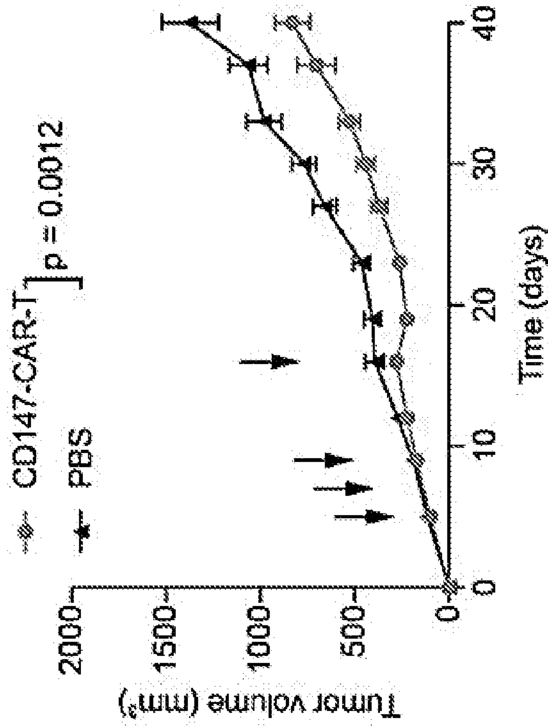
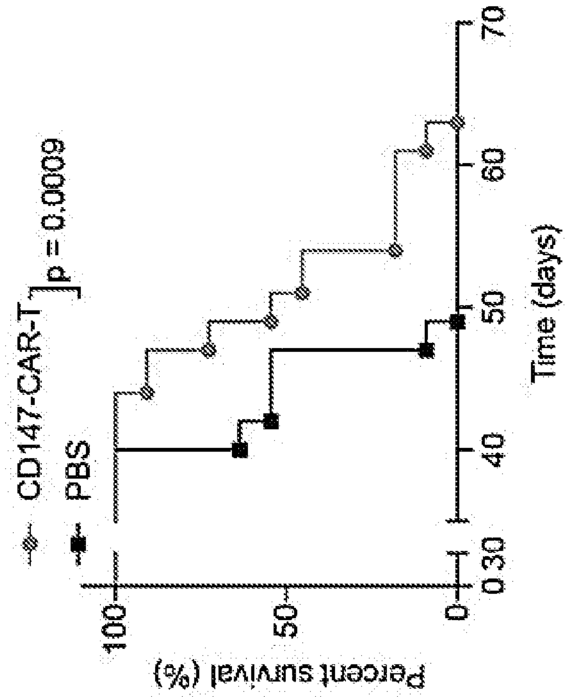


FIG. 11B



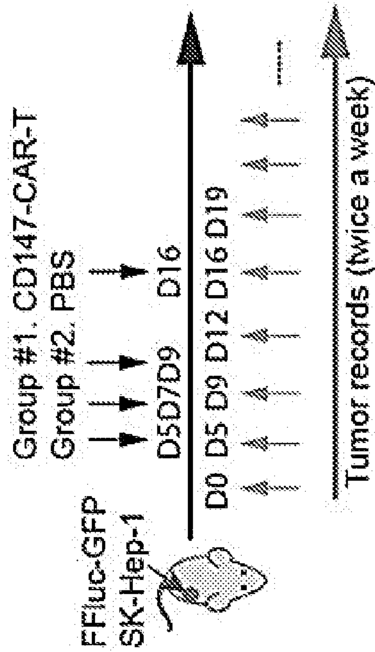


FIG. 11C

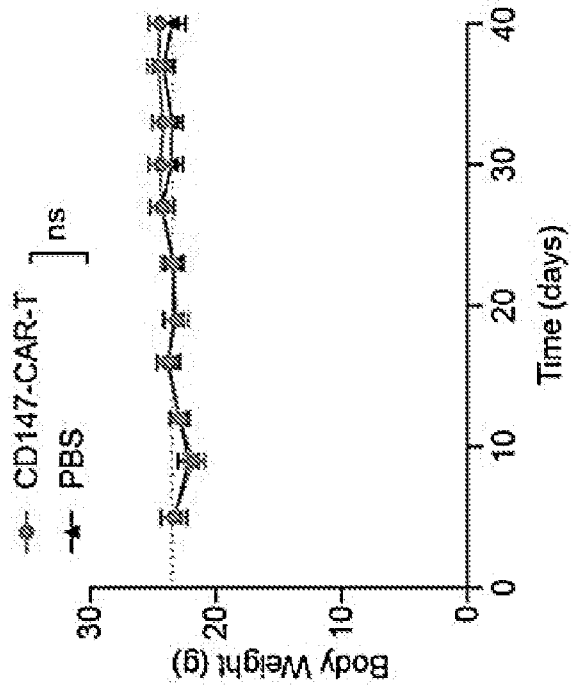


FIG. 11D

SUBSTITUTE SHEET (RULE 26)

FIG. 12A

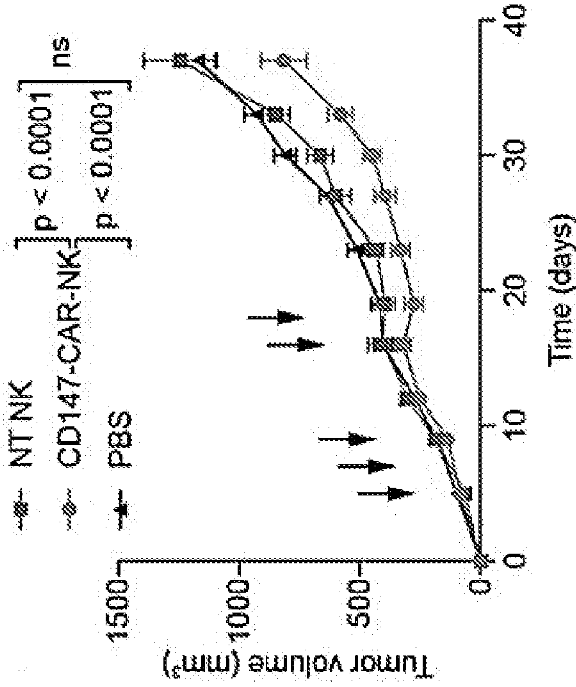
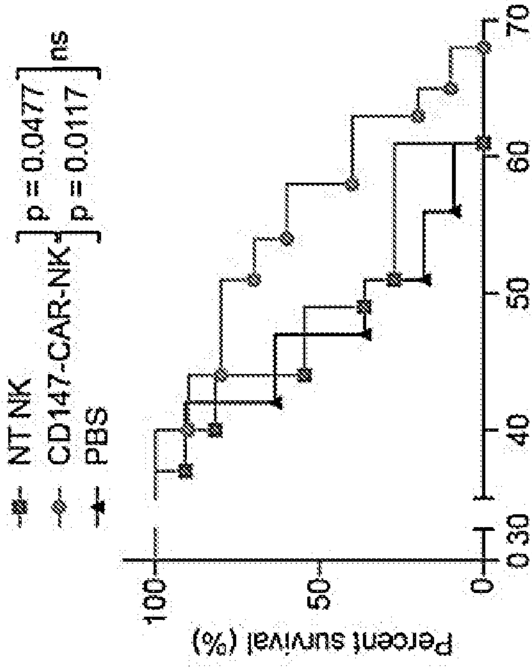


FIG. 12B



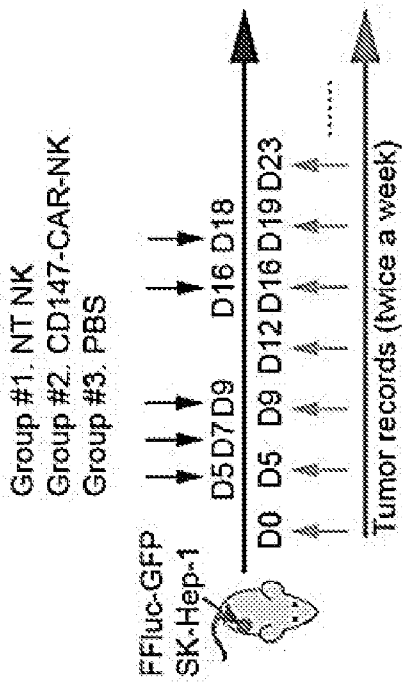


FIG. 12C

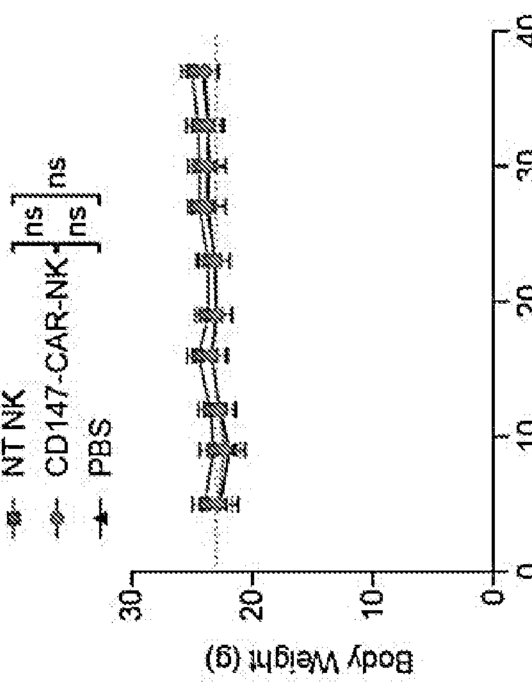


FIG. 12D

SUBSTITUTE SHEET (RULE 26)

FIG. 13

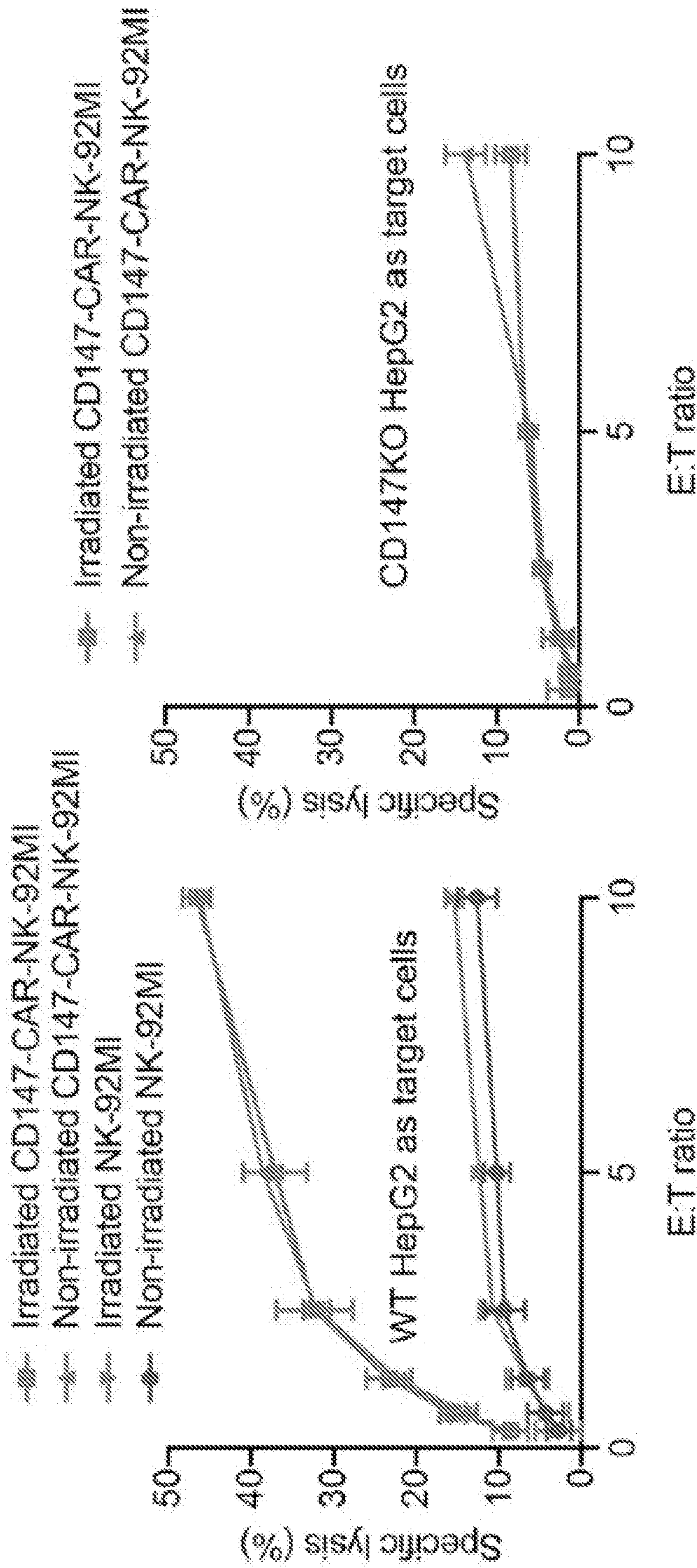


FIG. 14A

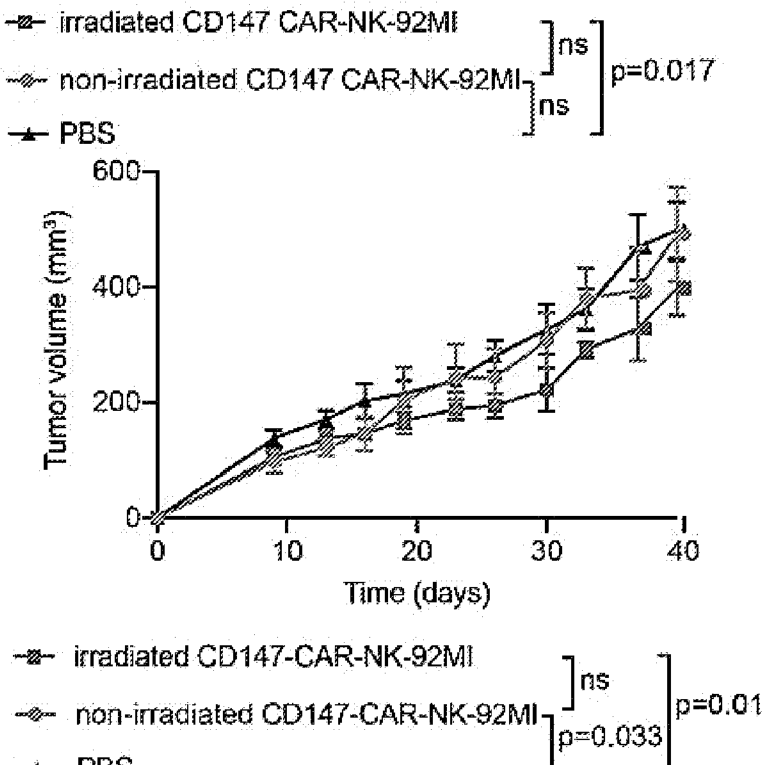
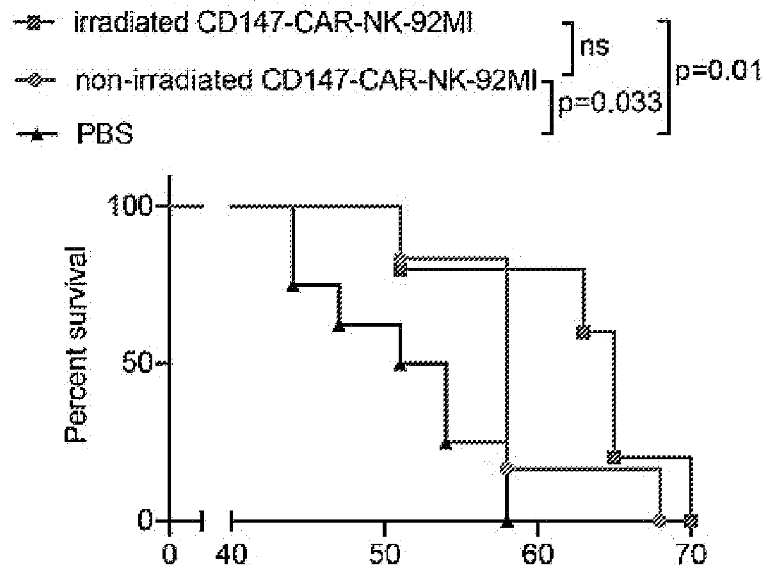


FIG. 14B



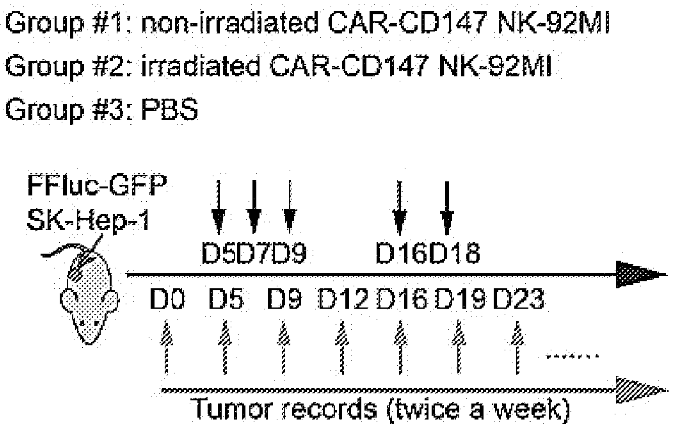


FIG. 14C

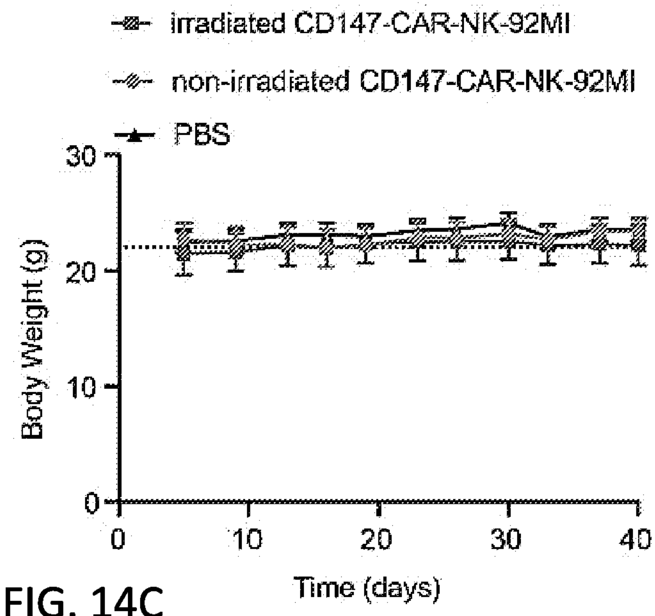


FIG. 14D

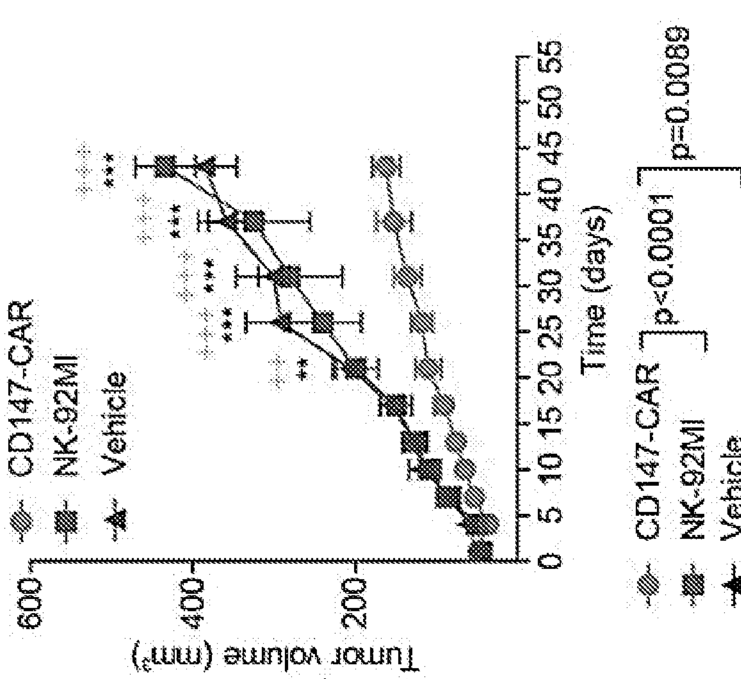


FIG. 15A

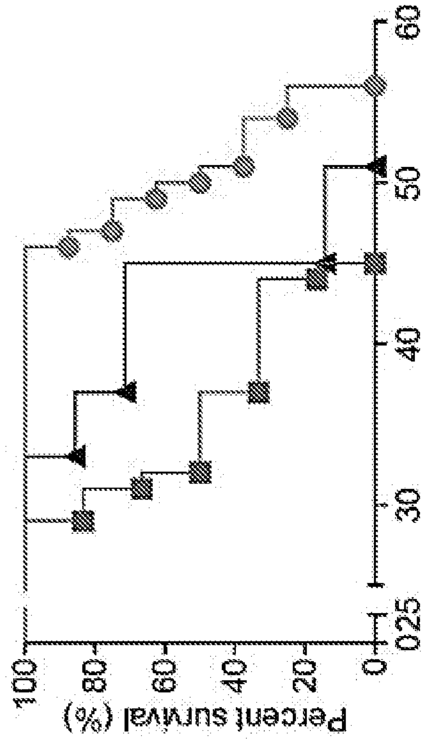


FIG. 15B

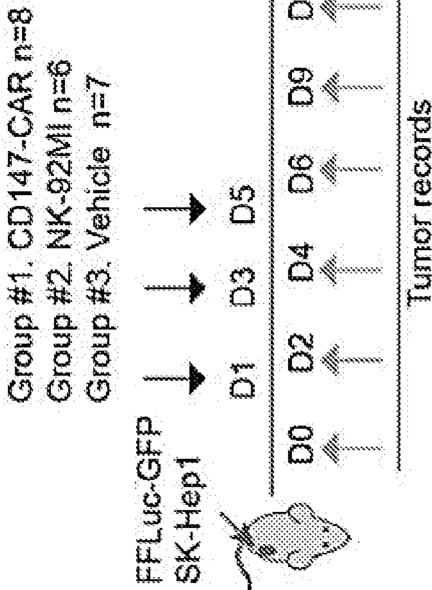


FIG. 15C

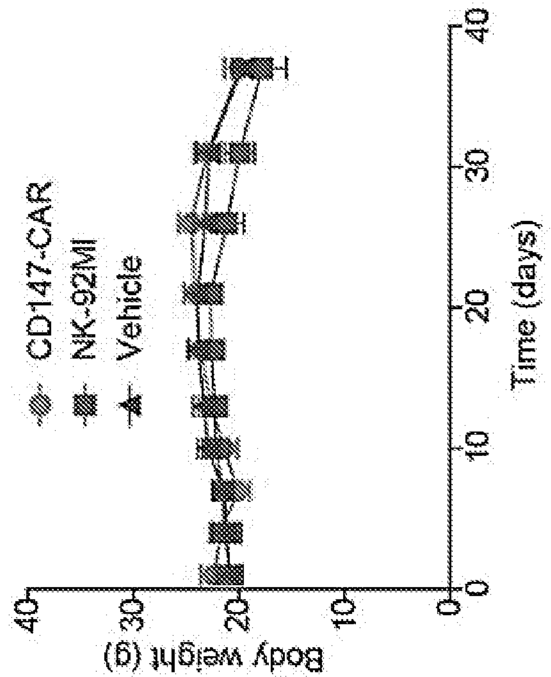


FIG. 15D

FIG. 16A

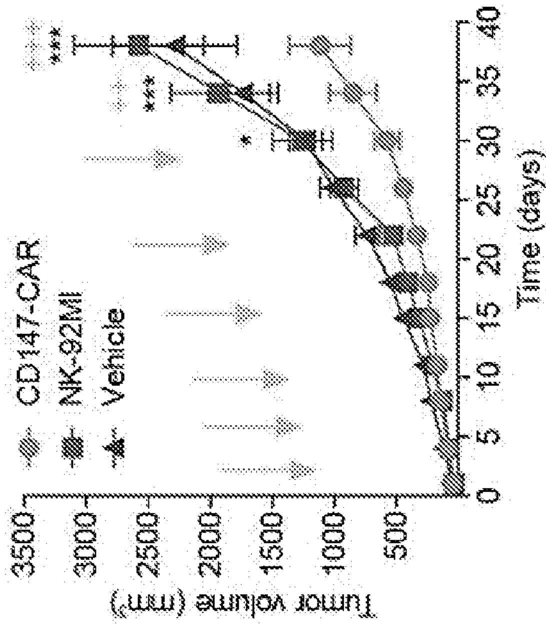
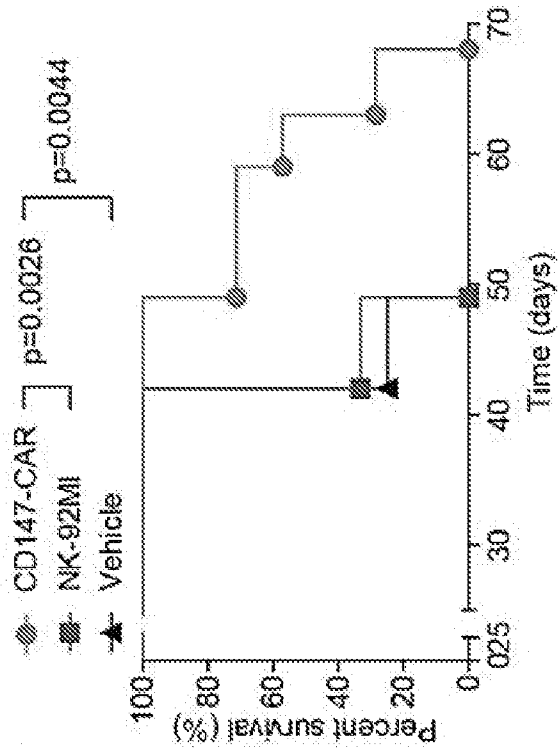


FIG. 16B



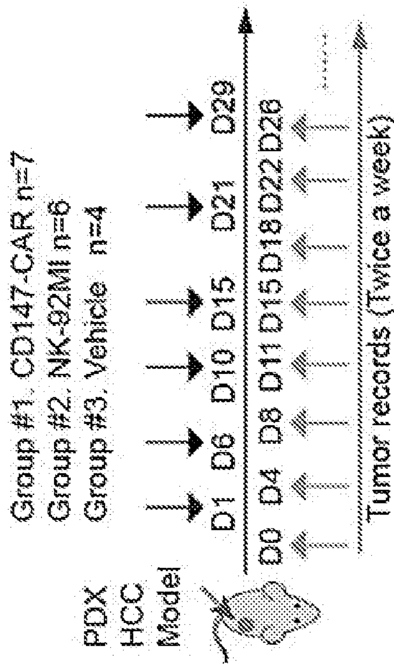


FIG. 16C

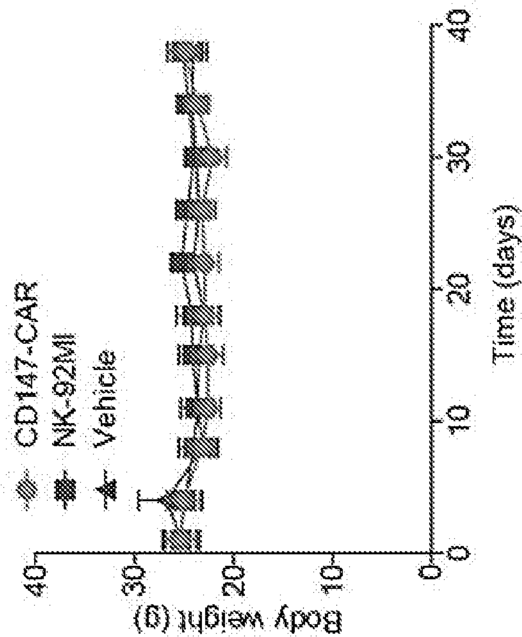


FIG. 16D

FIG. 17A

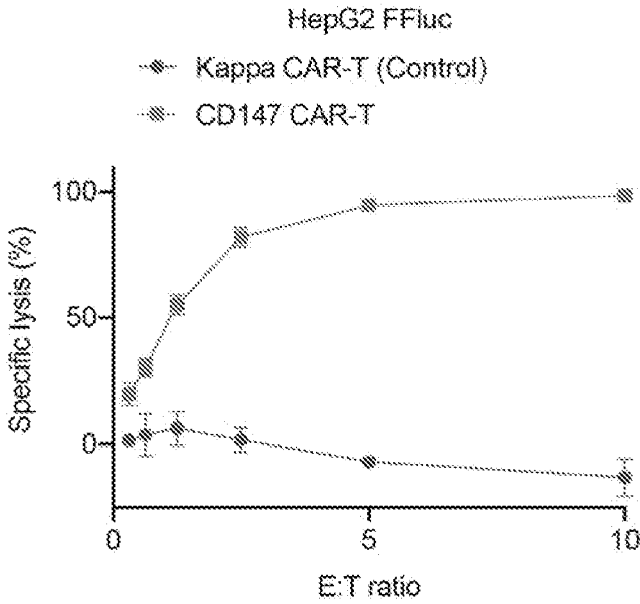
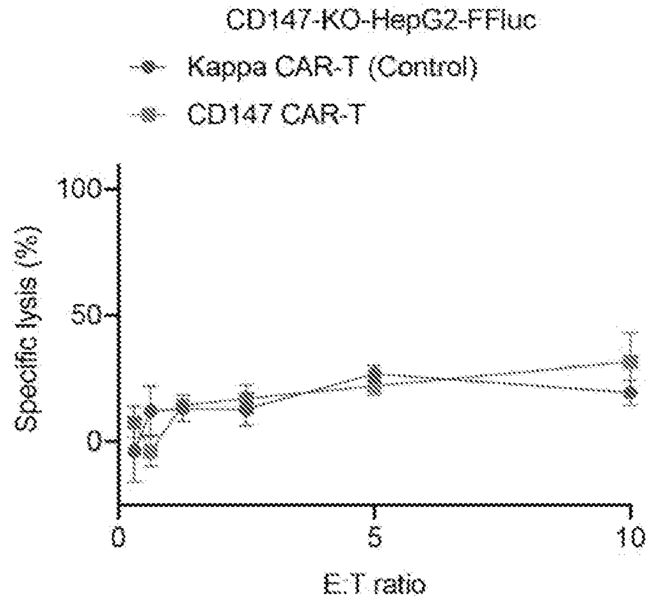


FIG. 17B



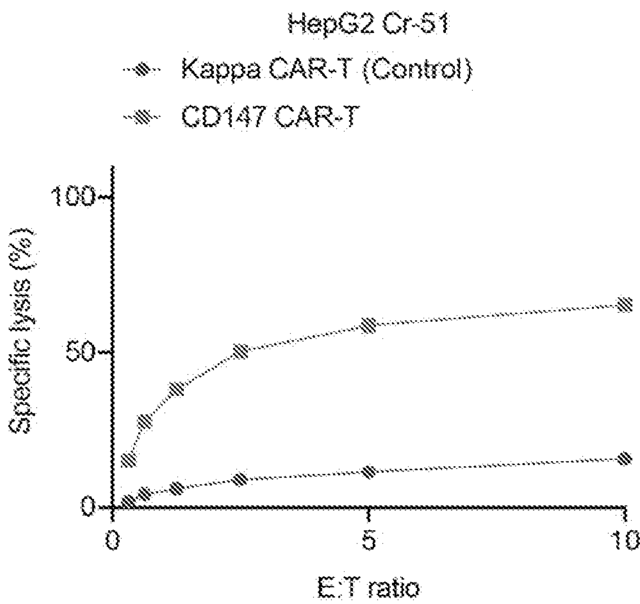


FIG. 17C

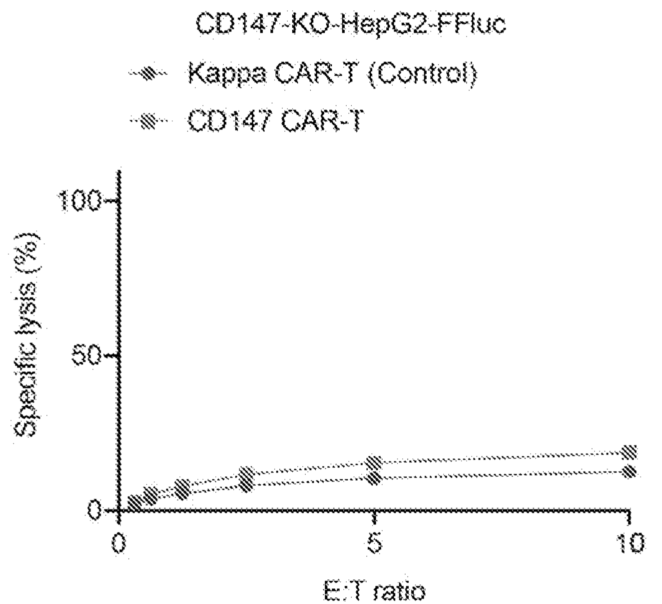


FIG. 17D

FIG. 18A

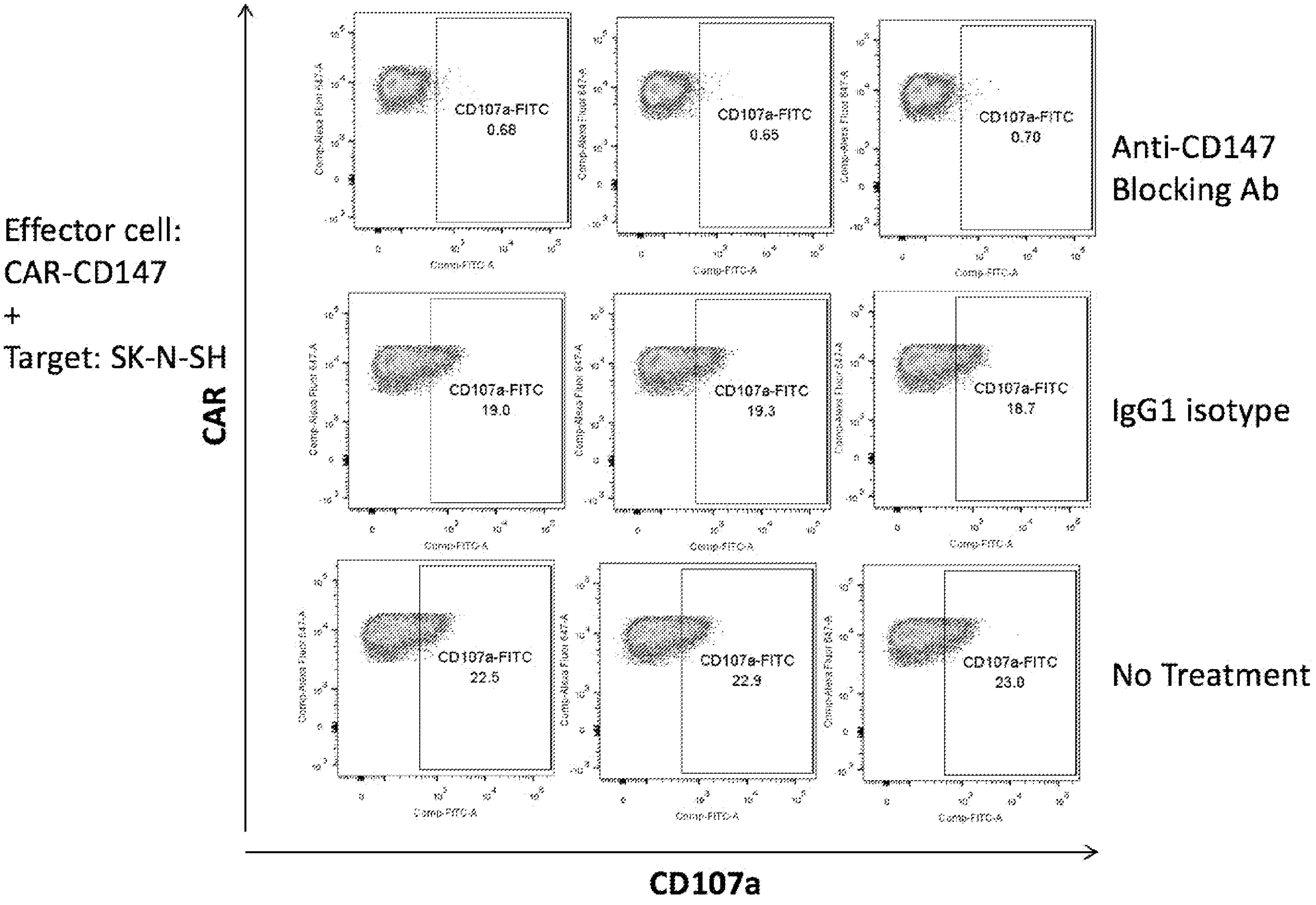


FIG. 18B

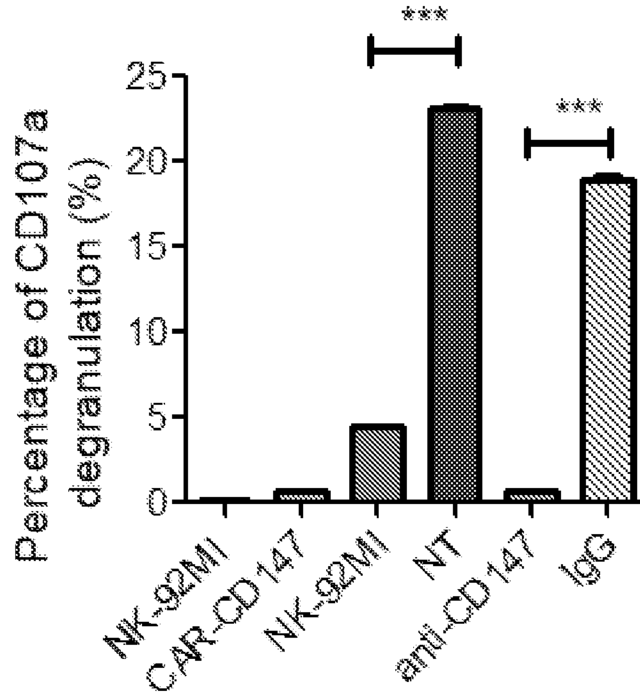


FIG. 19

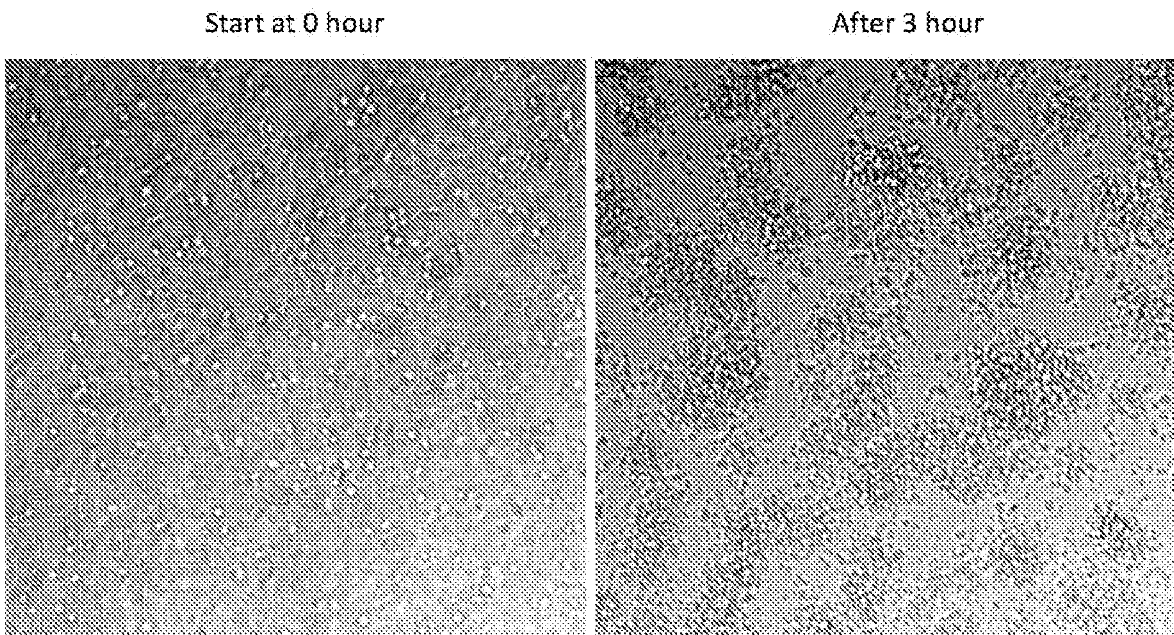


FIG. 20

SUBSTITUTE SHEET (RULE 26)

FIG. 21A

HCC

Healthy liver

Stage 1

Stage 2

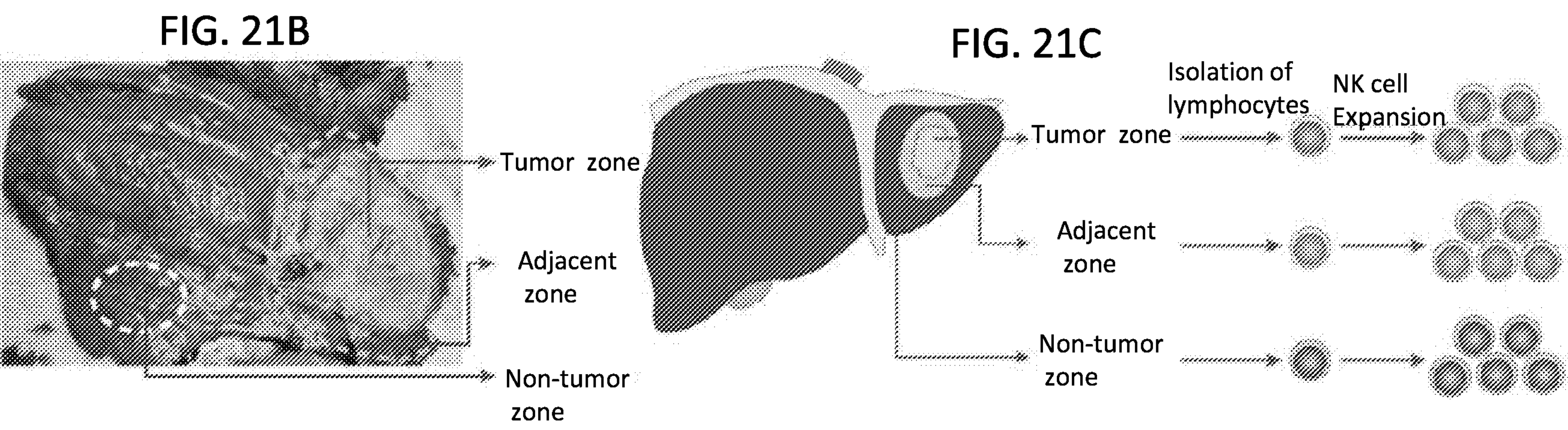
Stage 3

HE

CD147

FIG. 21B

FIG. 21C



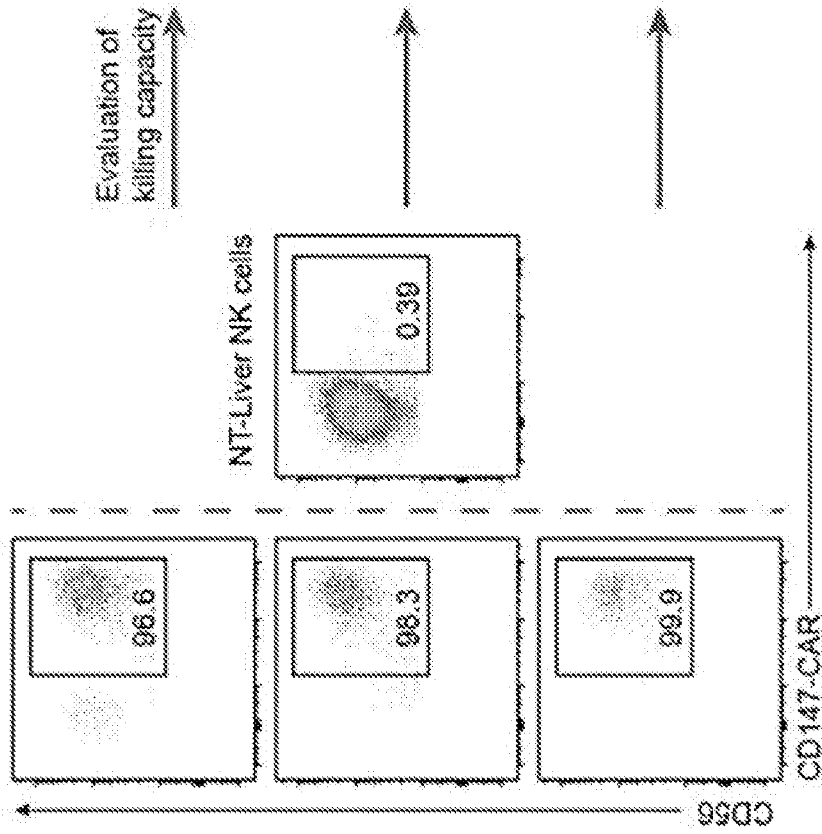


FIG. 21D

FIG. 21E

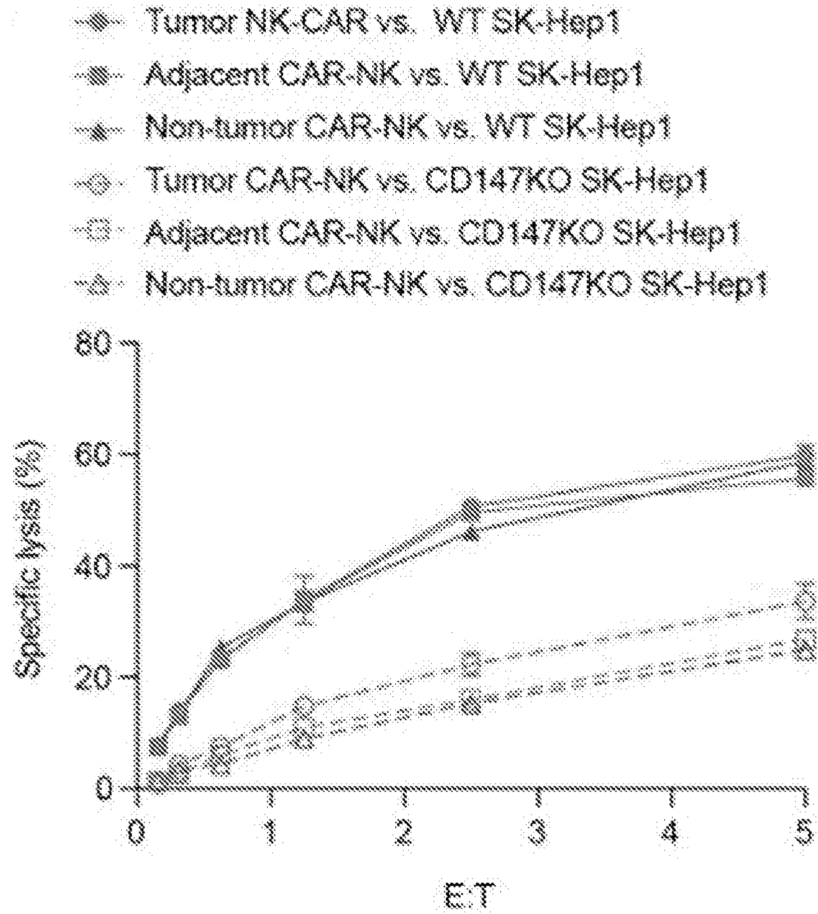


FIG. 22A

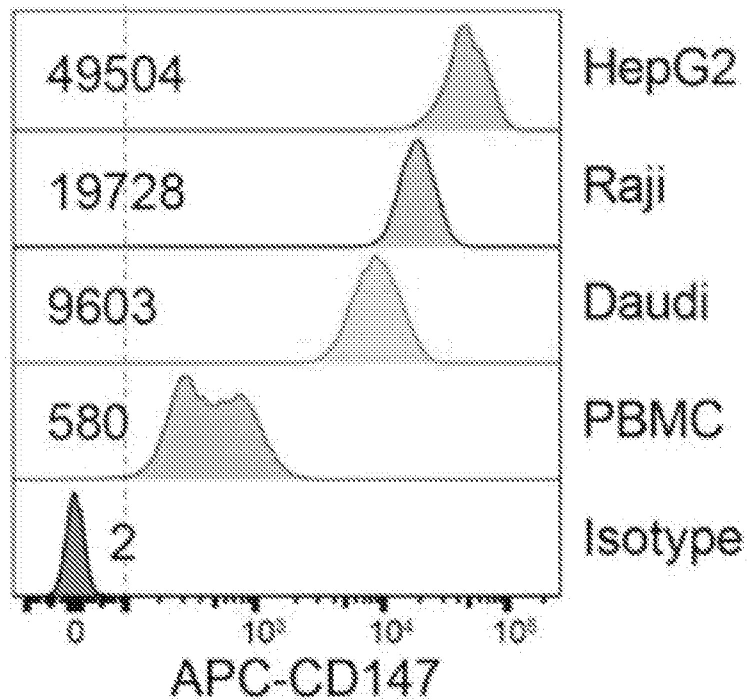
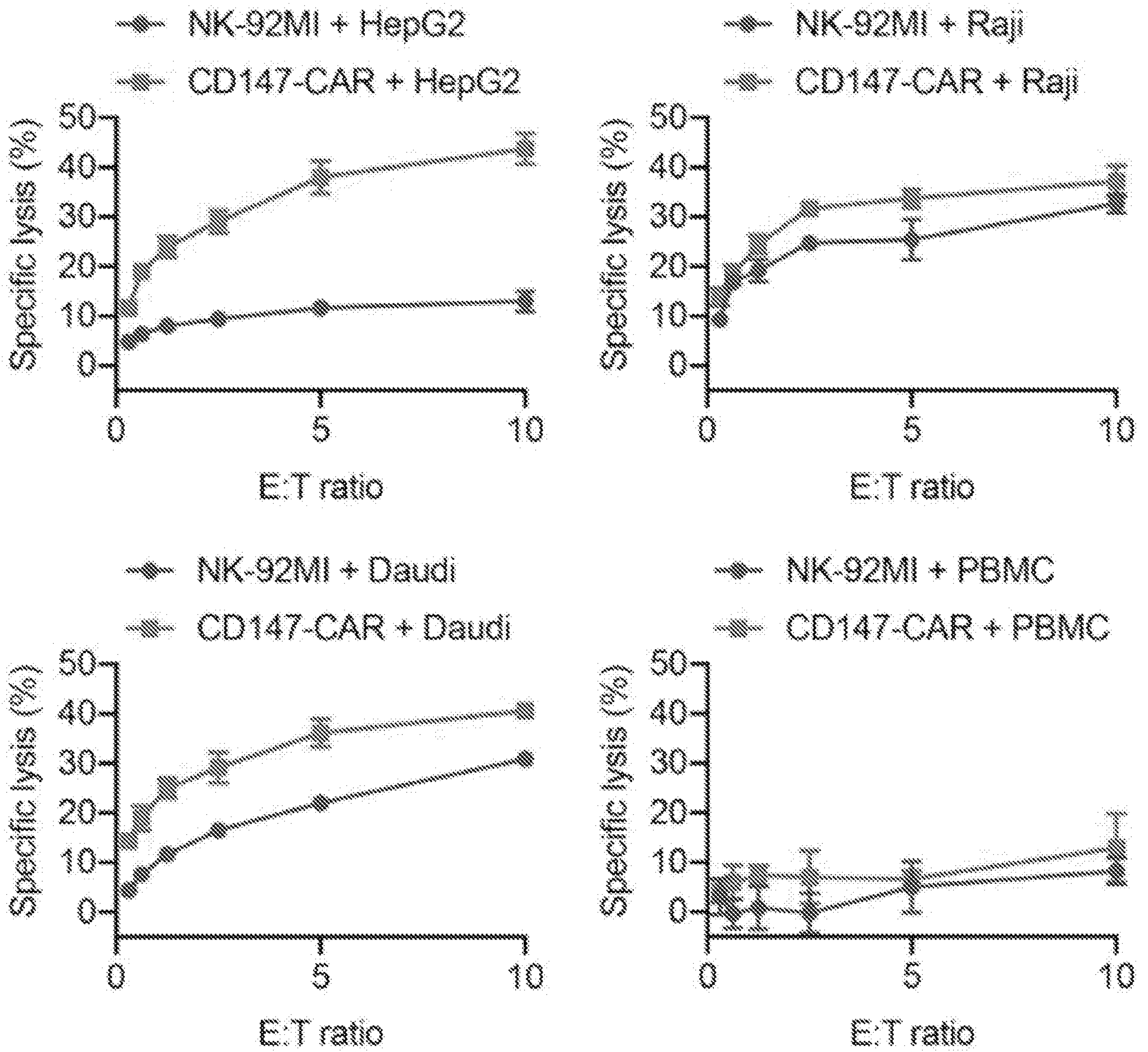


FIG. 22B



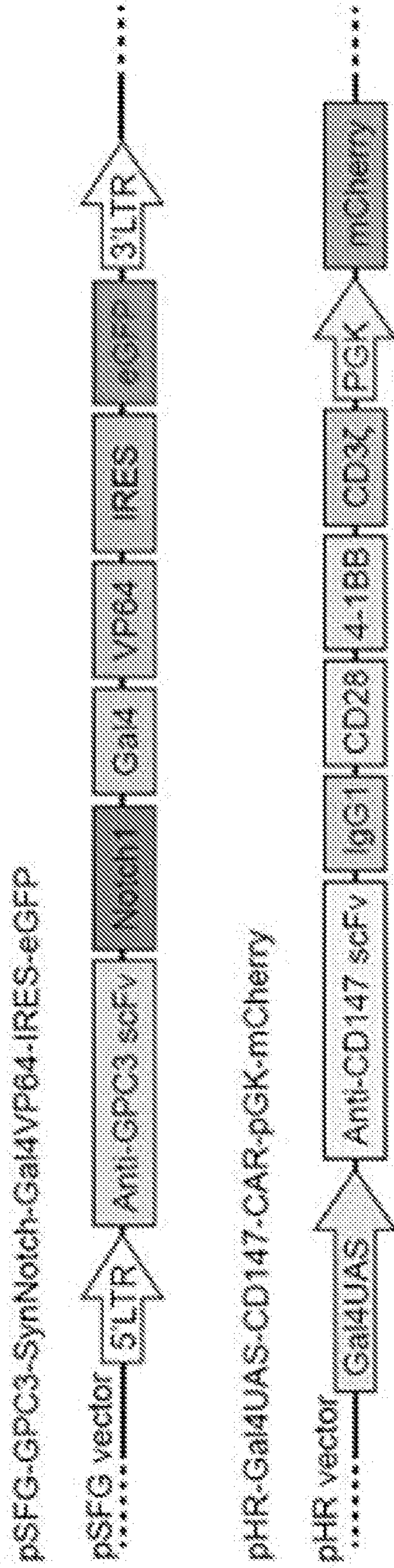


FIG. 23A

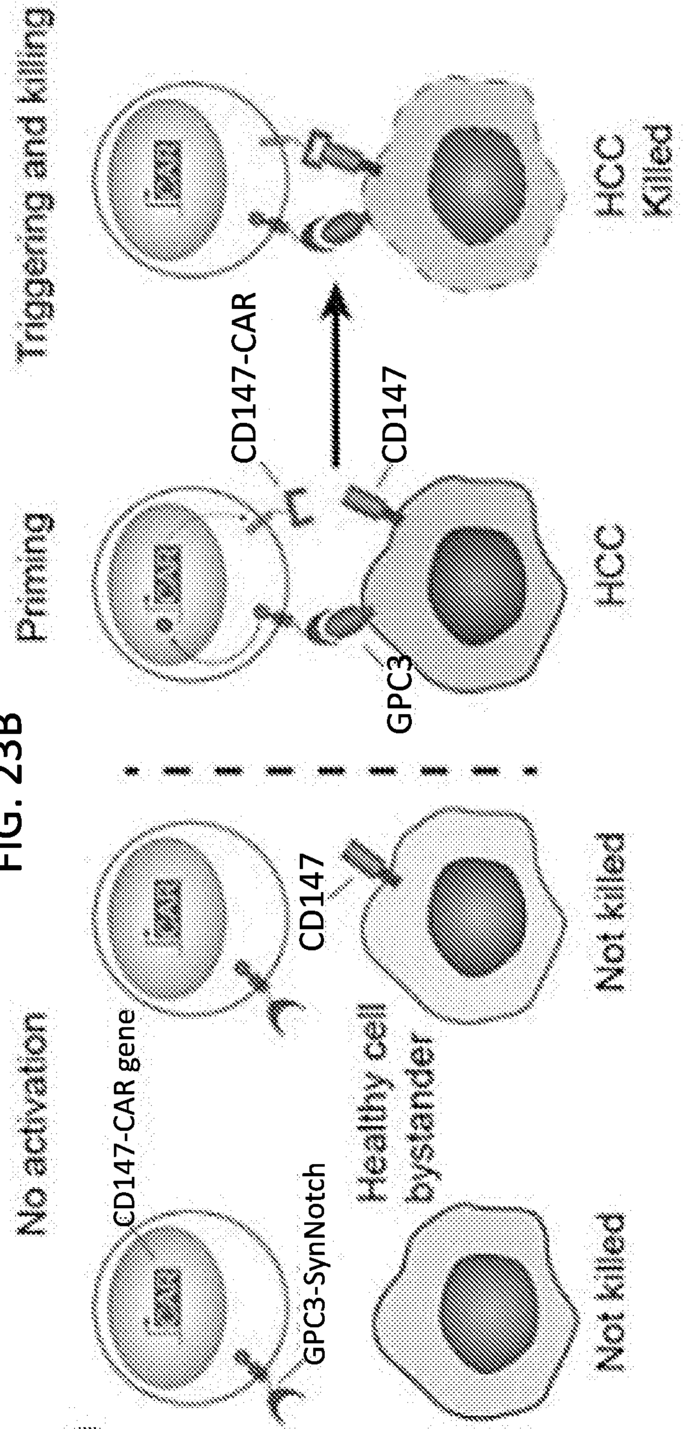


FIG. 23B

SUBSTITUTE SHEET (RULE 26)

FIG. 23C

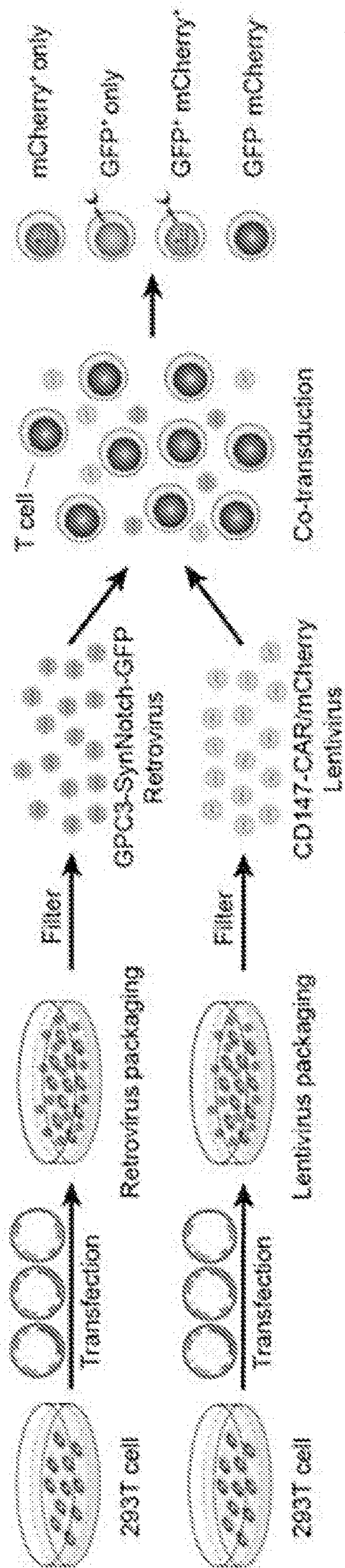
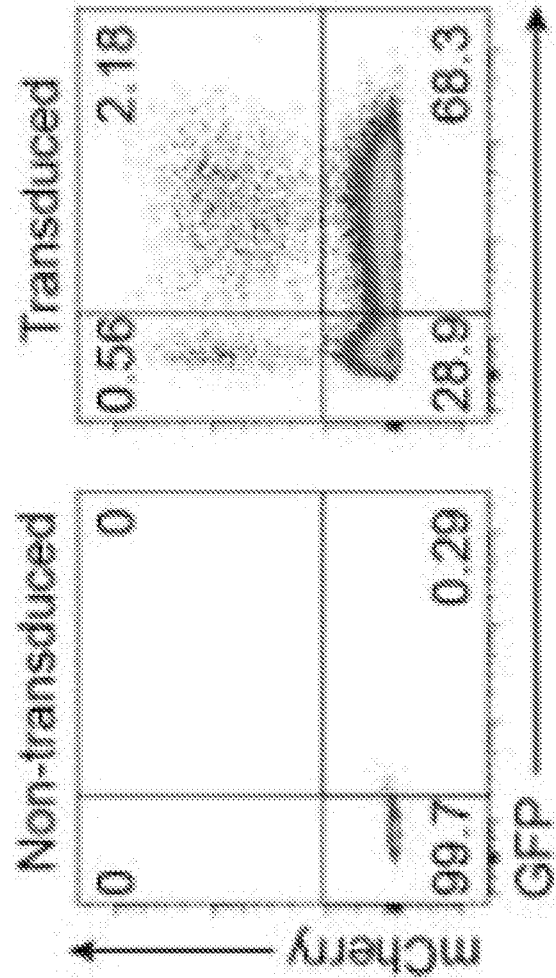


FIG. 23D



SUBSTITUTE SHEET (RULE 26)

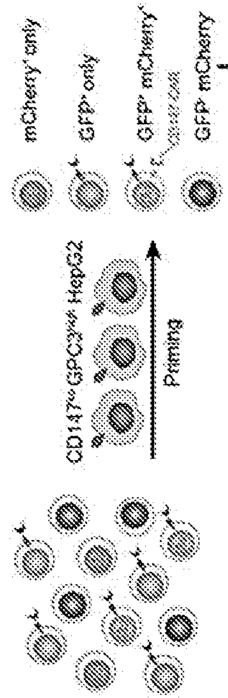


FIG. 23E

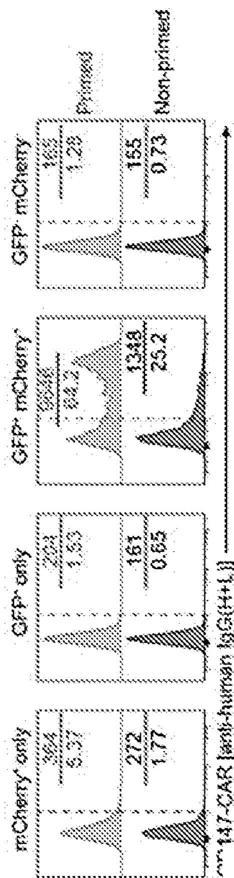


FIG. 23F

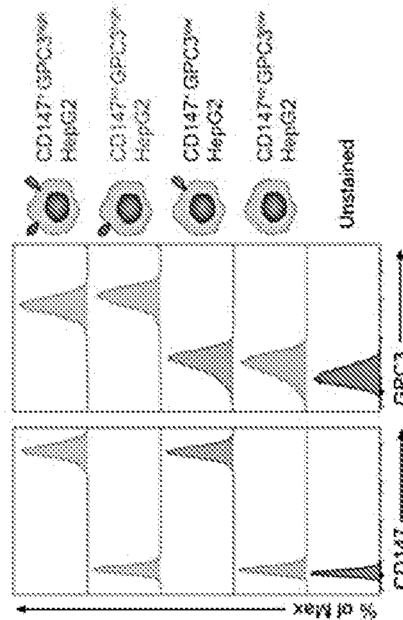


FIG. 23G

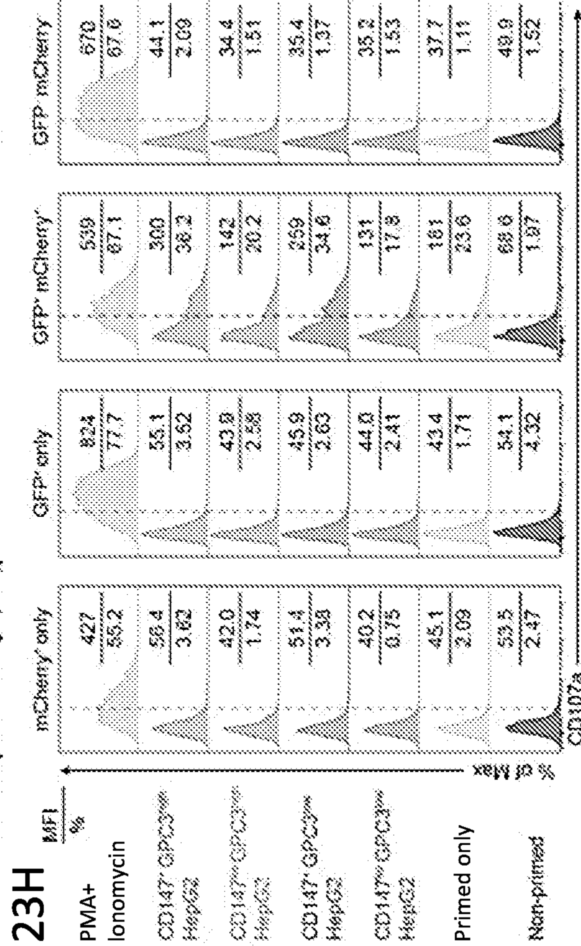


FIG. 23H

SUBSTITUTE SHEET (RULE 26)

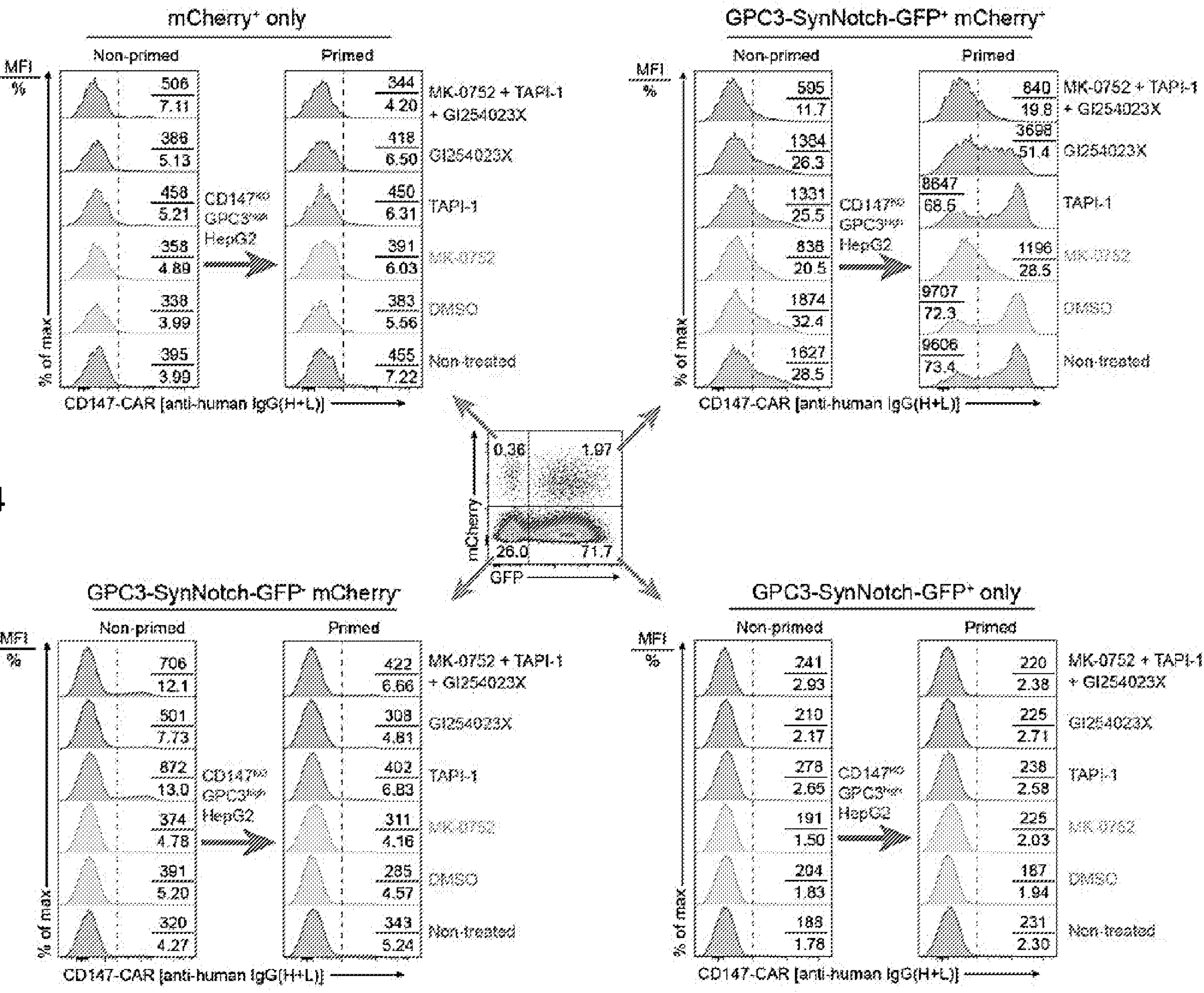


FIG. 24

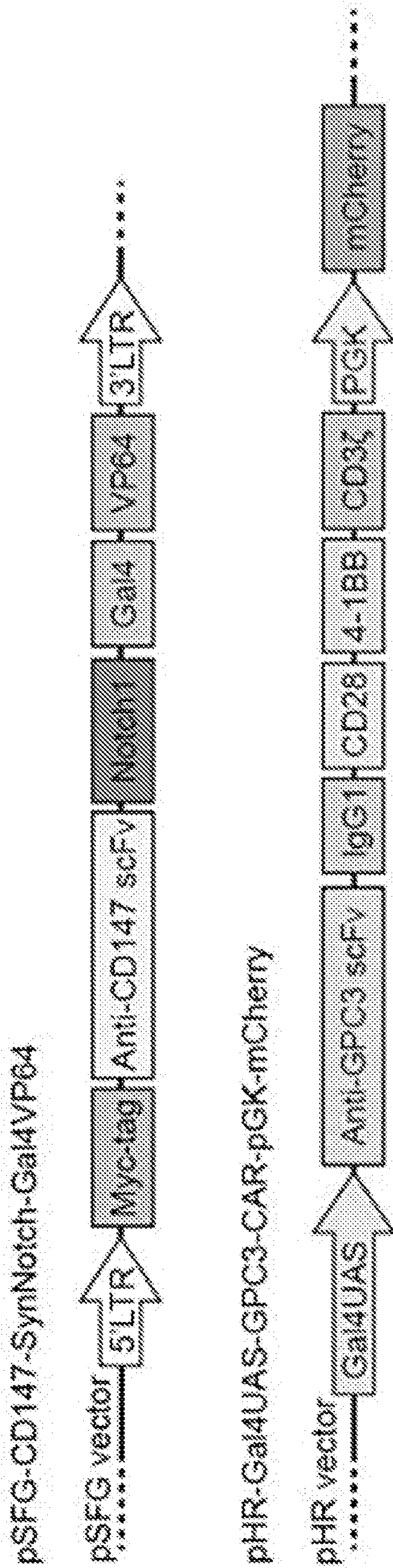


FIG. 25A

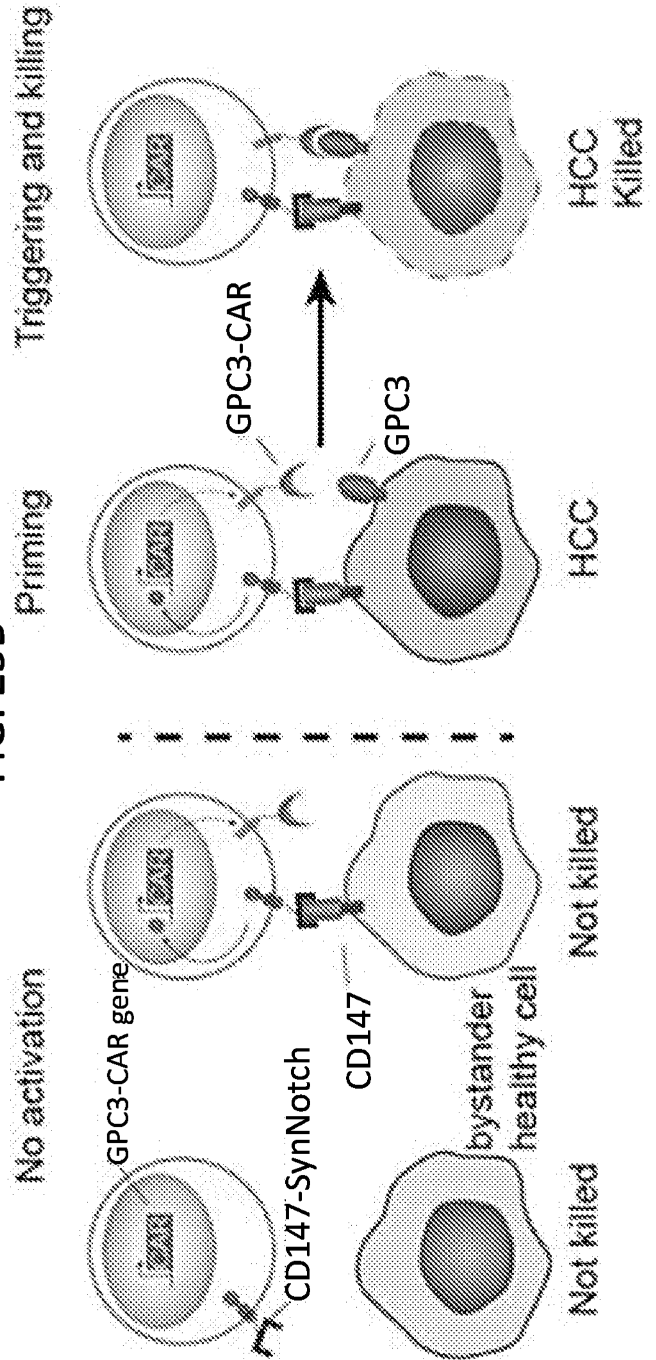


FIG. 25B

FIG. 25C

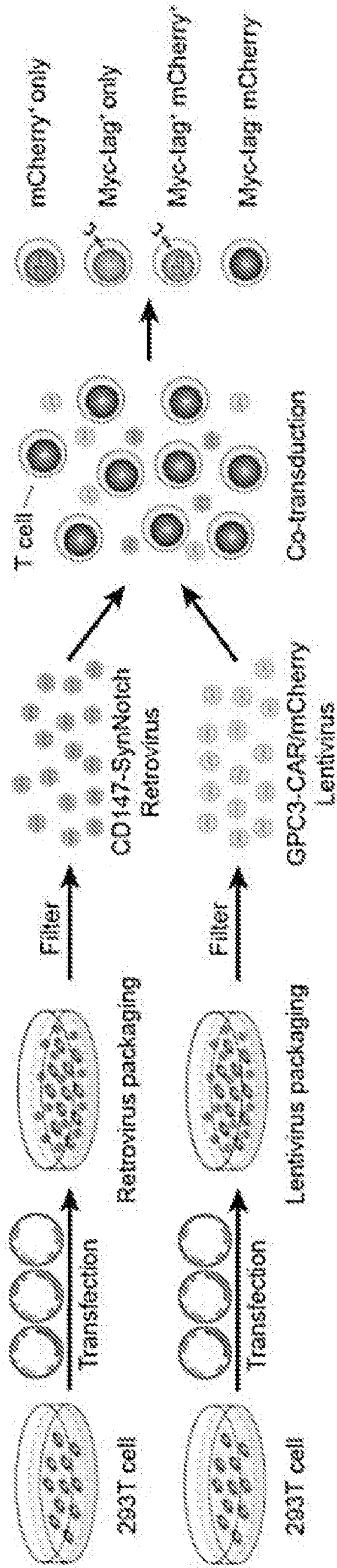
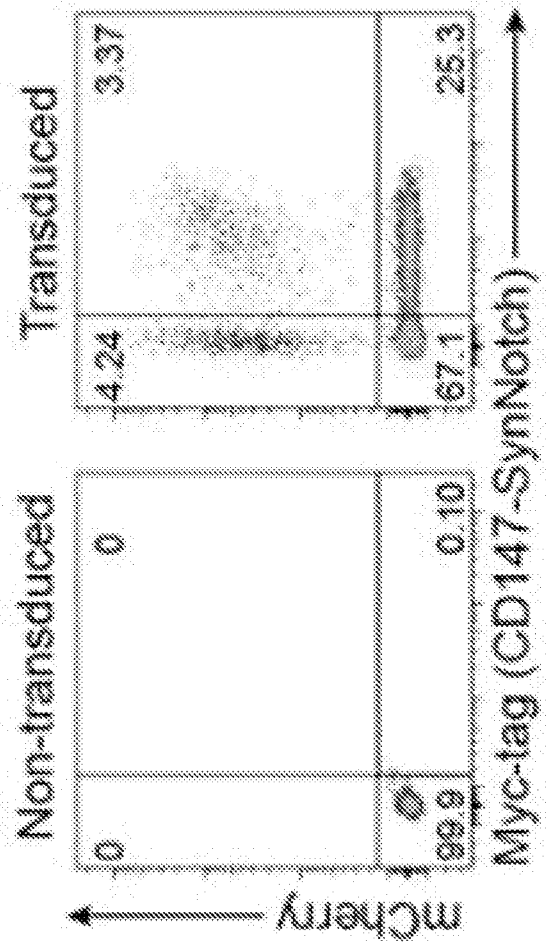


FIG. 25D



SUBSTITUTE SHEET (RULE 26)

FIG. 25E

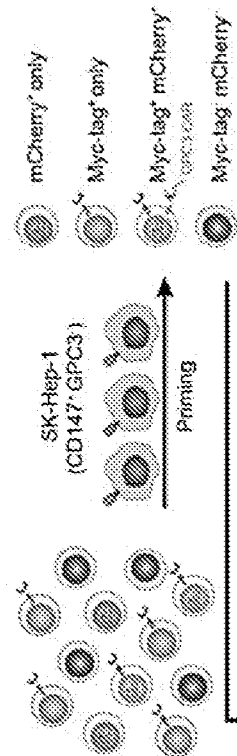


FIG. 25F

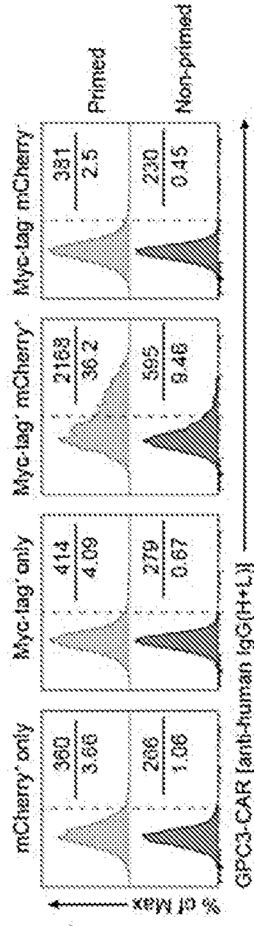


FIG. 25G

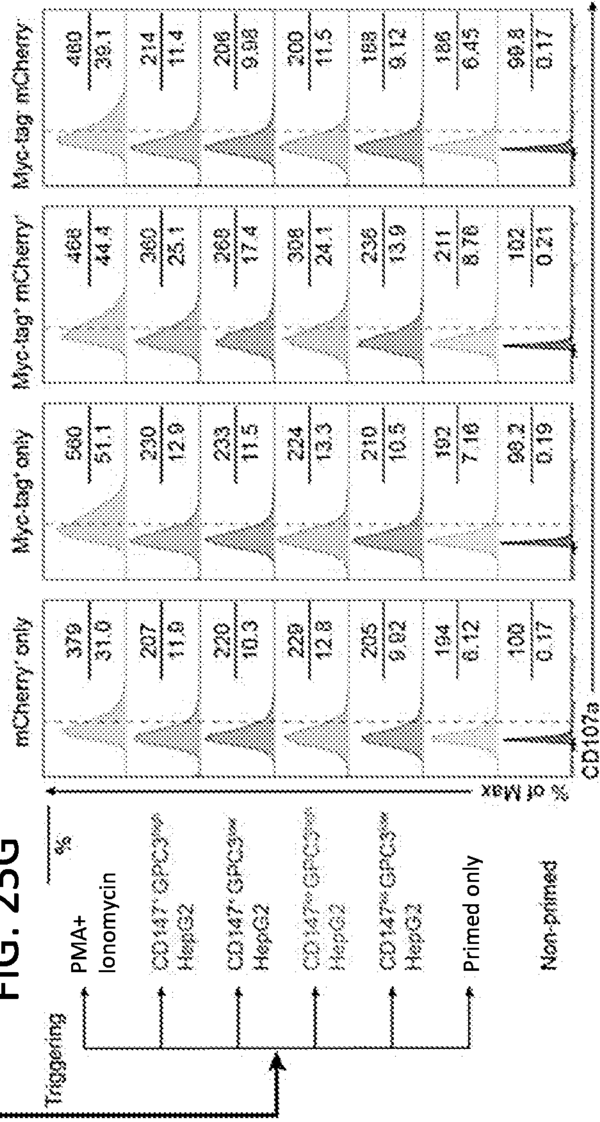
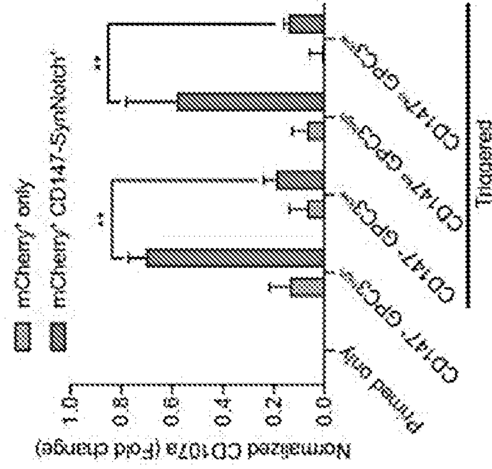


FIG. 25H



SUBSTITUTE SHEET (RULE 26)

FIG. 26A

FIG. 26B

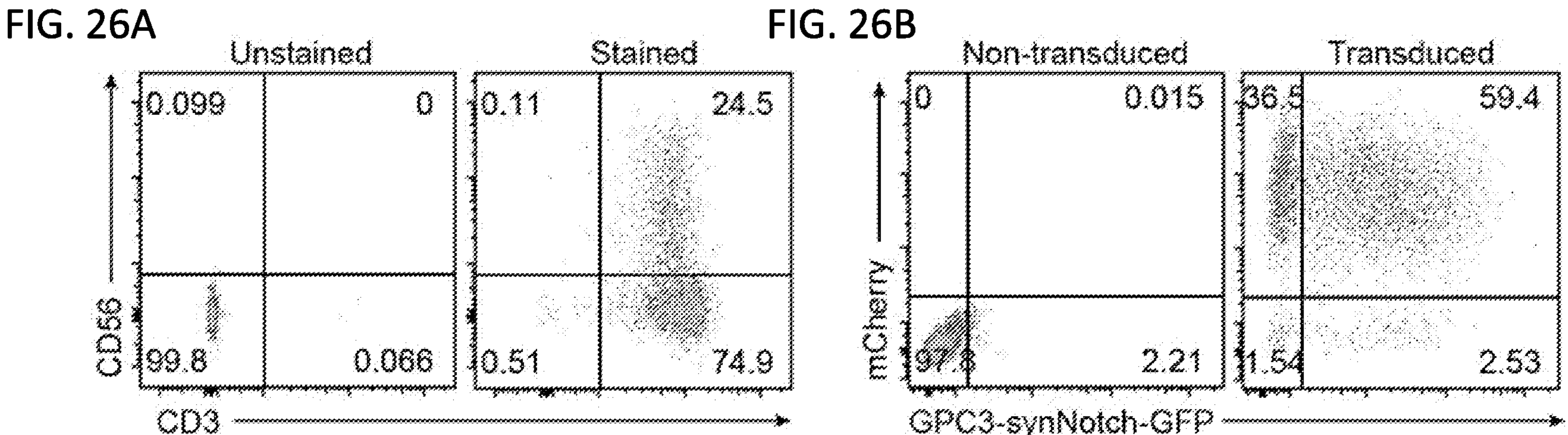
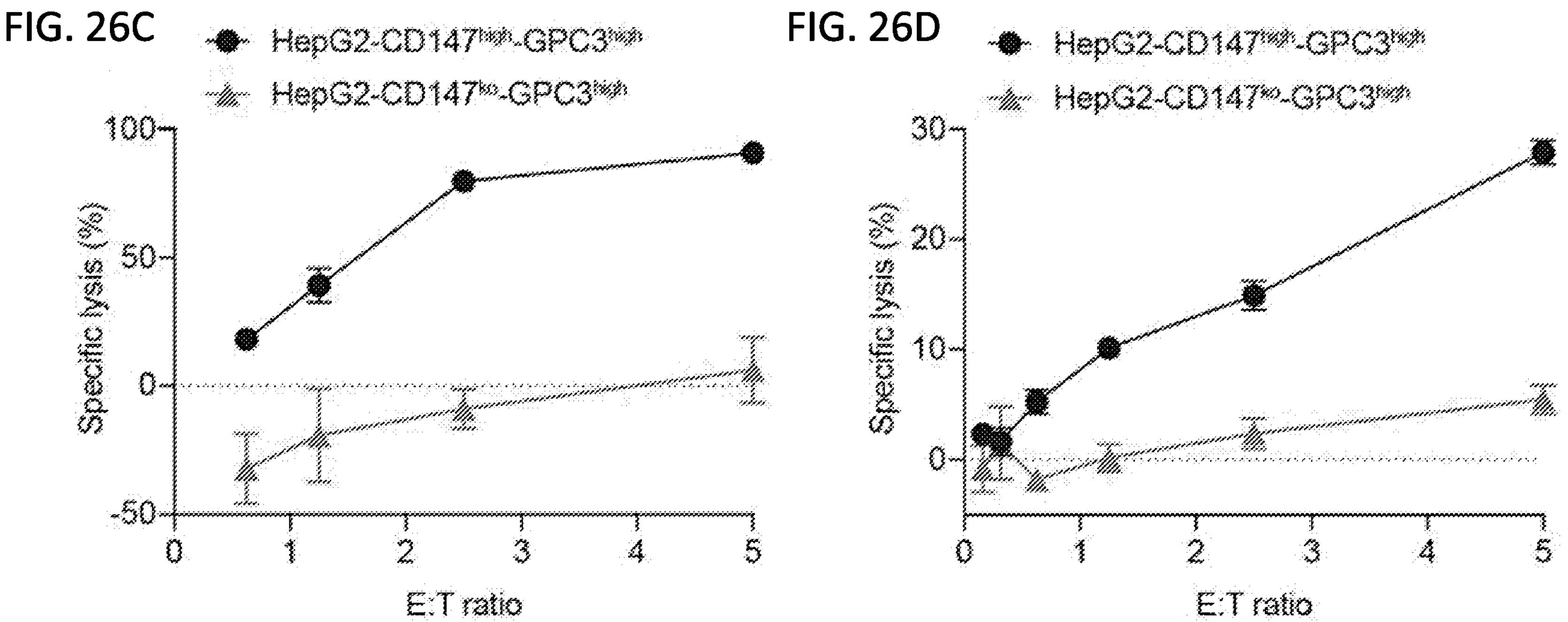


FIG. 26C

FIG. 26D



SUBSTITUTE SHEET (RULE 26)