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(54) Title: METHODS FOR PRODUCTION OF TISSUE RESIDENT MEMORY-LIKE T CELLS AND USE THEREOF

(57) Abstract: Provided herein are methods for the production of tissue resident memory-like T cells by the combination of hypoxia and TGFβ. Further provided herein are methods of using the tissue resident memory T cells as adoptive cell therapy.

DESCRIPTION

METHODS FOR PRODUCTION OF TISSUE RESIDENT MEMORY-LIKE T CELLS AND USE THEREOF

[001] This application claims the benefit of United States Provisional Patent Application Nos. 62/747,523, filed October 18, 2018 and 62/846,270, filed May 10, 2019, the entirety of both of which are incorporated herein by reference.

INCORPORATION OF SEQUENCE LISTING

[002] The sequence listing that is contained in the file named "UTFCP1408WO_ST25.txt", which is 8 KB (as measured in Microsoft Windows®) and was created on October 17, 2019, is filed herewith by electronic submission and is incorporated by reference herein.

BACKGROUND

1. Field

[003] The present invention relates generally to the fields of medicine and immunology. More particularly, it concerns methods for the production of tissue resident memory-like T cells and uses thereof.

2. Description of Related Art

[004] Tissue resident memory cells (T_{RM}) are a recently identified subset of memory T cells that are important in local frontline defense against viral diseases. Recent reports have also suggested that cells with this phenotype play an important role in anti-tumor immunity. Relatively little is known regarding T_{RM} differentiation and endogenous tissue resident memory cells are difficult to isolate, impeding their study in basic research and their application in adoptive cellular therapies. Thus, there is an unmet need for methods to produce tissue resident memory cells.

SUMMARY

[005] In one embodiment, the present disclosure provides an *in vitro* method for producing tissue resident memory-like T cells (T_{RM}-like T cells) comprising: (a) obtaining a starting population of T cells; (b) culturing the starting population of T cells in hypoxic

conditions or in the presence of a hypoxia-inducing agent to generate early effector cells; and (c) further culturing the early effector cells in the presence of transforming growth factor beta 1 (TGF-β1) to produce T_{RM}-like T cells.

[006] In another embodiment, the present disclosure provides an *in vitro* method for producing tissue resident memory-like T cells (T_{RM}-like T cells) comprising: (a) obtaining a starting population of T cells; (b) culturing the starting population of T cells in hypoxic conditions or in the presence of a hypoxia-inducing agent to generate early effector cells; and (c) further culturing the early effector cells in the presence of transforming growth factor beta 1 (TGF-β1), transforming growth factor beta 2 (TGF-β2) transforming growth factor beta 3 (TGF-β3) or transforming growth factor beta 4 (TGF-β4) to produce T_{RM}-like T cells. In some embodiments, culturing comprises activating the starting population of T cells to generate early effector cells.

[007] In yet another embodiment, the present disclosure provides an *in vitro* method for producing T_{RM}-like T cells comprising: (a) obtaining a starting population of T cells; (b) culturing the starting population of T cells in hypoxic conditions or in the presence of a hypoxia-inducing agent; and (c) further culturing the starting population of T cells in the presence of TGF-β1 to produce T_{RM}-like T cells.

[008] In some aspects, the starting population of T cells are $CD8^+$ peripheral blood T cells. In specific aspects, the $CD8^+$ peripheral blood T cells are human $CD8^+$ peripheral blood T cells. In certain aspects, obtaining the human $CD8^+$ peripheral blood T cells comprises selecting for $CD45RA^+CCR7^+CD8^+$ naïve T cells from a peripheral blood sample. In some aspects, the peripheral blood sample is obtained from a healthy subject. In some aspects, the peripheral blood sample is obtained from a subject diagnosed with cancer or suspected of having cancer. In some aspects, the peripheral blood sample is obtained from a subject diagnosed with a viral disease or is suspected of having a viral disease. In certain aspects, the starting population of T cells were generated by stimulation of naïve T cells by antigen presenting cells pulsed with peptide, full length antigen or cell lysate. In particular aspects, the T cells are obtained from a tumor site or are tumor infiltrating lymphocytes. In some aspects, T cells are naïve T cells. For example, the cell lysate is a tumor lysate. In specific aspects, the antigen is a cancer antigen. In some aspects, the peptide is a peptide from a protein that is differentially expressed in or highly expressed by cancer cells. In some aspects, the peptide is a peptide from a neoantigen or from a protein comprising a mutation. In certain aspects, the

starting population of T cells is enriched for T cells specific for an antigen of interest. In certain aspects, the starting population of T cells are purified to enrich for CD8-positive peptide MHC tetramer-positive cells. In some aspects, the starting population of T cells are purified by fluorescence activated cell sorting. In certain aspects, the starting population of T cells are engineered T cells. In some aspects, the engineered T cells are generated by introduction of a cloned T cell receptor (TCR) into a population of host cells. In certain aspects, the population of host cells are peripheral blood mononuclear cells. In some aspects, the cloned TCR is introduced into the population of host cells by non-viral methods, such as an episomal vector or transposon-transposase system. In particular aspects, the cloned TCR is introduced into the population of host cells by transduction. In some aspects, the population of host cells are transduced by a viral vector comprising TCR alpha and TCR beta chains. In certain aspects, the viral vector is a lentiviral vector. In some aspects, the transduced population of host cells are purified to enrich for CD8-positive peptide MHC tetramer-positive cells. In particular aspects, the engineered T cells expressed a chimeric antigen receptor. In specific aspects, the chimeric antigen receptor comprises a cloned TCR. In some aspects, the starting population of T cells are tumor infiltrating lymphocytes obtained from a subject.

[009] In certain aspects, hypoxic conditions are further defined as less than 5% oxygen, such as 4%, 3%, 2%, 1%, or less oxygen. In some aspects, the hypoxia-inducing agent is a hypoxia mimetic. In particular aspects, the hypoxia-inducing agent or hypoxia mimetic is cobalt chloride ($CoCl_2$), deferoxamine mesylate (DFOM), dimethyloxalylglycine (DMOG), or a prolyl hydroxylase inhibitor, such as a 2-OG analog. In some aspects, the prolyl hydroxylase inhibitor is Roxadustat (FG-4592).

[0010] In some aspects, the culturing of step (b) is in the presence of TCR stimulation and co-stimulation. In certain aspects, the TCR stimulation and co-stimulation comprises anti-CD3 and anti-CD28 antibodies, anti-CD3 and anti-CD28 beads, feeder cells, antigen presenting cells, artificial antigen presenting cells, peptide and/or protein antigens, or a combination thereof. In some aspects, the TCR stimulation and co-stimulation comprises anti-CD3 and anti-CD28 beads. In particular aspects, the culturing of step (b) is for 3-5 days, such as for 4 days. In certain aspects, the culturing of step (b) is performed at normoxia, such as 20% oxygen. In certain aspects, the step of culturing of step (b) is performed in the presence of IL-2, such as 25-100 IU/mL, such as 25, 50, or 75 IU/mL. In some aspects, the culturing of step (b) is performed in hypoxic conditions, such as 2% oxygen. In particular aspects, the culturing is in

the presence of IL-15. In some aspects, the IL-15 is present at a concentration of 5-20 ng/mL, such as 7-12 ng/mL, specifically 7, 8, 9, 10, 11, or 12 ng/mL.

[0011] In certain aspects, TGF-β1 is further defined as recombinant human TGF-β1 (rhTGF-β1). In some aspects, the rhTGF-β1 is present at a concentration of 0.1 to 5 ng/mL, such as 1 to 1.5 ng/mL, specifically 1.1, 1.15, 1.2, 1.25, 1.3, 1.35, 1.4, 1.45, or 1.5 ng/mL. In some embodiments, rhTGF-β1 is present at a concentration of about 2, 3, 4, 5, 6, 7, 8, 9, 10 ng/mL. In still other embodiments rhTGF-β1 is present at a concentration of about 15, 20, 25, 30, 35, 40, 45 or 50 ng/mL. In some aspects, the culturing of step (c) is in hypoxic conditions or in the presence of a hypoxia-inducing agent. In particular aspects, the culturing of step (c) is for 1-3 days, such as for 2 days.

[0012] In some aspects, the T_{RM}-like T cells are $CD69^+CD103^+$. In particular aspects, at least 30%, such as 40%, 45%, 50%, 55%, 60% or higher, of the cells produced in step (c) are $CD69^+CD103^+$ cells. In some aspects, the T_{RM}-like T cells express PD-1, CD101, and/or CD49a. In particular aspects, the T_{RM}-like T cell expression PD-1, CD101, and/or CD49a is measured as cell surface expression (*e.g.*, via flow cytometry). In certain aspects, the T_{RM}-like T cells have higher expression of CD69, ITGAE, PDCD1, and/or CD101, as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression of CD69, ITGAE, PDCD1, and/or CD101 is higher expression of CD69, ITGAE, PDCD1, and/or CD101 protein as compared to cells cultured in atmospheric oxygen conditions. In other aspects, higher expression of CD69, ITGAE, PDCD1, and/or CD101 is higher expression of CD69, ITGAE, PDCD1, and/or CD101 mRNA transcripts as compared to cells cultured in atmospheric oxygen conditions.

[0013] In certain aspects, the T_{RM}-like T cells have higher expression of TNFA, GZMB, SLC2A1, and/or VEGF as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression of TNFA, GZMB, SLC2A1, and/or VEGF is higher expression of TNFα, GZMB, GLUT1, and/or VEGF protein as compared to cells cultured in atmospheric oxygen conditions. In other aspects, higher expression of TNFA, GZMB, SLC2A1, and/or VEGF is higher expression of TNFA, GZMB, SLC2A1, and/or VEGF mRNA transcripts as compared to cells cultured in atmospheric oxygen conditions.

[0014] In some aspects, the T_{RM}-like T cells have decreased expression of S1PR1, KLF2, and/or SELL as compared to cells cultured in atmospheric oxygen conditions. In some

aspects, the T_{RM}-like T cells have decreased expression of S1PR1, KLF2, and/or CD62L protein as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have decreased expression of S1PR1, KLF2, and/or SELL mRNA transcripts as compared to cells cultured in atmospheric oxygen conditions.

[0015] In specific aspects, the T_{RM}-like T cells have essentially no expression of CXCR6 protein. In particular aspects, the T_{RM}-like T cells have essentially no detectable cell surface expression of CXCR6 protein.

[0016] In some aspects, the T_{RM}-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3 protein. In certain aspects, higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3 mRNA transcripts.

[0017] In some aspects, the T_{RM}-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 is higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 protein. In certain aspects, higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP,

RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 is higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 mRNA transcripts.

[0018] In some aspects, the T_{RM}-like T cells have higher expression of ITGAE, ITGA1, PDC1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression ITGAE, ITGA1, PDCD1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 is higher expression of ITGAE, ITGA1, PDCD1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 protein. In certain aspects, higher expression of ITGAE, ITGA1, PDCD1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 is higher expression of ITGAE, ITGA1, PDCD1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 mRNA transcripts.

[0019] In some aspects, the T_{RM}-like T cells have higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 is higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 protein. In certain aspects, higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 is higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 mRNA transcripts.

[0020] In some aspects, the T_{RM}-like T cells have lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 as compared to cells cultured in

atmospheric oxygen conditions. In certain aspects, lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3 TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 is lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 protein. In certain aspects, lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 is lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 mRNA transcripts.

[0021] In some aspects, the TRM-like T cells have lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 is lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 protein. In certain aspects, lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 is lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 mRNA transcripts.

[0022] In some aspects, the TRM-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 is higher expression of GNLY,

MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 protein. In certain aspects, higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 is higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 mRNA transcripts.

[0023] In some aspects, the T_{RM}-like T cells have higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions. In certain aspects, higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 is higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 protein. In certain aspects, higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 is higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 mRNA transcripts.

[0024] In additional aspects, the method further comprises producing T_{RM}-like T cells with specificity for an antigen of interest. In some aspects, the T_{RM}-like T cells with specificity for an antigen of interest are engineered to by transducing the T_{RM}-like T cells with a T cell receptor (TCR) specific for the antigen of interest. In other aspects, the T_{RM}-like T cells with specificity for an antigen of interest are produced by using a starting population of T cells with specificity for an antigen of interest. In some aspects, T_{RM}-like T cells are activated by culturing the starting population of T cells with peptide-pulsed antigen presenting cells (APCs), such as artificial APCs (aAPCs), during step (b). In some aspects, the APCs are mature dendritic cells. In specific aspects, steps (b) and (c) are repeated at least once. In some aspects, the T_{RM}-like T cells are cultured in the presence of a histone deacetylase (HDAC) inhibitor during step (b) and/or step (c). In particular aspects, the HDAC inhibitor is selected from the group consisting

of trichostatin A, trapoxin B, phenylbutyrate, valproic acid, vorinostat (suberanilohydroxamic acid or SAHA, marketed as Zolinza®), belinostat (PXD101, marketed as Beleodaq®), panobinostat (marketed as Farydaq®), dacinostat (LAQ824), entinostat (SNDX-275 or MS-275), tacedinaline (CI994), and mocetinostat (MGCD0103).

[0025] In some aspects, the antigen of interest is for targeting or treating lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, gastric or stomach cancer (including gastrointestinal cancer and gastrointestinal stromal cancer), pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, various types of head and neck cancer, or melanoma.

[0026] Further provided herein is a T_{RM}-like T cell with no expression, substantially no expression, or essentially no expression of CXCR6 protein. In some aspects, the no expression of CXCR6 protein is no cell surface expression of CXCR6 protein. In other aspects, the T_{RM}-like T cell expresses CXCR6 mRNA transcript but does not express CXCR6 protein or express CXCR6 protein on the cell surface. In some aspects, the T_{RM}-like T cells are specific for an antigen of interest. In another embodiment, there is provided a pharmaceutical composition comprising a population of T_{RM}-like T cells as provided above. In another embodiment, there is provided a pharmaceutical composition comprising a population of T_{RM}-like T cells with essentially no expression of CXCR6 protein and a pharmaceutically acceptable carrier. In some aspects, the T_{RM}-like T cells are produced by the methods of the present embodiments. In some aspects, the T_{RM}-like T cell(s) express PD-1, CD101, and/or CD49a. In particular aspects, at least 40%, at least 45%, at least 50%, at least 55%, at least 60% or more, of the cells are $CD69^{+}CD103^{+}$ cells. In certain aspects, T_{RM}-like T cell(s) are $CD69^{+}CD103^{+}$ cells. In some aspects, the T_{RM}-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP,

RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have higher expression of ITGAE, ITGA1, PDC1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have lower expression of KLF2, KLF3, SELL, FAM65B, and/or SERPINE2 as compared to cells cultured in atmospheric oxygen conditions. In some aspects, the T_{RM}-like T cells have lower expression of DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, ITGAM, FOS, KLF3, SLAMF7, TNFSF8, SLC6A8, KLF2, SOCS3, and/or PTGER2 as compared to cells cultured in atmospheric oxygen conditions.

[0027] In another embodiment, there is provided a composition comprising an effective amount of T_{RM}-like T cells with essentially no expression of CXCR6 protein, such as T_{RM}-like T cells produced by the methods of the present embodiments, for the treatment an immune-related disorder in a subject. In particular aspects, the T_{RM}-like T cells have specificity for an antigen of interest.

[0028] Further provided herein is the use of an effective amount of T_{RM}-like T cells with essentially no expression of CXCR6 protein, such as T_{RM}-like T cells produced by the methods of the present embodiments, for the treatment of an immune-related disorder in a subject. In particular aspects, the T_{RM}-like T cells have specificity for an antigen of interest.

[0029] In some aspects, the antigen of interest is for targeting or treating lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and

squamous carcinoma of the lung), cancer of the peritoneum, gastric or stomach cancer (including gastrointestinal cancer and gastrointestinal stromal cancer), pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, various types of head and neck cancer, or melanoma.

[0030] In a further embodiment, there is provided a method of treating an immune-related disorder in a subject comprising administering an effective amount of T_{RM}-like T cells with essentially no expression of CXCR6 protein, such as T_{RM}-like T cells produced by the methods of the present embodiments, to the subject. In some aspects, the subject is human.

[0031] In some aspects, the immune-related disorder is a cancer, autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition. In certain aspects, the subject has received a tissue or organ transplant.

[0032] In additional aspects, the method further comprises administering at least one therapeutic agent. In some aspects, the at least one second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy. In some aspects, the T_{RM}-like T cells and/or the at least one second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion. In certain aspects, the T_{RM}-like T cells are administered prior to the second therapeutic agent. In some aspects, the T_{RM}-like T cells are administered after the second therapeutic agent. In particular aspects, the T_{RM}-like T cells are administered concurrently with the second therapeutic agent. In specific aspects, the immunotherapy is a 4-1BB agonist. In particular aspects, the 4-1BB agonist is a 4-1BB antibody. In other aspects, the second therapeutic agent is an immune checkpoint inhibitor. In particular aspect, the immune checkpoint inhibitor is anti-CTLA-4, anti-PD1 or anti-PD-L1 inhibitor.

[0033] In another embodiment, there is provided a method of treating a viral infection in a subject comprising administering an effective amount of T_{RM}-like T cells with essentially no CXCR6 expression, such as T_{RM}-like T cells produced by the present methods, such as T_{RM}-like T cells with specificity for one or more viral antigens.

[0034] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The present disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0036] FIG. 1: Schematic depicting method for producing tissue resident memory cells.

[0037] FIGS. 2A-2E: Human $CD8^+$ T-cells exposed to hypoxia and TGF-β1 have a T_{RM}-like transcriptional profile. Naïve $CD8^+$ T-cells isolated from peripheral blood were activated in 20% or 2% O_2 (hypoxia) for 4 days and then for an additional 2 days with the addition of rhTGF-β1. Expression levels of genes associated with T_{RM} were analyzed via quantitative real-time PCR. (**A-E**) Fold change of gene transcript levels in hypoxia (2% O_2) + TGF-β1 over AtmosO_2 (~20% O_2) + TGF-β1. Canonical hypoxia responsive genes are shown in (**E**) as a control to indicate activation of cellular hypoxia response. CX3CR1 expression was not detectable. $n = 6$, 3 independent experiments; Paired t-test with Benjamini, Krieger and Yekutieli correction for multiple comparisons; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; $FDR < 0.05$, data are mean +/- SEM.

[0038] FIGS. 3A-3C: Hypoxia in combination with TGF-β1 induces a $CD69^+CD103^+$ population that expresses human T_{RM}-associated markers. Naïve $CD8^+$ T-cells isolated from peripheral blood of healthy human donors were activated in atmospheric oxygen (approximately 20%) or hypoxia (2% oxygen) for 4 days and then for an additional 2 days with the addition of rhTGF-β1. (**A**) The frequency of the $CD69^+CD103^+$ T_{RM}-like population and (**B** and **C**) expression of T_{RM}-associated markers was then assessed by flow cytometry. Representative results from 1 donor are shown in (**B**), gray histograms represent fluorescence

minus one (FMO) control. n = 7, 3 independent experiments; ratio paired t-test (**A**) or ANOVA (**C**); $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

[0039] FIGS. 4A-4B: Hypoxia and TGF-β1 synergize to induce $CD69^+CD103^+$ cells. Naïve $CD8^+$ T cells were activated as described in FIG. 2 with or without the addition of rhTGF-β1. (**A**) Frequency of the $CD69^+CD103^+$ population and (**B**) expression of T_{RM}-associated markers was assessed by flow cytometry, representative results shown for (**A**) 1 donor. $n = 4$, two-way ANOVA (**A**) or one-way ANOVA (**B**), $*P < 0.05$, $**P < 0.01$

[0040] FIGS. 5A-5F: Hypoxia and TGF-β1 induced T_{RM}-phenotype cells show transcriptional differences similar to those reported for endogenous T_{RM}. $CD69^-CD103^-$, $CD69^+CD103^-$, and $CD69^+CD103^+$ $CD8^+$ T-cells were generated as described earlier and sorted before isolation of RNA for transcriptome analysis via RNA-sequencing ($n = 3$). (**A**) Principal-component analysis (PCA) of paired $CD69^-CD103^-$, $CD69^+CD103^-$, and $CD69^+CD103^+$ $CD8^+$ T-cells based on the global transcriptome. (**B**) Unsupervised clustering of the top 150 differentially expressed genes for $CD69^-CD103^-$ (left), $CD69^+CD103^-$ (middle), and $CD69^+CD103^+$ (right) cells generated in 20% O_2, 2% O_2, and 2% O_2 + TGF-β1, respectively. Differential expression determined by $|\log2FC| \geq 1$ and FDR < 0.05. (**C**) Expression levels of selected differentially expressed T_{RM}-associated genes. GSEA of gene signatures derived from endogenous T_{RM} and TIL_{RM} in the transcriptome of (**D**) $CD69^+CD103^+$ vs. $CD69^-CD103^-$ and (**E**) $CD69^+CD103^+$ vs. $CD69^+CD103^-$ cells, presented as normalized enrichment score (NES). (**F**) Top 34 differentially expressed genes from TIL_{RM} GSEA shown in (**E**).

[0041] FIGS. 6A-6B: Pathways involved in metabolism, migration, and T_{RM} generation and maintenance are differentially regulated in hypoxia and TGF-β1 induced T_{RM}. (**A**) Top 30 canonical pathways from the Ingenuity Pathway Analysis (IPA) database that are enriched in $CD69^+CD103^+$ *in vitro* induced T_{RM}, shown as the frequency of differentially expressed genes encoding components of each pathway that are upregulated or downregulated in $CD69^+CD103^+$ cells relative to their expression in $CD69^-CD103^-$ cells, and negative-log-transformed *P* values (right vertical axis; Fisher's exact test); numbers above bars represent total genes in each pathway, bars are presented in the order of significance. (**B**) Differentially regulated IPA canonical pathways in hypoxia + TGF-β1 *in vitro* induced T_{RM} and endogenous human T_{RM}.

[0042] FIGS. 7A-7D: Differentiation of human $CD8^+$ T-cells in hypoxia and TGF-β1 results in induction of a $CD69^+CD103^+$ population. (**A**) Gating strategy used in flow cytometry analysis. (**B**) Cell viability determined by fixable viability dye (Invitrogen) in flow cytometry

analysis. (**C** and **D**) Changes in population frequencies comparing 20% O_2 + TGF-β1 and 2% O_2 + TGF-β1 conditions determined by flow cytometry. $n = 7$, 3 independent experiments; paired t-test (**B**), ratio paired t-test (**C**), or ANOVA (**D**); $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

[0043] FIGS. 8A-8F: Human $CD8^+$ T-cells differentiated in 10% O_2 and TGF-β1 do not have a T_{RM}-like transcriptional profile. Naïve $CD8^+$ T-cells isolated from peripheral blood were activated in 20% O_2 ($AtmosO_2$) or 10% O_2 ($circulationO_2$) for 4 days and then for an additional 2 days with the addition of rhTGF-β1. Expression levels of T_{RM}-associated genes were analyzed via quantitative real-time PCR. (**A-E**) Fold change of gene transcript levels in 10% O_2 + TGF-β1 over 20% O_2 + TGF-β1. (**F**) The frequency of the $CD69^+CD103^+$ T_{RM}- like population was then assessed by flow cytometry. Representative results from 1 donor are shown in (**F**). $n = 3$, 2 independent experiments; (**A-E**) Paired t-test with Benjamini, Krieger and Yekutieli correction for multiple comparisons; FDR < 0.05, data are mean +/- SEM; (**F**) unpaired t-test, $^*P < 0.05$.

[0044] FIGS. 9A-9B: Hypoxia and TGF-β1 induced T_{RM}-phenotype cells show transcriptional differences similar to those reported for endogenous T_{RM}. $CD69^-CD103^-$, $CD69^+CD103^-$, and $CD69^+CD103^+$ T-cells were generated as described in FIG. 4 and sorted before isolation of RNA for transcriptome analysis via RNA-sequencing ($n = 3$). (**A**) Heatmap showing expression of selected genes commonly reported in transcriptome analyses of endogenous human T_{RM}. (**B**) Heatmap comparing transcriptional differences (log2FC) in $CD8^+CD69^+$ versus $CD8^+CD69^-$ T cells from human lung (see Kumar *et al.*, 2017, incorporated herein by reference) and $CD69^+CD103^+$ hypoxia and TGF-β1 *in-vitro* induced T_{RM} (i-T_{RM}) versus $CD69^-CD103^-$ cells from normal cell culture conditions (20% O_2 without TGF-β1). Differential expression determined by $log2FC \geq |1|$ and FDR < 0.05.

[0045] FIGS. 10A-10C: (**A**) Naïve $CD8^+$ T-cells isolated from peripheral blood were activated in 20% O_2 ($AtmosO_2$) in the presence of the HIF prolyl hydroxylase inhibitor FG-4592 (Roxadustat) for 4 days and then for an additional 2 days with rhTGF-β1. Cells activated in 2% O_2 (hypoxia) with addition of rhTGF-β1 on day 4 are shown for comparison. Frequency of the $CD69^+CD103^+$ population was assessed by flow cytometry, representative results shown in FACS plots for 1 donor. $n = 3$ (**B**) Stimulation with MART-1 peptide-pulsed autologous dendritic cells. Naïve $CD8^+$ T-cells were stimulated with autologous monocyte-derived dendritic cells pulsed with MART-1 (M27) peptide for 7 days in 20% O_2 ($AtmosO_2$) or 2% O_2

with the addition of rhTGF-β1 on day 4 to generate $CD69^+CD103^+$ antigen-specific ($Tetramer^+$) T-cells detected by flow cytometry. Representative results shown in FACS plots for 1 donor. $n = 4$, unpaired t-test, $^{**}P < 0.01$. Data are mean +/- SEM. (C) Modified rapid expansion protocol induces T_{RM} phenotype in antigen-specific T cells. Hypoxia and rhTGF-β1 were used in a modified rapid expansion protocol to induce TRM phenotype in antigen-specific T cells. Antigen-specific T-cells were generated via stimulation with autologous MART-1 peptide-pulsed dendritic cells (ETC) or transduction of gp100-specific TCR (TCRT), labeled with fluorochrome-conjugated tetramer, and sorted. The sorted antigen-specific T cells were then stimulated with anti-CD3 (OKT3) and irradiated feeder cells in 20% O_2 and supplemented with IL-2 (conventional REP) or 2% O_2 supplemented with IL-15 and the addition of rhTGF-β1 from day 4 onwards. Bar graph shows results for MART-1 ETC, data are mean +/- SEM, $n = 3$, unpaired t-test, $^{**}P < 0.01$

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0046] Tissue resident memory cells (T_{RM}) are non-recirculating memory T cells that reside in tissues, lack the molecules enabling egress from tissues and migration to lymph nodes, and act as frontline responders (Mami-Chouaib and Tartour, 2019). Relatively little is known about T_{RM} differentiation. Effector T cells that enter tissue can become T_{RM} by up- or downregulating genes allowing tissue retention. In the present studies, it was found that hypoxia and TGF-β1 can induce a T_{RM}-like phenotype in human peripheral blood $CD8^+$ T-cells. The present studies showed that when human peripheral blood T cells, such as $CD8^+$ T cells or $CD4^+$ T cells, are differentiated in hypoxia and TGF-β1 *in vitro* they develop a T_{RM} phenotype and express protein markers and genes commonly associated with tissue resident memory cells (Table 1). These findings identify a previously unreported cue for T_{RM} differentiation and enable a facile means of generating T_{RM}-phenotype cells for basic studies and translational applications such as adoptive cellular therapies.

[0047] Accordingly, certain embodiments of the present disclosure provide methods for the production of T_{RM}-phenotype cells. The terms "T_{RM}-phenotype cells" and "T_{RM}-like cells" are used interchangeably herein to refer to the cells provided by the present methods. The method can comprise culturing peripheral blood T cells in hypoxic conditions or in the presence of agents which induce or mimic hypoxia, exemplary hypoxia mimetics include but are not limited to cobalt chloride ($CoCl_2$), deferoxamine mesylate (DFOM), dimethyloxalylglycine (DMOG), or a prolyl hydroxylase inhibitor, such as Roxadustat. During

this period, the cells can be polyclonally activated, such as by anti-CD3 and anti-CD28 beads, to produce early effector cells. The term "early effector cell" refers to cells that are within one week of activation from the naïve state. The activation may comprise culturing in the presence of TCR stimulation and co-stimulation, including but not limited to anti-CD3/anti-CD28 antibodies, anti-CD3/anti-CD28 beads, feeder cells, antigen presenting cells, artificial antigen presenting cells, peptide and/or protein antigens, or combinations of these. After the activation to produce early effector cells, the cells are further cultured in the presence of TGF-β1 to produce the T_{RM}-phenotype cells. Thus, hypoxia and TGF-β1 can be used to induce a $CD8^+CD69^+CD103^+$ cell population that expresses human T_{RM}-associated markers. Human $CD8^+$ T-cells differentiated in hypoxia and TGF-β1 have a T_{RM}-like transcriptional profile.

[0048] The T_{RM}-like cells may be rendered antigen-specific. One method may comprise polyclonal activation of naïve T cells under the conditions described herein to generate T_{RM}-like cells followed by transduction to express an antigen-specific TCR. In a modified version of ETC stimulation method, the naïve T cells may be activated via peptide-pulsed antigen presenting cells (or artificial antigen presenting cells) in hypoxia followed by further culture in the presence of rh TGF-β1. This activation may be performed for 2 rounds to generate the antigen-specific cells. In another method, the antigen-specific T_{RM}-like cells may be produced by combination of hypoxia and TGF-β1 with epigenetic modifying agents such as HDAC inhibitors to differentiate already expanded antigen-specific cells to the T_{RM} phenotype.

[0049] The present T cells, such as the starting population of T cells, may be engineered T cells. In certain embodiments, the engineered T cells comprise T cells expressing a chimeric antigen receptor (CAR T cells). In certain embodiments, the engineered T cells comprise T cells expressing a recombinant T cell receptor capable of binding tumor-specific epitopes or neoepitopes. In some embodiments, the engineered T cells are constructed using any of the many well-established gene transfer methods known to those skilled in the art. In certain embodiments, the engineered cells are constructed using viral vector-based gene transfer methods to introduce nucleic acids encoding a chimeric antigen receptor specific for a desired target tumor antigen or encoding a recombinant TCR specific for a desired tumor-specific epitope or neoepitope. In certain embodiments, the engineered cells are constructed using non-viral vector-based gene transfer methods to introduce nucleic acids encoding a chimeric antigen receptor specific for a desired target tumor antigen or encoding a recombinant TCR specific for a desired tumor-specific epitope or neoepitope. In certain embodiments, the viral vector-

based gene transfer method comprises a lentiviral vector. In certain embodiments, the viral vector-based gene transfer method comprises a retroviral vector. In certain embodiments, the viral vector-based gene transfer method comprises an adenoviral or an adeno-associated viral vector. The non-viral vector-based gene transfer method may comprise an episomal vector or a transposon-transposase system. For example, the transposon-transposase system could be the well-known Sleeping Beauty, the Frog Prince transposon-transposase system, or the TTAA-specific transposon PiggyBac system. In certain embodiments, the non-viral vector-based gene transfer method comprises a gene-editing method selected from the group consisting of a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TALENs), and a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) nuclease. In certain embodiments, the non-viral vector-based gene editing method comprises a transfection or transformation method selected from the group consisting of lipofection, nucleofection, biolistics, virosomes, liposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA.

[0050] In certain embodiments, the CAR T cell expresses a CAR construct comprising an extracellular antigen-binding domain, an optional spacer sequence, a transmembrane domain, one or more intracellular signaling domains, and one or more optional regulatory sequences for activating or inactivating the CAR T cell.

[0051] In certain embodiments, the extracellular antigen-binding domain comprises a moiety capable of specifically binding a desired target. In certain embodiments, the moiety capable of specifically binding a desired target comprises a monoclonal antibody or antigen-binding fragment thereof. In certain embodiments, the antigen-binding fragment thereof comprises a single-chain variable fragment (scFv) of a monoclonal antibody capable of specifically binding a desired target. In certain embodiments, the desired target is a tumor-specific antigen. In certain embodiments, the tumor-specific antigen is selected from the group consisting of CD19, CD20, CD22, carcinoembryonic antigen, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, melanoma-associated antigen (MAGE) (*e.g.*, MAGE-1, MAGE-11, or MAGE-A), mutated p53, mutated ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD123, CD23, CD30, CD56, c-Met, mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, EGFRvIII, VEGFR2, and human papilloma virus (HPV). In certain embodiments, the desired target is a tumor-specific neoepitope. In certain embodiments, the tumor-specific neoepitope is identified by *in silico* analysis. In certain

embodiments, the tumor-specific neoepitope is identified and purified from a population of autologous TILs derived from a human subject.

[0052] In certain embodiments, the transmembrane domain comprises any synthetic or natural amino acid sequence capable of forming a structure able to span a cell membrane. In certain embodiments, the structure able to span a cell membrane comprises an alpha helix. In certain embodiments, the transmembrane region is derived from a naturally occurring transmembrane protein selected from the group consisting of CD3ζ, CD3ε, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, 4-1BB/CD137, CD154, inducible T cell costimulator (ICOS)/CD278, glucocorticoid-induced TNFR-related protein (GITR)/CD357, NKG2D, TCRα and TCRβ. In certain embodiments, the transmembrane region derived from a naturally occurring transmembrane protein comprises one or more amino acid substitutions in sequences known to be involved in interactions with other signaling proteins.

[0053] In certain embodiments, the one or more intracellular signaling domains comprise one or more intracellular tyrosine-based activation motifs ("ITAMs"). In certain embodiments, the one or more ITAMs are present on a CD3-zeta (CD3ζ) molecule. In certain embodiments, the one or more intracellular signaling domains further comprise a costimulatory signaling domain selected from the group consisting of CD28, 4-1BB/CD137, ICOS, OX40, CD2, CD40L, CD27, Light-R, GITR, or combinations thereof.

[0054] In certain embodiments, the T cells comprise a recombinant T cell receptor capable of binding tumor-specific epitopes or neoepitopes. In certain embodiments, the recombinant T cell receptor comprises a naturally occurring TCR cloned from a T cell isolated from a subject. In certain embodiments, the recombinant TCR comprises a heterodimer comprising a TCR alpha (TCRα) polypeptide and a TCR beta (TCRβ) polypeptide (*i.e.*, a TCRαβ). In certain embodiments, the recombinant TCR comprises a heterodimer comprising a TCR gamma (TCRγ) polypeptide and a TCR delta (TCRδ) polypeptide (*i.e.*, a TCRγδ).

[0055] In certain embodiments, the recombinant TCRαβ comprises a cloned TCRαβ isolated from a subject and specific for a peptide antigen derived from a desired target. In certain embodiments, the subject is a mammal. In certain embodiments, the mammal is a human. In certain embodiments, the desired target is a tumor-specific antigen selected from the group consisting of CD19, CD20, CD22, carcinoembryonic antigen, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated

ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD123, CD23, CD30, CD56, c-Met, mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, EGFRvIII, and VEGFR2. In certain embodiments, the recombinant TCRγδ comprises a cloned TCRγδ isolated from a subject and specific for a peptide antigen derived from a desired target. In certain embodiments, the subject is a mammal. In certain embodiments, the mammal is a human. In certain embodiments, the desired target is a tumor-specific antigen selected from the group consisting of CD19, CD20, CD22, carcinoembryonic antigen, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD123, CD23, CD30, CD56, c-Met, mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, EGFRvIII, and VEGFR2.

[0056] Further provided herein are methods for the use of the T_{RM}-like cells provided herein for adoptive cellular therapies, such as for treating cancer or viral disease. The cells may be used for immunosuppression, such as for subjects with graft versus host disease (GVHD), tissue or organ rejection, or an autoimmune condition.

[0057] Table 1. Tissue resident memory cell-associated genes assessed in transcriptional analysis.

	Gene	**Expression in T_{RM}**	**Significance/ Proposed Function**	**References**
Human T_{RM} Core Signature	CD69	upregulated	Constitutively expressed by T_{RM}; may promote tissue retention via S1PR1 antagonism	[3, 4, 5]
	ITGAE (CD103)	upregulated	Constitutively expressed by T_{RM}; induced by TGF-β; may promote tissue retention via interaction with e-cadherin	[3, 4, 5]
	ITGA1 (CD49a)	upregulated	May promote tissue retention via interaction with collagen IV	[3, 4, 5]

	PDCD1 (PD-1)	upregulated	High expression could dampen response to prevent tissue damage	[3, 4, 5]
	CD101	upregulated	High expression could dampen response to prevent tissue damage	[3]
	CXCR6	upregulated	Unclear	[3, 4, 5]
	CX3CR1	downregulated	Unclear	[3, 4, 5]
	CTLA4	upregulated	High expression could dampen response to prevent tissue damage	[4]
Tissue Retention	S1PR1	downregulated	Downregulation inhibits recirculation	[3, 4, 6]
	KLF2	downregulated	Downregulation suppressed S1PR1 and SELL	[3, 6]
	SELL (CD62L)	downregulated	Downregulation inhibits recirculation	[3]
	CCR7	downregulated	Downregulation inhibits recirculation	[7]
Transcription Factors	EOMES	downregulated	Downregulation required for TGF-β responsiveness	[4, 7, 8]
	TBX21 (T-bet)	downregulated	Downregulation required for TGF-β responsiveness	[7, 8]
	TCF7 (TCF1)	downregulated	Downregulation may prevent development of circulating memory T-cells	[7, 9]
	IRF4	upregulated	Undefined	[3]
	RUNX3	upregulated?	Key regulator of TRM differentiation	[10]
Effector Molecules	IFNG	upregulated	Constitutive expression could enable more rapid effector response	[3, 5]
	TNFA	upregulated		[5]
	GZMB	upregulated		[5]

I. Definitions

[0058] As used herein, "essentially free," in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0059] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one.

[0060] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more. The terms "about", "substantially" and "approximately" mean, in general, the stated value plus or minus 5%.

[0061] An "autoimmune disease" refers to a disease in which the immune system produces an immune response (for example, a B-cell or a T-cell response) against an antigen that is part of the normal host (that is, an autoantigen), with consequent injury to tissues. An autoantigen may be derived from a host cell, or may be derived from a commensal organism such as the micro-organisms (known as commensal organisms) that normally colonize mucosal surfaces.

[0062] The term "Graft-Versus-Host Disease (GVHD)" refers to a common and serious complication of bone marrow or other tissue transplantation wherein there is a reaction of donated immunologically competent lymphocytes against a transplant recipient's own tissue. GVHD is a possible complication of any transplant that uses or contains stem cells from either a related or an unrelated donor. In some embodiments, the GVHD is chronic GVHD (cGVHD).

[0063] As used herein, the terms "chimeric antigen receptor", "CAR", "chimeric T cell receptor", "artificial T cell receptor" or "chimeric immunoreceptor" refer to an engineered chimeric receptor construct grafting a desired non-MHC-restricted antigen-binding specificity onto an immune effector cell, *e.g.*, an effector T cell. CARs may comprise, for example, an

extracellular antigen-binding domain (*e.g.*, an antibody or an antibody fragment such as, for example, a single-chain variable fragment (scFv) having the desired antigen specificity), a spacer sequence, a transmembrane domain, and one or more intracellular signaling domains. Exemplary intracellular signaling domains may comprise one or more intracellular tyrosine-based activation motifs ("ITAMs"), such as CD3-zeta (CD3ζ), and/or one or more costimulatory signaling domains, such as, for example, CD28, 4-1BB/CD137, ICOS, OX40, or combinations thereof.

[0064] As used herein, the terms "treat", "treatment", "treating", and the like refer to the process of ameliorating, lessening, or otherwise mitigating the symptoms of a disease or condition in a subject by, for example, administering a therapeutic agent to the subject, or by performing a surgical, clinical, or other medical procedure on the subject.

[0065] As used herein, the terms "subject" or "patient" are used interchangeably herein to refer to an individual, *e.g.*, a human or a non-human organism, such as a primate, a mammal, or a vertebrate.

[0066] The term "therapeutic benefit" or "therapeutically effective" as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the size of a tumor, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

[0067] As generally used herein "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0068] "Pharmaceutically acceptable salts" means salts of compounds disclosed herein which are pharmaceutically acceptable, as defined above, and which possess the desired pharmacological activity. Such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or with organic acids such as 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid,

2-naphthalenesulfonic acid, 3-phenylpropionic acid, 4,4′-methylenebis(3-hydroxy-2-ene-1-carboxylic acid), 4-methylbicyclo[2.2.2]oct-2-ene-1carboxylic acid, acetic acid, aliphatic mono- and dicarboxylic acids, aliphatic sulfuric acids, aromatic sulfuric acids, benzenesulfonic acid, benzoic acid, camphorsulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclopentanepropionic acid, ethanesulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, heptanoic acid, hexanoic acid, hydroxynaphthoic acid, lactic acid, laurylsulfuric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, muconic- acid, *o*-(4-hydroxybenzoyl)benzoic acid, oxalic acid, *p*-chlorobenzenesulfonic- acid, phenyl-substituted alkanoic acids, propionic acid, *p*-toluenesulfonic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, tartaric acid, tertiarybutylacetic acid, trimethylacetic- acid, and the like. Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, *N*-methylglucamine- and the like. It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (P. H. Stahl & C. G. Wermuth eds., Verlag Helvetica Chimica Acta, 2002).

[0069] A "pharmaceutically acceptable carrier," "drug carrier," or simply "carrier" is a pharmaceutically acceptable substance formulated along with the active ingredient medication that is involved in carrying, delivering and/or transporting a chemical agent. Drug carriers may be used to improve the delivery and the effectiveness of drugs, including for example, controlled-release technology to modulate drug bioavailability, decrease drug metabolism, and/or reduce drug toxicity. Some drug carriers may increase the effectiveness of drug delivery to the specific target sites. Examples of carriers include: liposomes, microspheres (*e.g.*, made of poly(lactic-co-glycolic) acid), albumin microspheres, synthetic polymers, nanofibers, protein-DNA complexes, protein conjugates, erythrocytes, virosomes, and dendrimers.

[0070] The term "culturing" refers to the *in vitro* maintenance, differentiation, and/or propagation of cells in suitable media. By "enriched" is meant a composition comprising cells

present in a greater percentage of total cells than is found in the tissues where they are present in an organism

[0071] An "isolated" biological component (such as a portion of hematological material, such as blood components) refers to a component that has been substantially separated or purified away from other biological components of the organism in which the component naturally occurs. An isolated cell is one which has been substantially separated or purified away from other biological components of the organism in which the cell naturally occurs.

II. Methods of Use

[0072] In some embodiments, the present disclosure provides methods for adoptive cell therapy comprising administering an effective amount of the T_{RM} cells of the present disclosure. In certain embodiments of the present disclosure, cancer or viral disease is treated by adoptive transfer of a T_{RM} cell population that elicits an immune response. In some embodiments, T_{RM} cell population itself will mediate an immune response. Once activate *in vivo* the T_{RM} cells may produce various pro-inflammatory factors, such as chemokines and cytokines, that would elicit an immune response. the Provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount a T_{RM} cell population. The present methods may be applied for the treatment of immune disorders, solid cancers, hematologic cancers, and viral infections. For example, a viral infection for treatment according to the embodiments may be an HIV, HBV or Herpes virus infection.

[0073] Tumors for which the present treatment methods are useful include any malignant cell type, such as those found in a solid tumor or a hematological tumor. Exemplary solid tumors can include, but are not limited to, a tumor of an organ selected from the group consisting of pancreas, colon, cecum, stomach, brain, head, neck, ovary, kidney, larynx, sarcoma, lung, bladder, melanoma, prostate, and breast. Exemplary hematological tumors include tumors of the bone marrow, T or B cell malignancies, leukemias, lymphomas, blastomas, myelomas, and the like. Further examples of cancers that may be treated using the methods provided herein include, but are not limited to, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, gastric or stomach cancer (including gastrointestinal cancer and gastrointestinal stromal cancer), pancreatic cancer, cervical cancer, ovarian cancer, liver

cancer, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, various types of head and neck cancer, and melanoma.

[0074] The cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; lentigo malignant melanoma; acral lentiginous melanomas; nodular melanomas; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant;

dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; hodgkin's disease; hodgkin's; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-hodgkin's lymphomas; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; hairy cell leukemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML); and chronic myeloblastic leukemia.

[0075] Particular embodiments concern methods of treatment of leukemia. Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation (production by multiplication) of blood cells, usually white blood cells (leukocytes). It is part of the broad group of diseases called hematological neoplasms. Leukemia is a broad term covering a spectrum of diseases. Leukemia is clinically and pathologically classified as acute and chronic disease.

[0076] In certain embodiments of the present disclosure, T_{RM} cells are delivered to an individual in need thereof, such as an individual that has cancer or an infection, such as a

bacterial or viral infection. The cells then enhance the individual's immune system to attack the respective cancer or pathogenic cells. In some cases, the individual is provided with one or more doses of the cells. In cases where the individual is provided with two or more doses of the immune cells, the duration between the administrations should be sufficient to allow time for propagation in the individual, and in specific embodiments the duration between doses is 1, 2, 3, 4, 5, 6, 7, or more days.

[0077] Certain embodiments of the present disclosure provide methods for treating or preventing an immune-mediated disorder. In one embodiment, the subject has an autoimmune disease. Non-limiting examples of autoimmune diseases include: alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac spate-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, type 1 or immune-mediated diabetes mellitus, myasthenia gravis, nephrotic syndrome (such as minimal change disease, focal glomerulosclerosis, or mebranous nephropathy), pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, ulcerative colitis, uveitis, vasculitides (such as polyarteritis nodosa, takayasu arteritis, temporal arteritis/giant cell arteritis, or dermatitis herpetiformis vasculitis), vitiligo, and Wegener's granulomatosis. Thus, some examples of an autoimmune disease that can be treated using the methods disclosed herein include, but are not limited to, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosis, type I diabetes mellitus, Crohn's disease; ulcerative colitis, myasthenia gravis, glomerulonephritis, ankylosing spondylitis, vasculitis, or psoriasis. The subject can also have an allergic disorder such as Asthma.

[0078] In yet another embodiment, the subject is the recipient of a transplanted organ or stem cells and T_{RM} cells are used to prevent and/or treat rejection. In particular embodiments, the subject has or is at risk of developing graft versus host disease. GVHD is a possible complication of any transplant that uses or contains stem cells from either a related or an unrelated donor. There are two kinds of GVHD, acute and chronic. Acute GVHD appears within the first three months following transplantation. Signs of acute GVHD include a reddish skin rash on the hands and feet that may spread and become more severe, with peeling or blistering skin. Acute GVHD can also affect the stomach and intestines, in which case cramping, nausea, and diarrhea are present. Yellowing of the skin and eyes (jaundice) indicates that acute GVHD has affected the liver. Chronic GVHD is ranked based on its severity: stage/grade 1 is mild; stage/grade 4 is severe. Chronic GVHD develops three months or later following transplantation. The symptoms of chronic GVHD are similar to those of acute GVHD, but in addition, chronic GVHD may also affect the mucous glands in the eyes, salivary glands in the mouth, and glands that lubricate the stomach lining and intestines. Any of the populations of immune cells disclosed herein can be utilized. Examples of a transplanted organ include a solid organ transplant, such as kidney, liver, skin, pancreas, lung and/or heart, or a cellular transplant such as islets, hepatocytes, myoblasts, bone marrow, or hematopoietic or other stem cells. The transplant can be a composite transplant, such as tissues of the face. Immune cells can be administered prior to transplantation, concurrently with transplantation, or following transplantation. In some embodiments, the immune cells are administered prior to the transplant, such as at least 1 hour, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, or at least 1 month prior to the transplant. In one specific, non-limiting example, administration of the therapeutically effective amount of immune cells occurs 3-5 days prior to transplantation.

[0079] In some embodiments, the subject can be administered nonmyeloablative lymphodepleting chemotherapy prior to the T_{RM} cell population. The nonmyeloablative lymphodepleting chemotherapy can be any suitable such therapy, which can be administered by any suitable route. The nonmyeloablative lymphodepleting chemotherapy can comprise, for example, the administration of cyclophosphamide and fludarabine, particularly if the cancer is melanoma, which can be metastatic. An exemplary route of administering cyclophosphamide and fludarabine is intravenously. Likewise, any suitable dose of cyclophosphamide and fludarabine can be administered. In particular aspects, around 60 mg/kg of cyclophosphamide

is administered for two days after which around 25 mg/m^2 fludarabine is administered for five days.

[0080] In certain embodiments, a growth factor that promotes the growth and activation of the T_{RM} cell population is administered to the subject either concomitantly with the T_{RM} cell population or subsequently to the immune cells. The growth factor can be any suitable growth factor that promotes the growth and activation of the T_{RM} cell population. Examples of suitable immune cell growth factors include interleukin (IL)-2, IL-7, IL-15, and IL-12, which can be used alone or in various combinations, such as IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, IL-2, IL-7 and IL-15, IL-12 and IL-7, IL-12 and IL-15, or IL-12 and IL2.

[0081] Therapeutically effective amounts of T_{RM} cells can be administered by a number of routes, including parenteral administration, for example, intravenous, intraperitoneal, intramuscular, intrasternal, or intraarticular injection, or infusion.

[0082] The therapeutically effective amount of T_{RM} cells for use in adoptive cell therapy is that amount that achieves a desired effect in a subject being treated. For instance, this can be the amount of T_{RM} cells necessary to inhibit advancement, or to cause regression of an autoimmune or alloimmune disease, or which is capable of relieving symptoms caused by an autoimmune disease, such as pain and inflammation. It can be the amount necessary to relieve symptoms associated with inflammation, such as pain, edema and elevated temperature. It can also be the amount necessary to diminish or prevent rejection of a transplanted organ.

[0083] The T_{RM} cells can be administered in treatment regimens consistent with the standard of care for treating the disease, for example a single or a few doses over one to several days to ameliorate a disease state or periodic doses over an extended time to inhibit disease progression and prevent disease recurrence. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. The therapeutically effective amount of immune cells will be dependent on the subject being treated, the severity and type of the affliction, and the manner of administration. In some embodiments, doses that could be used in the treatment of human subjects range from at least 3.8×10^4, at least 3.8×10^5, at least 3.8×10^6, at least 3.8×10^7, at least 3.8×10^8, at least 3.8×10^9, or at least 3.8×10^{10} immune cells/m^2. In a certain embodiment, the dose used in the treatment of human subjects ranges from about 3.8×10^9 to about 3.8×10^{10} immune cells/m^2. In

additional embodiments, a therapeutically effective amount of immune cells can vary from about 5×10^6 cells per kg body weight to about 7.5×10^8 cells per kg body weight, such as about 2×10^7 cells to about 5×10^8 cells per kg body weight, or about 5×10^7 cells to about 2×10^8 cells per kg body weight. The exact amount of immune cells is readily determined by one of skill in the art based on the age, weight, sex, and physiological condition of the subject. Effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0084] The T_{RM} cells may be administered in combination with one or more other therapeutic agents for the treatment of the immune-mediated disorder. Combination therapies can include, but are not limited to, one or more anti-microbial agents (for example, antibiotics, anti-viral agents and anti-fungal agents), anti-tumor agents (for example, fluorouracil, methotrexate, paclitaxel, fludarabine, etoposide, doxorubicin, or vincristine), immune-depleting agents (for example, fludarabine, etoposide, doxorubicin, or vincristine), immunosuppressive agents (for example, azathioprine, or glucocorticoids, such as dexamethasone or prednisone), anti-inflammatory agents (for example, glucocorticoids such as hydrocortisone, dexamethasone or prednisone, or non-steroidal anti-inflammatory agents such as acetylsalicylic acid, ibuprofen or naproxen sodium), cytokines (for example, interleukin-10 or transforming growth factor-beta), hormones (for example, estrogen), or a vaccine. In addition, immunosuppressive or tolerogenic agents including but not limited to calcineurin inhibitors (*e.g.*, cyclosporin and tacrolimus); mTOR inhibitors (*e.g.*, Rapamycin); mycophenolate mofetil, antibodies (*e.g.*, recognizing CD3, CD4, CD40, CD154, CD45, IVIG, or B cells); chemotherapeutic agents (*e.g.*, Methotrexate, Treosulfan, Busulfan); irradiation; or chemokines, interleukins or their inhibitors (*e.g.*, BAFF, IL-2, anti-IL-2R, IL-4, JAK kinase inhibitors) can be administered. Such additional pharmaceutical agents can be administered before, during, or after administration of the immune cells, depending on the desired effect. This administration of the cells and the agent can be by the same route or by different routes, and either at the same site or at a different site.

A. Combination Therapies

[0085] In certain embodiments, the methods provided herein further comprise a step of administering at least one additional therapeutic agent to the subject. All additional therapeutic agents disclosed herein will be administered to a subject according to good clinical practice for

each specific composition or therapy, taking into account any potential toxicity, likely side effects, and any other relevant factors.

[0086] In certain embodiments, the additional therapy may be immunotherapy, radiation therapy, surgery (*e.g.*, surgical resection of a tumor), chemotherapy, bone marrow transplantation, or a combination of the foregoing. The additional therapy may be targeted therapy. In certain embodiments, the additional therapy is administered before the primary treatment (*i.e.*, as adjuvant therapy). In certain embodiments, the additional therapy is administered after the primary treatment (*i.e.*, as neoadjuvant therapy.

[0087] In certain embodiments, the additional therapy comprises an immunotherapy. In certain embodiments, the immunotherapy comprises an immune checkpoint inhibitor. In certain embodiments, the immune checkpoint inhibitor inhibits an immune checkpoint protein selected from the group consisting of programmed cell death pathway 1 (PD-1/CD279) and its ligands (PD-L1/CD274 and PD-L2/CD273), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4/CD152), lymphocyte-activation gene 3 (LAG-3/CD223), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains (TIGIT), T cell immunoglobulin domain and mucin domain 3 (TIM-3/HAVcr2), killer immunoglobulin-like receptor (KIR/CD158), V-domain immunoglobulin suppressor of T cell activation (VISTA), and the adenosine A2a receptor (A2aR). In some aspects, the immunotherapy is a 4-1BB agonist. Exemplary 4-1BB agonists include but are not limited to 4-1BB agonist antibodies (e.g., Utomilumab), recombinant 4-1BB (including but not limited to soluble, matrix-bound, scaffold bound forms), and 4-1BB aptamers.

[0088] In certain embodiments, the immune checkpoint inhibitor is a PD-1 binding antagonist. In certain embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In certain embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and CT-011. In certain embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PDL1 or PDL2 fused to an immunoglobulin constant region (*e.g.*, an Fc region of an immunoglobulin sequence).

[0089] In certain embodiments, the immune checkpoint inhibitor is a CTLA-4 binding antagonist. In certain embodiments, the CTLA-4 binding antagonist is an anti-CTLA-

4 antibody. In certain embodiments, the anti-CTLA-4 antibody is selected from the group consisting of ipilimumab and tremelimumab.

[0090] In certain embodiments, the additional therapeutic agent comprises treatment with radiotherapy. In certain embodiments, the radiotherapy is selected from the group consisting of gamma rays (γ-rays), X-rays, microwaves, proton beam irradiation, ultraviolet irradiation, and the directed delivery of radioisotopes to the tumor. In certain embodiments, the radiotherapy comprises treatment with X-rays. In certain embodiments, the X-rays are administered in daily doses of 50 to 200 roentgens over a period of three to four weeks. In certain embodiments, the X-rays are administered in a single dose of 2000 to 6000 roentgens. In certain embodiments, the radiotherapy comprises directed delivery of radioisotopes to the tumor. Dosage ranges for radioisotopes vary widely depending on the half-life of the isotope, the strength and type of radiation emitted, and the degree of uptake by tumor cells, but determination of an appropriate therapeutically effective dose is within the level of ordinary skill in the art.

[0091] In certain embodiments, the additional therapeutic agent comprises administration of agents for the treatment of side-effects associated with the primary treatment (*e.g.*, nausea, cachexia, and the like). In certain embodiments, the additional therapy comprises an immunotherapy. In certain embodiments, the additional therapy comprises radiation therapy. In some embodiments, the radiotherapy comprises gamma irradiation. In certain embodiments, the additional therapy comprises surgery. In certain embodiments, the additional therapy comprises a combination of radiation therapy and surgery. In certain embodiments, the additional therapy comprises treatment with a class of chemotherapeutic agent selected from the group consisting of alkylating agents, anthracyclines, cytoskeletal disruptors, epothilones, histone deacetylase inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, kinase inhibitors, nucleotide analogs and nucleotide precursor analogs, peptide antibiotics, platinum-based compounds, retinoids, vinca alkaloids and derivatives thereof.

[0092] The additional therapies contemplated herein may be administered before, after, or concurrently with administration of the compositions provided herein. In certain embodiments, the additional therapy is administered before the compositions provided herein. In certain embodiments, the additional therapy is administered after the compositions provided herein. In certain embodiments, the additional therapy is administered at one or more intervals before or after administration of the compositions provided herein. Determination of an

appropriate interval for administration of an additional therapy such that the subject being treated benefits from the combination therapy is within the level of ordinary skill in the art.

B. Pharmaceutical Compositions

[0093] In another aspect, provided herein are pharmaceutical compositions and formulations comprising T_{RM} cells and a pharmaceutically acceptable carrier.

[0094] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (such as an antibody or a polypeptide) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 22nd edition, 2012), in the form of aqueous solutions, such as normal saline (*e.g.*, 0.9%) and human serum albumin (*e.g.*, 10%). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zinc-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

III. Kits

[0095] In some embodiments, a kit that can include, for example, one or more media and components for the production of T_{RM} cells is provided. Such formulations may comprise a cocktail of factors, in a form suitable for combining with T_{RM} cells. The reagent system may be packaged either in aqueous media or in lyophilized form, where appropriate. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there is more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately

placed. However, various combinations of components may be comprised in a vial. The components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means. The kits also will typically include a means for containing the kit component(s) in close confinement for commercial sale. Such containers may include injection or blow molded plastic containers into which the desired vials are retained. The kit can also include instructions for use, such as in printed or electronic format, such as digital format.

IV. Examples

[0096] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Production of Tissue Resident Memory T Cells

[0097] Peripheral blood samples were obtained from healthy human subjects. The blood was sorted using FACS to isolate naïve ($CD45RA^+CCR7^+$) $CD8^+$ T cells. The T cells were then polyclonally activated for 4 days in atmospheric oxygen (approximately 20%) or hypoxia (2% O_2) to generate "early effectors". The early effectors were then cultured for an additional 2 days in the presence of 1.25 ng/mL rhTGF-β1. The cells were then harvested and analyzed for expression of T_{RM}-associated genes and surface markers.

[0098] It was found that hypoxia in combination with TGF-β1 induced a $CD69^+CD103^+$ population that expressed human T_{RM}-associated markers, including CD69 and CD103 (FIG. 3). The T_{RM}-like T cells were further analyzed for changes in expression of additional genes. It was found that the cells cultured in hypoxia versus atmospheric oxygen conditions had changes in gene expression associated with the T_{RM} phenotype (FIG. 2).

[0099] Naïve $CD8^+$ T-cells were activated by hypoxia with or without the addition of rhTGF-β1. The frequency of the $CD69^+CD103^+$ population was assessed by flow cytometry. It was observed that hypoxia and TGF-β1 synergize to induce $CD69^+CD103^+$ cells (FIG. 4).

[00100] Further, it was found that hypoxia and TGF-β1 induced T_{RM}-phenotype cells show transcriptional differences similar to those reported for endogenous T_{RM}. $CD69^-CD103^-$, $CD69^+CD103^-$, and $CD69^+CD103^+$ T-cells were generated and sorted before isolation of RNA for transcriptome analysis via RNA-sequencing ($n = 3$). FIG. 9A shows a heatmap showing expression of selected genes commonly reported in transcriptome analyses of endogenous human T_{RM}. FIG. 9B shows a heatmap comparing transcriptional differences (log2FC) in $CD8^+CD69^+$ versus $CD8^+CD69^-$ T-cells from human lung and $CD69^+CD103^+$ hypoxia and TGF-β1 *in-vitro* induced T_{RM} (i-T_{RM}) versus $CD69^-CD103^-$ cells from normal cell culture conditions (20% O_2 without TGF-β1). Differential expression determined by $|log2FC| \geq 1$ and $FDR < 0.05$.

[00101] The present studies showed that hypoxia and TGF-β1 synergize to induce a $CD8^+CD69^+CD103^+$ cell population that expresses human T_{RM}-associated markers and has a transcriptional profile similar to that of endogenous human T_{RM}. This work reveals another possible cue for T_{RM} differentiation *in vivo*, and provides the basis for an *in vitro* method to generate antigen-specific T_{RM}-like cells that would enable the development of adoptive cellular therapies utilizing this unique cell type. Thus, the present methods can be used to produce cells with a T_{RM} phenotype.

Example 2 – Materials and Methods

[00102] **Cell isolation and *in vitro* cell culture:** Healthy donor peripheral blood mononuclear cells (PBMCs) were collected by leukapheresis and stored in liquid nitrogen until use. All human sample collection was performed with informed consent and approved by the institutional review board (IRB) of UT MD Anderson Cancer Center. $CD8^+$ T cells were enriched from healthy donor PBMCs using StemCell EasySep™ kits. Cells were then stained with fluorochrome-conjugated antibodies against CD8, CD45RA, and CCR7. Naïve CD8+CD45RA+CCR7+ cells were sorted using a FACSAria™ III or Fusion cell sorter (BD Biosciences). Sorted naïve cells were resuspended in cell culture media (RPMI with 10% FBS, L-glutamine, and penicillin-streptomycin) with 10 IU/ml IL-2 (Prometheus) and equilibrated overnight to 2% oxygen in a hypoxic chamber (Coy Laboratory Products) or atmospheric oxygen (approx. 20%) in a standard cell culture incubator (Thermo Fisher). After equilibration

cells were activated with anti-CD3/anti-CD28 beads (Dynabeads®, Gibco) for four days. On day four 1.25 ng/ml recombinant human TGF-β1 (Biolegend) was added and cells were cultured for an additional two days. Antigen-specific $CD8^+$ T cells generated via stimulation with autologous peptide pulsed dendritic cells or TCR transduction followed by tetramer-based sorting were expanded using a conventional Rapid Expansion Protocol (REP) or a TRM modifying REP. Tetramer-positive cells were expanded for 10-14 days using 30 ng/mL anti-CD3 (OKT3) and 200x irradiated allogeneic PBMCs and LCLs as feeder cells in either i) 20% oxygen and supplemented with IL-2 (50 IU/ml) in the case of conventional REP or ii) in 2% oxygen and supplemented with IL-15 (10 ng/ml) and rhTGF-β1 (1.25 ng/ml, beginning on day 4) in the case of modified REP to induce TRM phenotype.

[00103] Flow cytometry: For analysis of human TRM-associated markers beads were removed from cells and cells were washed once in staining buffer and stained with Live/Dead Fixable Aqua (Life Technologies) and fluorochrome-conjugated antibodies against CD8, CD69, CD103, PD-1, CD101, CXCR6, and CD49a (all Biolegend). After staining cells were fixed in 4% paraformaldehyde Fixation Buffer (Biolegend), washed, and stored in staining buffer until analysis. Stained cells were analyzed using an ACEA Novocyte® 3000 flow cytometer. Single fluorochrome-stained compensation beads (UltraComp™, eBioscience) and fluorescence minus one (FMO) samples were used as controls. Data were analyzed using FlowJo software (BD Biosciences).

[00104] Quantitative real-time PCR (qRT-PCR or qPCR): For analysis of human T_{RM}-associated gene expression beads were removed from cells and cells were washed once in PBS. RNA was isolated using the Qiagen RNeasy® Plus Mini Kit according to manufacturer's instructions. When necessary RNA was further purified and/or concentrated using the Qiagen RNeasy® MinElute Cleanup Kit according to manufacturer's instructions. First strand cDNA was synthesized using M-MLV Reverse Transcriptase (Thermo Fisher). Quantitative real-time PCR was performed using the QuantStudio® 5 Real-Time PCR System and PowerUp™ SYBR® Green Master Mix (Applied Biosystems, ThermoFisher Scientific). Relative mRNA gene expression was normalized to the housekeeping gene RPL13A. Primers used are listed in the table below.

[00105] Table 2: Primer sequences.

Gene	Primer	SEQ ID NO
CD69 F	ATTGTCCAGGCCAATACACATT	1
CD69 R	CCTCTCTACCTGCGTATCGTTTT	2
ITGAE R	AGCCATGCAACACGTCTTAGA	3
ITGAE F	TCCTCGAATATGCCACCATCG	4
ITGA1 R	CTGGACATAGTCATAGTGCTGGA	5
ITGA1 F	ACCTGTGTCTGTTTAGGACCA	6
CXCR6 F	CAAGAGCCTACTGGGCATCTACAC	7
CXCR6 R	TGGCCTTAACCACTACAATGAAAC	8
CX3CR1 F	TCACCGTCATCAGCATTGATAGG	9
CX3CR1 R	GTTTCCACATTGCGGAGCAC	10
PDCD1 F	ACGAGGGACAATAGGAGCCA	11
PDCD1 R	GGCATACTCCGTCTGCTCAG	12
CD101 F	CAGCCAGTGACGTACAGCTC	13
CD101 R	CCATTCCGTTGCCTCACAGAA	14
S1PR1 F	GCCTCTTCCTGCTAATCAGCG	15
S1PR1 R	GCAGTACAGAATGACGATGGAG	16
KLF2 F	CATCTGAAGGCGCATCTG	17
KLF2 R	CGTGTGCTTTCGGTAGTGG	18
EOMES F	GCCCACGTCTACCTGTGCAA	19
EOMES R	GGGCAGTGGGATTGAGTCCG	20
TBX21 F	CAACACAGGAGCGCACTGGA	21
TBX21 R	GTGTTGGAAGCGTTGCAGGC	22
TCF7 F	TGCAGCTATACCCAGGCTGG	23
TCF7 R	CCTCGACCGCCTCTTCTTC	24
IRF4 F	CCCGTACCAATGTCCCATGA	25
IRF4 R	CCTGTCACCTGGCAACCATTT	26
RUNX3 F	AGCACCACAAGCCACTTCAG	27
RUNX3 R	GGGAAGGAGCGGTCAAACTG	28
CCR7 F	CAAGCTGTCCTGTGTGGGCA	29
CCR7 R	CGCTCAAAGTTGCGTGCCTG	30
SELL F	ATGGAACGATGACGCCTGCC	31
SELL R	GGCCTCCAAAGGCTCACACT	32
IFNG F	TGGAAAGAGGAGAGTGACAGAAA	33
IFNG R	TCCTTGATGGTCTCCACACTC	34
TNFA F	GGCGCTCCCCAAGAAGACAG	35
TNFA R	CAGGCTTGTCACTCGGGGTT	36
GZMB F	CAACCAATCCTGCTTCTGCT	37

GZMB R	CCGCACCTCTTCAGAGACTT	38
CTLA4 F	TGGACACGGGACTCTACATCT	39
CTLA4 R	GGCACGGTTCTGGATCAATTACA	40
SLC2A1 F	TCTGGCATCAACGCTGTCTTC	41
SLC2A1 R	CGATACCGGAGCCAATGGT	42
VEGF F	AAATGCTTTCTCCGCTCTGA	43
VEGF R	CCCACTGAGGAGTCCAACAT	44
RPL13A F	CCTCAAGGTCGTGCGTCTGA	45
RPL13A R	TCCACGTTCTTCTCGGCCTG	46

[00106] **RNA-sequencing transcriptome analysis:** Cells were sorted using a FACSAria™ IIIu cell sorter (BD Biosciences) before RNA isolation using the Qiagen RNeasy® Plus Mini Kit followed by the Qiagen RNeasy® MinElute Cleanup Kit. The library was constructed using the Illumina TruSeq Stranded mRNA kit. RNA sequencing was carried out using the Illumina NextSeq®500 platform. The raw reads were mapped to the *Homo sapiens* reference genome and transcriptome (GRCh38, GENCODEV23) by HISAT2 (version: 2.1.0). Htseq-count (version: 2.1.0) was used to get the counts for genes. R and Bioconductor packages DESeq2 (version 1.14.1) was used to identify the differentially expressed genes. The genes (mRNA only, taking the protein-coding genes for p-value adjustment) with FDR < 0.05 and |fold-change| > 2 were considered differentially expressed. R and Bioconductor package fgsea (version 1.10.0) was used to determine whether an *a priori* defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes). Gene sets were derived from several previously published T cell signatures. The T_{RM} signature was constructed from several studies. Lung T_{RM} and Breast Cancer TIL signatures were downloaded from the Gene Expression Omnibus (GEO), GSE61397 and GSE110938, respectively. False-discovery rate (FDR) adjusted p-values less than 0.05 were considered significant or "true".

[00107] Functional analysis of significantly differentially expressed genes (FDR < 0.05 and |fold-change| > 2) was done with Ingenuity® Pathway Analysis (IPA) software (version 48207413, Qiagen) using all genes in the Ingenuity Knowledge Base as the reference set and right-tailed Fisher's exact test in a core analysis to determine if pathways are significantly altered between conditions (−log10[p value] > 1.3).

[00108] **Statistical analysis:** Graphical presentation and statistical analysis of the data was performed using GraphPad Prism (Version 7, GraphPad software, San Diego,

CA). Data are displayed as mean ± SEM. Results between experimental groups were compared using statistical tests described in the figure legends (ANOVA always followed by Tukey's multiple comparisons test). $p < 0.05$ was considered statistically significant.

Example 3 - Hypoxia acts as an environmental cue for human tissue resident memory T-cell differentiation

[00109] Human $CD8^+$ T-cells differentiated in hypoxia and TGF-β1 acquire a T_{RM}-like phenotype: Given the relative hypoxia in inflamed tissues, it was postulated that low oxygen tension could provide additional cues to T_{RM} differentiation. To determine whether hypoxia can contribute to induction of a T_{RM} phenotype naïve ($CD45RA^+$ $CCR7^+$) CD8+ T-cells were sorted from human peripheral blood, activated them for 4 days in hypoxia (2% O_2) or normal cell culture conditions (atmospheric oxygen, approximately 20% O_2) to generate "early effectors" and then cultured an additional 2 days in the presence of rhTGF-β1.

[00110] Quantitative real-time PCR (qPCR) was used to assess the bulk populations for expression of a panel of genes associated with T_{RM} transcriptional profile. Cells differentiated in 2% O_2 + TGF-β1 showed upregulation of most of the genes identified by Kumar *et al.* 2017 (which is incorporated herein by reference) as the core transcriptional signature of human T_{RM}, namely *CD69*, *ITGAE* (CD103), *PDCD1* (PD-1), *CD101*, and *CXCR6* (FIG. 2A). Notably, no difference was observed in transcript levels of *ITGA1* (CD49a). In addition, transcripts of genes important in T-cell recirculation (*S1PR1*, *KLF2*, *SELL* (CD62L)) were downregulated, further suggesting a resident memory phenotype (FIG. 2B). Previous reports in mouse models have demonstrated that downregulation of *S1PR1* and *KLF2* are critical to T_{RM} differentiation, and decreased levels of these genes have also been observed in endogenous human T_{RM}. Transcripts for the transcription factor Eomes were dramatically decreased (FIG. 2C). Studies in mice have demonstrated that extinguishment of Eomes is necessary for TGF-β1 responsiveness and establishment of T_{RM}. *IRF4* and *RUNX3* were upregulated. The specific role of *IRF4* in T_{RM} remains undefined but its upregulation has been reported in human T_{RM}. The transcription factor *RUNX3* has recently been identified as a key regulator of T_{RM} differentiation. Finally, elevated levels of transcripts encoding effector molecules TNF-α and granzyme b were observed, similar to findings reported in human lung T_{RM} (FIG. 2D). Elevated expression of the canonical hypoxia responsive genes *SLC2A1* (Glut-1) and *VEGF* confirmed the cells were responding to hypoxic conditions (FIG. 1E). In totality,

these results indicate that when human $CD8^+$ T-cells are differentiated in hypoxia in combination with TGF-β1 they acquire a tissue resident memory-like transcriptional profile.

[00111] Flow cytometric analysis to evaluate protein-level expression of markers considered to be the core signature of human T_{RM}. In all healthy donors tested there was an increase in $CD69^+CD103^+$ cells in the 2% O_2 + TGF-β1 condition compared to 20% O_2 + TGF-β1 (FIG. 2A). Cell viability was comparable or better in the 2% O_2 + TGF-β1 cells versus the 20% O_2 + TGF-β1 cells (FIG. 7B). These $CD69^+CD103^+$ cells expressed PD-1, CD101, and CD49a (FIG. 3B). Notably, CXCR6 surface protein expression was not observed despite transcriptional upregulation. Although expression of both CD69 and CD103 is now commonly used to define T_{RM} there is still debate regarding which of these surface markers is best to use to identify T_{RM}, in part due to heterogeneous expression of CD69 and CD103 in endogenous resident memory cells. Thus, the expression levels of the T_{RM}-associated markers PD-1, CD101, and CD49a were compared among the $CD69^-CD103^+$, $CD69^+CD103^+$, and $CD69^+CD103^-$ populations from the 2% O_2 + TGF-β1 condition. As expected, the CD69+$CD103^+$ population had the highest levels of PD-1 and CD101 surface expression (FIG. 3C). Expression of CD49a was also high but equivalent to expression levels observed in the $CD69^+CD103^-$ population. In comparing different oxygen conditions, the most dramatic increase in population fold change was found to be in the $CD69^+CD103^+$ population (FIG. 7C, 7D). On the basis of these results, it was chosen to focus further analysis on the $CD69^+CD103^+$ population as the *in vitro* induced T_{RM} cells.

[00112] Hypoxia and TGF-β1 exposure are synergistic cues for T_{RM} phenotype acquisition: Since the atmospheric oxygen levels in normal tissue culture conditions are higher than that experienced by T cells *in vivo* the effect of 10% O_2 was evaluated, which is a physiologically relevant, non-hypoxic oxygen level T-cells are exposed to in circulation. Although there was a slight increase in $CD69^+CD103^+$ T-cells in 10% O_2 + TGF-β1 compared to 20% O_2 + TGF-β1, correcting for multiple comparisons uncovered no significant differences between the two conditions in expression of T_{RM} signature genes (FIG. 8A-E). In addition, the fold-increase of the $CD69^+CD103^+$ population (over 20% O_2 + TGF-β1) was significantly greater in the 2% O_2 + TGF-β1 condition versus 10% O_2 + TGF-β1 (FIG. 8F).

[00113] To assess the individual contributions of hypoxia and TGF-β1 to induction of T_{RM} phenotype cells *in vitro* differentiation experiments were performed in 2% or 20% O_2 with or without the addition of rhTGF-β1. Hypoxia primarily induced $CD69^+$ cells

whereas TGF-β1 induced $CD103^+$ cells, in congruence with published reports that hypoxia and TGF-β can drive expression of these markers, respectively. Although hypoxia or TGF-β1 alone does induce a modest population of $CD69^+CD103^+$ cells, the combination of hypoxia and TGF-β1 appears to synergize induction of the resident phenotype as the combination effect was markedly greater than the additive effects of either condition alone (FIG. 4A). The $CD69^+CD103^+$ cells induced by hypoxia and TGF-β1 expressed high levels of the T_{RM} markers PD-1, CD101, and CD49a, compared to the majority populations in the 20% O_2 and 2% O_2 conditions ($CD69^-CD103^-$ and $CD69^+CD103^-$, respectively) (FIG. 4B).

[00114] *In vitro* induced T_{RM} show enrichment for endogenous human T_{RM} gene signatures: Since differences in T_{RM} marker expression among the $CD69^-CD103^-$ (20% O_2), $CD69^+CD103^-$ (2% O_2) and $CD69^+CD103^+$ (2% O_2 + TGF-β1) cells suggested that these represent distinct populations, each phenotype was sorted and their transcriptional profile analyzed via RNA sequencing. Principal component analysis (PCA) confirmed that these three populations are distinct from one another (FIG. 5A). Unsupervised hierarchical clustering showed distinct gene signatures for $CD69^-CD103^-$ and $CD69^+CD103^+$ cells, whereas $CD69^+CD103^-$ cells had a somewhat intermediate transcriptional profile (FIG. 5B, 5C). Comparison of the top differentially expressed genes between $CD69^+CD103^+$ and $CD69^-CD103^-$ cells revealed gene expression patterns consistent with those reported for endogenous human T_{RM}, including increased expression of *ITGAE*, *EGR2*, *GNLY*, *BMF*, *RASGEF1B*, and *NR4A1*, and decreased expression of *SELL*, *KLF2*, and *KLF3*, indicating a non-recirculating transcriptional program (FIG. 5B, 5C). *S1PR1*, a KLF2-target gene, was also downregulated but did not meet the threshold for fold change. $CD69^+CD103^+$ cells demonstrated increased expression of *ITGA1*, *PDCD1*, *CD101*, and *TNFRSF9*, all of which are consistently reported as upregulated in endogenous human T_{RM}. Elevated levels of the transcription factor NOTCH1 were observed, which is known to contribute to maintenance of endogenous lung T_{RM} *in vivo*, as well as *RBPJ*, which plays a central role in Notch signaling (FIG. 5C).

[00115] Endogenous T_{RM} often express various chemokines, likely as part of their 'alarm function' in recruiting other immune cells to local tissues. Consistent with the chemokine profile of endogenous T_{RM}, *in vitro* induced $CD69^+CD103^+$ cells in the study upregulated *CXCL13* and *CCL20*, as well as *CCL4*, *CCL5*, and *CCL22* (FIG. 5C). Changes in expression of genes that have undefined roles but are consistently reported in endogenous

human T_{RM} were also observed, such as upregulation of *MYO7A* and *RGS1* and downregulation of *SERPINE2*, *RAP1GAP2*, *RASGRP2*, and *FAM65B*.

[00116] To gain insight on the physiological relevance of the findings Gene Set Enrichment Analysis (GSEA) was performed using gene signatures from published analyses on endogenous human $CD103^+$ T_{RM} compared with $CD103^-$ effector memory cells from peripheral blood or $CD103^-$ T-cells within the same tissue site. The results revealed that the transcriptional profile of $CD69^+CD103^+$ cells compared to $CD69^-CD103^-$ cells is similar to the signature of T_{RM} compared to blood T_{EM} and local $CD103^-$ T-cells, whereas $CD69^+CD103^+$ cells compared to $CD69^+CD103^-$ is only similar to the signature of T_{RM} versus $CD103^-$ T-cells within the same tissue (FIG. 5D). These results reflect the degree of difference in oxygen tension in circulation versus local tissue. TIL within the same tumor would also experience more similar oxygen levels than T cells in tissues versus those in circulation, and multiple recent profiles of TIL in various solid tumor types have reported the presence of $CD103^+$ resident memory-like TIL (TIL_{RM}). Thus, $CD69^+CD103^+$ cells induced in hypoxia + TGF-β1 were compared with $CD69^+CD103^-$ cells induced in hypoxia alone and observed enrichment of the gene signature reported for $CD8^+CD69^+CD103^+$ versus $CD8^+CD69^+CD103^-$ breast cancer TIL (FIG. 5E, 5F). As hypoxia and TGF-β are common features of the tumor microenvironment, the results indicate that these conditions may contribute to generation of $CD103^+$ TIL *in vivo*.

[00117] Ingenuity Pathway Analysis (IPA) comparing $CD69^+CD103^+$ cells with $CD69^-CD103^-$ cells revealed that many of the differentially expressed genes are components of glycolysis, gluconeogenesis, and TGF-β signaling pathways (FIG. 6A). Given that the cells were exposed to hypoxia and TGF-β and that hypoxia is a major regulator of cellular metabolism, these results were expected. There was also an enrichment of genes in the Notch Signaling pathway, which has been reported in endogenous human lung T_{RM} (FIG. 6A). These findings raise questions regarding the role of metabolism in T_{RM} differentiation. Hombrink *et al.* suggested that a major role of Notch signaling in lung T_{RM} is regulation of metabolic programs, as chemical inhibition of Notch signaling affected genes involved in glycolysis, oxidative phosphorylation, and fatty acid metabolism pathways. It has also been suggested that deletion of the purinergic receptor P2RX7, which, like hypoxia, is a known modulator of aerobic glycolysis, impairs T_{RM} formation via dysregulation of metabolism, as P2RX7 deficient

cells displayed decreased mitochondrial mass and function, defective aerobic glycolysis, and impaired glucose uptake.

[00118] Enrichment of differentially expressed genes involved in Leukocyte Extravasation Signaling, Epithelial Adherens Junction Signaling, Integrin Signaling, and Paxilin Signaling was observed, all involved in focal adhesion signaling and suggesting changes in migratory programming (FIG. 6B). Multiple pathways involved in inositol phosphate signaling were also enriched, namely 3-phosphoinositide Biosynthesis, Superpathway of Inositol Phosphate Compounds, D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis, and D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis; consistent with a previous report that PI(3)K (phosphatidylinositol-3-OH kinase) signaling is implicated in cytokine-induced downregulation of *KLF2* and may play a role in generation of T_{RM} *in vivo*.

[00119] To better understand the functional relevance of the hypoxia + TGF-β induced T_{RM} transcriptional profile pathway analysis was also run on transcriptional data published by Kumar *et al.* and it was found that changes in the Th1 and Th2 activation and Granulocyte Adhesion and Diapedesis pathways were common to their endogenous lung T_{RM} and the *in vitro* induced T_{RM}. Remarkably, the Axonal Guidance pathway was also highly significantly differentially regulated in all of the datasets. Axonal guidance, while at first seemingly unrelated to resident memory T-cells and unreported in current T_{RM} literature, is a process whereby environmental cues influence migratory patterns of cells. Many of the same factors governing axon guidance are also known to regulate immune cell trafficking and can be regulated by hypoxia and/or TGF-β.

[00120] A further study was conducted to assess the effect of the HIF prolyl hydroxylase inhibitor FG-4592 (Roxadustat) in combination with TGFB1. Naïve $CD8^+$ T-cells isolated from peripheral blood were activated in 20% O_2 ($AtmosO_2$) in the presence of the HIF prolyl hydroxylase inhibitor FG-4592 (Roxadustat) for 4 days and then for an additional 2 days with rhTGF-β1. Cells activated in 2% O_2 (hypoxia) with addition of rhTGF-β1 on day 4 are shown for comparison in FIG 10A. The combination was observed to induce $CD69^+CD103^+$ cells (FIG. 10A).

[00121] Next, naïve $CD8^+$ T-cells were stimulated with autologous monocyte-derived dendritic cells pulsed with MART-1 (M27) peptide for 7 days in 20% O_2 ($AtmosO_2$) or 2% O_2 with the addition of rhTGF-β1 on day 4 to generate $CD69^+CD103^+$ antigen-specific

(Tetramer$^+$) T-cells detected by flow cytometry (FIG. 10B). Hypoxia and rhTGF-β1 were used in a modified rapid expansion protocol to induce TRM phenotype in antigen-specific T cells. Antigen-specific T-cells were generated via stimulation with autologous MART-1 peptide-pulsed dendritic cells (ETC) or transduction of gp100-specific TCR (TCRT), labeled with fluorochrome-conjugated tetramer, and sorted. The sorted antigen-specific T cells were then stimulated with anti-CD3 (OKT3) and irradiated feeder cells in 20% O_2 and supplemented with IL-2 (conventional REP) or 2% O_2 supplemented with IL-15 and the addition of rhTGF-β1 from day 4 onwards. Thus, it was also shown that the modified rapid expansion protocol induces T_{RM} phenotype in antigen-specific T cells (FIG. 10C).

[00122] Thus, the present studies recapitulated the T_{RM} phenotype *in vitro* from human peripheral blood derived T cells, as well as identified hypoxia as a potential cue for T_{RM} differentiation. While there are obvious limitations to experiments that can be conducted in humans, the studies described provide compelling evidence that hypoxia is an environmental cue that can contribute to acquisition of a T_{RM} phenotype, supported by the observation that hypoxia + TGF-β induced T_{RM} recapitulate the transcriptional and proteomic landscape of endogenous T_{RM} as well as pathways associated with migration and metabolism.

* * *

[00123] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

WHAT IS CLAIMED IS:

1. An *in vitro* method for producing tissue resident memory-like T cells (T_{RM}-like T cells) comprising:

(a) obtaining a starting population of T cells;

(b) culturing the starting population of T cells in hypoxic conditions or in the presence of a hypoxia-inducing agent to generate early effector cells; and

(c) further culturing the early effector cells in the presence of transforming growth factor beta 1 (TGF-β1) to produce T_{RM}-like T cells.

2. The method of claim 1, wherein the T cells of the starting population are $CD8^+$ peripheral blood T cells.

3. The method of claim 2, wherein the $CD8^+$ peripheral blood T cells are human $CD8^+$ peripheral blood T cells.

4. The method of claim 3, wherein obtaining the human $CD8^+$ peripheral blood T cells comprises selecting for $CD45RA^+CCR7^+CD8^+$ naïve T cells from a peripheral blood sample.

5. The method of claim 4, wherein the peripheral blood sample is obtained from a healthy subject.

6. The method of claim 4, wherein the peripheral blood sample is obtained from a subject diagnosed with cancer or suspected of having cancer.

7. The method of claim 4, wherein the peripheral blood sample is obtained from a subject diagnosed with a viral disease or is suspected of having a viral disease.

8. The method of claim 1, wherein the T cells of the starting population are stimulated by antigen presenting cells pulsed with peptide, full length antigen or cell lysate prior to culturing.

9. The method of claim 8, wherein the T cells are obtained from a tumor site or are tumor infiltrating lymphocytes.

10. The method of claim 9, wherein the T cells are naïve T cells.

11. The method of claim 8, wherein the cell lysate is a tumor lysate.

12. The method of claim 8, wherein the antigen is a cancer antigen or a viral antigen.

13. The method of claim 8, wherein the peptide is a peptide from a protein that is differentially expressed in or highly expressed by cancer cells.

14. The method of claim 8, wherein the peptide is a peptide from a neoantigen or from a protein comprising a mutation.

15. The method of claim 8, wherein the starting population of T cells is enriched for T cells specific for an antigen of interest.

16. The method of claim 8, wherein the starting population of T cells is purified to enrich for cells that are CD8-positive and peptide MHC tetramer-positive.

17. The method of claim 16, wherein the starting population of T cells is purified by fluorescence activated cell sorting.

18. The method of claim 1, wherein the starting population of T cells is a population of engineered T cells.

19. The method of claim 18, wherein the engineered T cells are generated by introduction of a cloned T cell receptor (TCR) into a population of host cells.

20. The method of claim 19, wherein the host cells are peripheral blood mononuclear cells.

21. The method of claim 19, wherein the cloned TCR is introduced into the population of host cells by non-viral methods.

22. The method of claim 21, wherein the cloned TCR is introduced into the population of host cells by an episomal vector or transposons.

23. The method of claim 19, wherein the cloned TCR is introduced into the population of host cells by transduction.

24. The method of claim 23, wherein the population of host cells are transduced by a viral vector comprising TCR alpha and TCR beta chains.

25. The method of claim 24, wherein the viral vector is a lentiviral vector.

26. The method of claim of claim 24, wherein the transduced population of host cells is purified to enrich for CD8-positive/peptide MHC tetramer-positive cells.

27. The method of claim 18, wherein the engineered T cells express a chimeric antigen receptor.

28. The method of claim 27, wherein the chimeric antigen receptor comprises an antigen recognition domain that specifically binds a peptide from a cancer antigen, a viral antigen, a neoantigen or a protein comprising a mutation.

29. The method of claim 1, wherein the T cells of the starting population are tumor infiltrating lymphocytes obtained from a subject.

30. The method of claim 1, wherein hypoxic conditions are further defined as less than 5% oxygen.

31. The method of claim 1, wherein hypoxic conditions are further defined as 2% oxygen.

32. The method of claim 1, wherein the hypoxia-inducing agent is a hypoxia mimetic.

33. The method of claim 32, wherein the hypoxia-inducing agent or hypoxia mimetic is cobalt chloride ($CoCl_2$), deferoxamine mesylate (DFOM), dimethyloxalylglycine (DMOG), or a prolyl hydroxylase inhibitor.

34. The method of claim 33, wherein the prolyl hydroxylase inhibitor is a 2-OG analog.

35. The method of claim 33, wherein the prolyl hydroxylase inhibitor is Roxadustat (FG-4592).

36. The method of claim 1, wherein the culturing of step (b) is in the presence of TCR stimulation and co-stimulation.

37. The method of claim 36, wherein the TCR stimulation and co-stimulation comprises anti-CD3 and anti-CD28 antibodies, anti-CD3 and anti-CD28 beads, feeder cells, antigen presenting cells, artificial antigen presenting cells, peptide and/or protein antigens, or a combination thereof.

38. The method of claim 36, wherein the TCR stimulation and co-stimulation comprises anti-CD3 and anti-CD28 beads.

39. The method of claim 1, wherein the culturing of step (b) is for 3-5 days.

40. The method of claim 1, wherein the culturing of step (b) is for 4 days.

41. The method of claim 36, wherein the culturing of step (c) is in the presence of IL-15.

42. The method of claim 41, wherein the IL-15 is present at a concentration of 5-20 ng/mL.

43. The method of claim 41, wherein the IL-15 is present at a concentration of 10 ng/mL.

44. The method of claim 1, wherein TGF-β1 is further defined as recombinant human TGF-β1 (rhTGF-β1).

45. The method of claim 44, wherein the rhTGF-β1 is present at a concentration of 0.1 to 5 ng/mL.

46. The method of claim 45, wherein the rhTGF-β1 is present at a concentration of 1 to 1.5 ng/mL.

47. The method of claim 45, wherein the rhTGF-β1 is present at a concentration of 1.25 ng/mL.

48. The method of claim 1, wherein the culturing of step (c) is in hypoxic conditions or in the presence of a hypoxia-inducing agent.

49. The method of claim 48, wherein the culturing of step (c) is in the presence of IL-15.

50. The method of claim 49, wherein the IL-15 is present at a concentration of 5-20 ng/mL.

51. The method of claim 49, wherein the IL-15 is present at a concentration of 10 ng/mL.

52. The method of claim 1, wherein the culturing of step (c) is for 1-3 days.

53. The method of claim 1, wherein the culturing of step (c) is for 2 days.

54. The method of claim 1, wherein the T_{RM}-like T cells are $CD69^+CD103^+$.

55. The method of claim 54, wherein at least 30% of the cells produced in step (c) are $CD69^+CD103^+$ cells.

56. The method of claim 54, wherein at least 40% of the cells produced in step (c) are $CD69^+CD103^+$ cells.

57. The method of claim 1, wherein the T_{RM}-like T cells express PD-1, CD101, and/or CD49a.

58. The method of claim 1, wherein the T_{RM}-like T cells have higher expression of CD69, ITGAE, PDCD1 and/or CD101 as compared to cells cultured in atmospheric oxygen conditions.

59. The method of claim 58, wherein the higher expression of CD69, ITGAE, PDCD1 and/or CD101, is higher expression of CD69, ITGAE, PDCD1 and/or CD101 mRNA transcripts.

60. The method of claim 58, wherein the higher expression of CD69, ITGAE, PDCD1 and/or CD101 is higher expression of CD69, ITGAE, PDCD1 and/or CD101 protein.

61. The method of claim 1, wherein the T_{RM}-like T cells have higher expression of TNFα, GZMB, SLC2A1, and/or VEGF as compared to cells cultured in atmospheric oxygen conditions.

62. The method of claim 1, wherein the T_{RM}-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B,

NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions.

63. The method of claim 62, wherein the T_{RM}-like T cells have higher expression of GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions.

64. The method of claim 62, wherein the T_{RM}-like T cells have higher expression of ITGAE, ITGA1, PDC1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1 as compared to cells cultured in atmospheric oxygen conditions.

65. The method of claim 62, wherein the T_{RM}-like T cells have higher expression of MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3 as compared to cells cultured in atmospheric oxygen conditions.

66. The method of claim 1, wherein the T_{RM}-like T cells have lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2 as compared to cells cultured in atmospheric oxygen conditions.

67. The method of claim 66, wherein the T_{RM}-like T cells have lower expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3,

RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8 as compared to cells cultured in atmospheric oxygen conditions.

68. The method of claim 66, wherein the T_{RM}-like T cells have lower expression of KLF2, KLF3, SELL, FAM65B, and/or SERPINE2 as compared to cells cultured in atmospheric oxygen conditions.

69. The method of claim 66, wherein the T_{RM}-like T cells have lower expression of DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, ITGAM, FOS, KLF3, SLAMF7, TNFSF8, SLC6A8, KLF2, SOCS3, and/or PTGER2 as compared to cells cultured in atmospheric oxygen conditions.

70. The method of claim 1, wherein the T_{RM}-like T cells have decreased expression of S1PR1, KLF4 and/or SELL as compared to cells cultured in atmospheric oxygen conditions.

71. The method of claim 1, wherein the T_{RM}-like T cells have essentially no expression of CXCR6 protein.

72. The method of claim 1, wherein the T_{RM}-like T cells are antigen specific.

73. The method of claim 1, further comprising producing antigen-specific T_{RM}-like T cells.

74. The method of claim 73, wherein producing antigen-specific T_{RM}-like T cells comprises transducing the T_{RM}-like T cells with an antigen-specific T cell receptor (TCR).

75. The method of claim 73, wherein producing antigen-specific T_{RM}-like T cells comprises culturing the starting population of T cells with peptide-pulsed antigen presenting cells (APCs) during step (b).

76. The method of claim 75, wherein the APCs are mature dendritic cells.

77. The method of claim 75, wherein the APCs are artificial APCs (aAPCs).

78. The method of claim 77, wherein steps (b) and (c) are repeated at least once.

79. The method of claim 73, wherein producing antigen-specific T_{RM}-like T cells comprises culturing the cells in the presence of a histone deacetylase (HDAC) inhibitor during step (b) and/or step (c).

80. The method of claim 79, wherein the HDAC inhibitor is selected from the group consisting of trichostatin A, trapoxin B, phenylbutyrate, valproic acid, vorinostat (suberanilohydroxamic acid or SAHA, marketed as Zolinza®), belinostat (PXD101, marketed as Beleodaq®), panobinostat (marketed as Farydaq®), dacinostat (LAQ824), entinostat (SNDX-275 or MS-275), tacedinaline (CI994), and mocetinostat (MGCD0103).

81. A T_{RM}-like T cell, wherein the T cell has essentially no expression of CXCR6 protein.

82. The T_{RM}-like T cell of claim 81, wherein the T_{RM}-like T cell is produced by the method of any one of claims 1-80.

83. The T_{RM}-like T cell of claim 81, wherein the T_{RM}-like T cell expresses PD-1, CD101, and/or CD49a.

84. The T_{RM}-like T cell of claim 81, wherein the T_{RM}-like T cell is $CD69^{+}CD103^{+}$.

85. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell expresses GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3.

86. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell expresses GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1.

87. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell expresses ITGAE, ITGA1, PDCD1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1.

88. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell expresses MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3.

89. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell does not express or has essentially no expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2.

90. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell does not express or has essentially no expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8.

91. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell does not express or has essentially no expression of KLF2, KLF3, SELL, FAM65B, and/or SERPINE2.

92. The T_{RM}-like T cell of claim 81, wherein T_{RM}-like T cell does not express or has essentially no expression of DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, ITGAM, FOS, KLF3, SLAMF7, TNFSF8, SLC6A8, KLF2, SOCS3, and/or PTGER2.

93. A pharmaceutical composition comprising a population of T_{RM}-like T cells with essentially no expression of CXCR6 protein and a pharmaceutically acceptable carrier.

94. The composition of claim 93, wherein the T_{RM}-like T cells are produced by the method of any one of claims 1-80.

95. The composition of claim 93, wherein the T_{RM}-like T cells express PD-1, CD101, and/or CD49a.

96. The composition of claim 93, wherein at least 40% of the cells are $CD69^{+}CD103^{+}$ cells.

97. The composition of claim 93, wherein T_{RM}-like T cells are $CD69^{+}CD103^{+}$ cells.

98. The composition of claim 93, wherein T_{RM}-like T cell expresses GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, RGS1, ITGA1, CD101, TNFRSF9 (4-1BB), CCL4, CCL5, NOTCH1, RBPJ, STRIP2, ARHGEF40, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CDK14, LMCD1, ILDR2, and/or ADCY3.

99. The composition of claim 93, wherein the T_{RM}-like T cells express GNLY, MYO7A, ITGAE, EGR2, CCL20, ATP1B1, NR4A3, PERP, RASGEF1B, NR4A1, BMF, EGR1, CXCL13, PDCD1, ITGA1, CCL22, CA10, and/or RGS1.

100. The composition of claim 93, wherein the T_{RM}-like T cells express ITGAE, ITGA1, PDC1, CD101, TNFRSF9 (4-1BB), CXCL13, CCL20, NOTCH1, RBPJ, NR4A1, EGR2, and/or RGS1.

101. The composition of claim 93, wherein the T_{RM}-like T cells express MYO7A, STRIP2, ARHGEF40, ITGAE, DBH, SRGAP3, CSGALNACT1, GPR25, RGS16, DAPK2, NCS1, COL6A3, GDPD4, SLC1A4, CXCL13, CDK14, LMCD1, ILDR2, and/or ADCY3.

102. The composition of claim 93, wherein the T_{RM}-like T cells express do not express or have essentially no expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2,

TBX21, ITGAL, SELL, KLF3, KLF2, RASGRP2, FAM65B, SERPINE2, ITGAM, KLRB1, TGFBR3, SMAD3, TNFSF8, DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, SLAMF7, SLC6A8, SOCS3, and/or PTGER2.

103. The composition of claim 93, wherein the T_{RM}-like T cells express do not express or have essentially no expression of CD58, NR3C1, RAP1GAP2, SELP, CXCR2, TBX21, ITGAL, SELL, KLF3, RASGRP2, ITGAM, KLRB1, TGFBR3, SMAD3, and/or TNFSF8.

104. The composition of claim 93, wherein the T_{RM}-like T cells express do not express or have essentially no expression of KLF2, KLF3, SELL, FAM65B, and/or SERPINE2.

105. The composition of claim 93, wherein the T_{RM}-like T cells express do not express or have essentially no expression of DUSP2, PLEK, GOLGA2P7, FOSB, PLCG2, ITGAM, FOS, KLF3, SLAMF7, TNFSF8, SLC6A8, KLF2, SOCS3, and/or PTGER2.

106. A composition comprising an effective amount of T_{RM}-like T cells with essentially no expression of CXCR6 protein for the treatment an immune-related disorder in a subject.

107. The composition of claim 106, wherein the T_{RM}-like T cells are produced by the method of any one of claims 1-80.

108. The use of an effective amount of T_{RM}-like T cells with essentially no expression of CXCR6 protein for the treatment of an immune-related disorder in a subject.

109. The use of claim 108, wherein the T_{RM}-like T cells are produced by the method of any one of claims 1-80.

110. A method of treating an immune-related disorder in a subject comprising administering an effective amount of T_{RM}-like T cells produced by the method of any one of claims 1-80 to the subject.

111. The method of claim 110, wherein the immune-related disorder is a cancer, autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition.

112. The method of claim 111, wherein the subject has received a tissue or organ transplant.

113. The method of claim 110, further comprising administering at least a second therapeutic agent.

114. The method of claim 113, wherein the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy.

115. The method of claim 113, wherein the T_{RM}-like T cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

116. The method of claim 113, wherein the T_{RM}-like T cells are administered prior to the second therapeutic agent.

117. The method of claim 113, wherein the T_{RM}-like T cells are administered after the second therapeutic agent.

118. The method of claim 113, wherein the T_{RM}-like T cells are administered concurrently with the second therapeutic agent.

119. The method of claim 114, wherein the immunotherapy is a 4-1BB agonist.

120. The method of claim 120, wherein the 4-1BB agonist is a 4-1BB antibody.

121. The method of claim 114, wherein the immunotherapy is an immune checkpoint therapy.

122. The method of claim 121, wherein the immune checkpoint therapy is CTLA-4, PD-1, or PD-L1 blockade or inhibition.

123. The method of claim 110, wherein the subject is human.

124. A method of treating a viral infection in a subject comprising administering an effective amount of T_{RM}-like T cells produced by the method of any one of claims 1-80 to the subject.

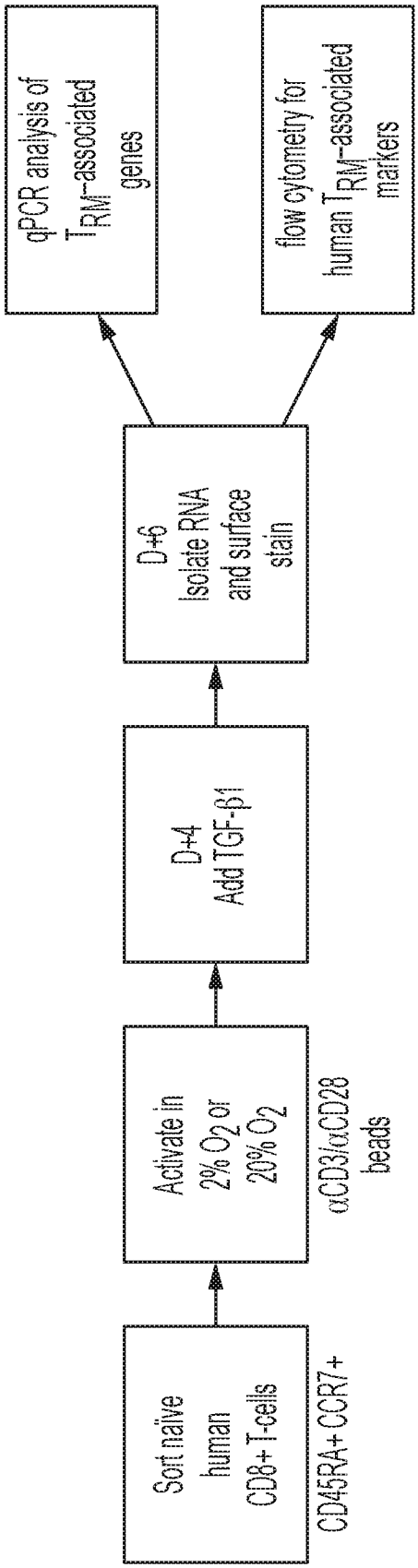
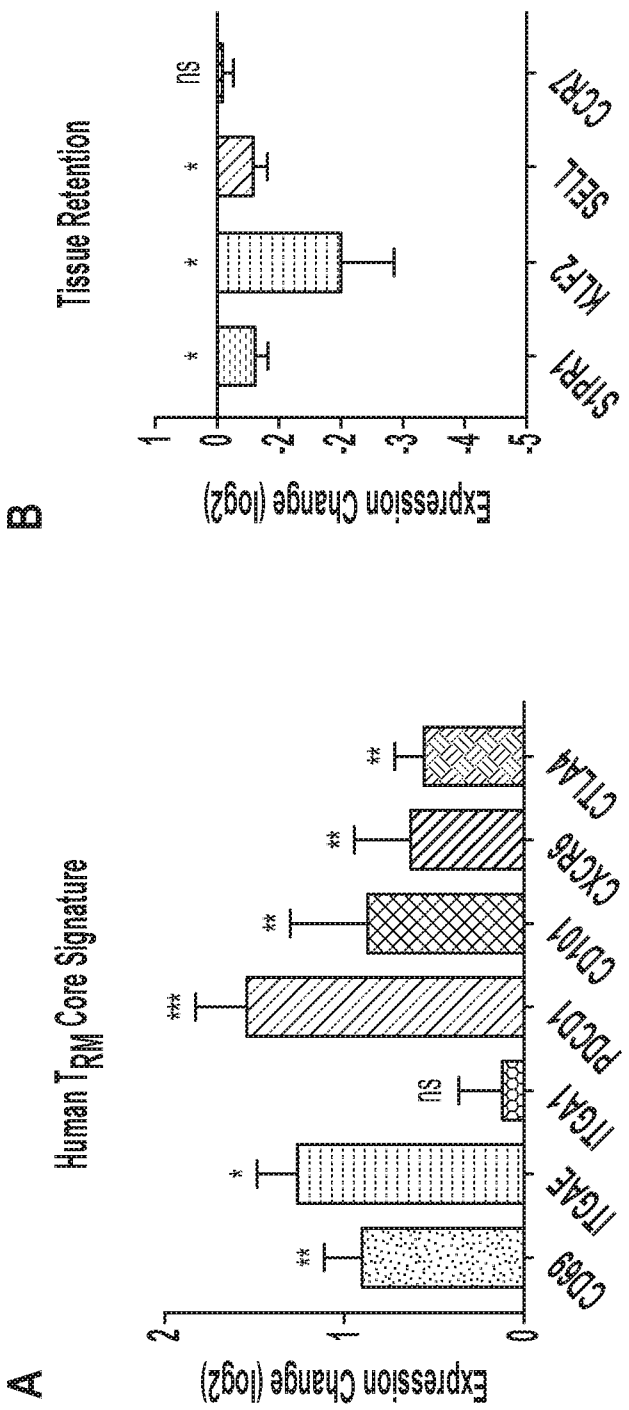
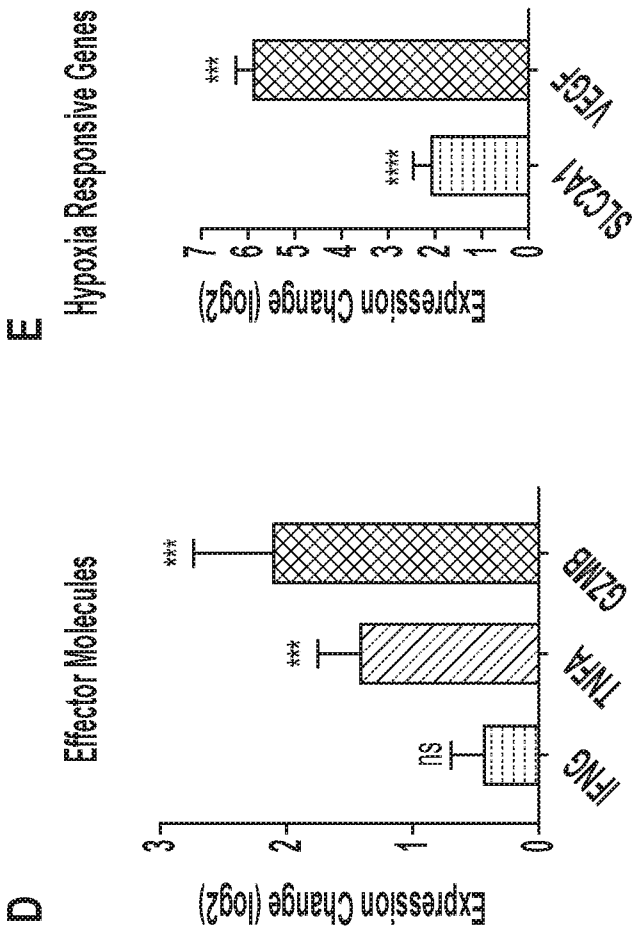

Sort naïve human CD8+ T-cells
CD45RA+ CCR7+
Activate in 2% O_2 or 20% O_2
αCD3/αCD28 beads
D+4 Add TGF-β1
D+6 Isolate RNA and surface stain
qPCR analysis of T_{RM}-associated genes
flow cytometry for human T_{RM}-associated markers

FIG. 1


A
Human TRM Core Signature
Expression Change (log2)
2
1
0
CD69
ITGAE
ITGA1
PDCD1
CD101
CXCR6
CTLA4
**
*
ns
***
**
**
**
B
Tissue Retention
Expression Change (log2)
1
0
-.2
-.2
-.3
-.4
-.5
S1PR1
KLF2
SELL
CCR7
*
*
*
ns

FIGS. 2A-2B


C
Transcription Factors
Expression Change (log2)
2
1
0
-1
-2
-3
-4
****
ns
*
****
***
EOMES
TBX21
TCF7
IRF4
RUNX3
D
Effector Molecules
Expression Change (log2)
3
2
1
0
ns
**
***
IFNG
TNFA
GZMB
E
Hypoxia Responsive Genes
Expression Change (log2)
7
6
5
4
3
2
1
0
****
***
SLC2A1
VEGF

FIGS. 2C-E

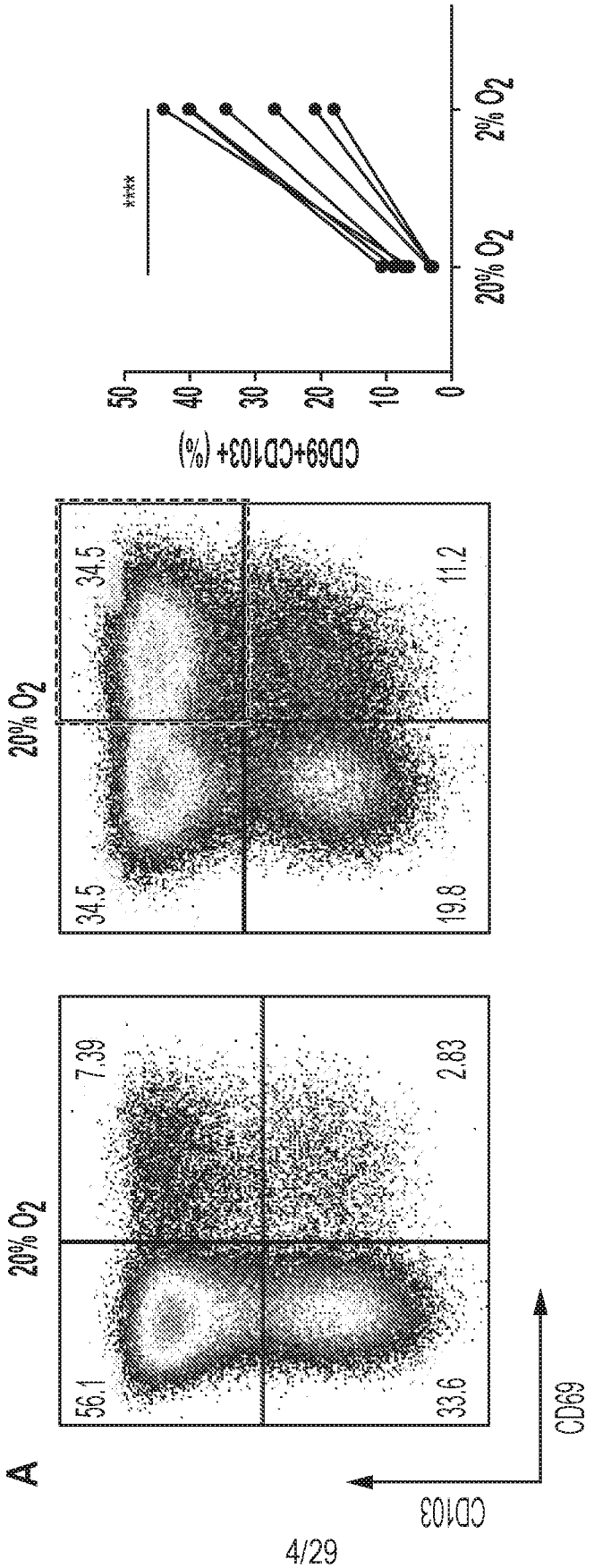

A
20% O2
56.1
7.39
33.6
2.83
CD103
CD69
20% O2
34.5
34.5
11.2
19.8
CD69+CD103+ (%)
50
40
30
20
10
0
****
20% O2
2% O2

FIG. 3A

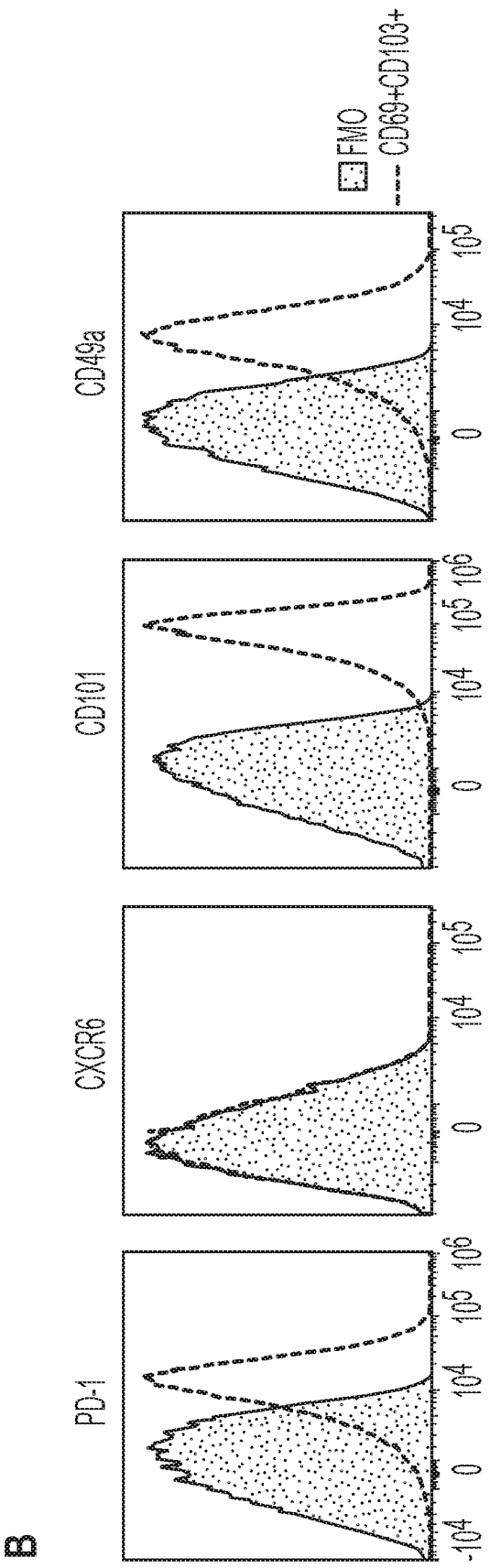


FIG. 3B

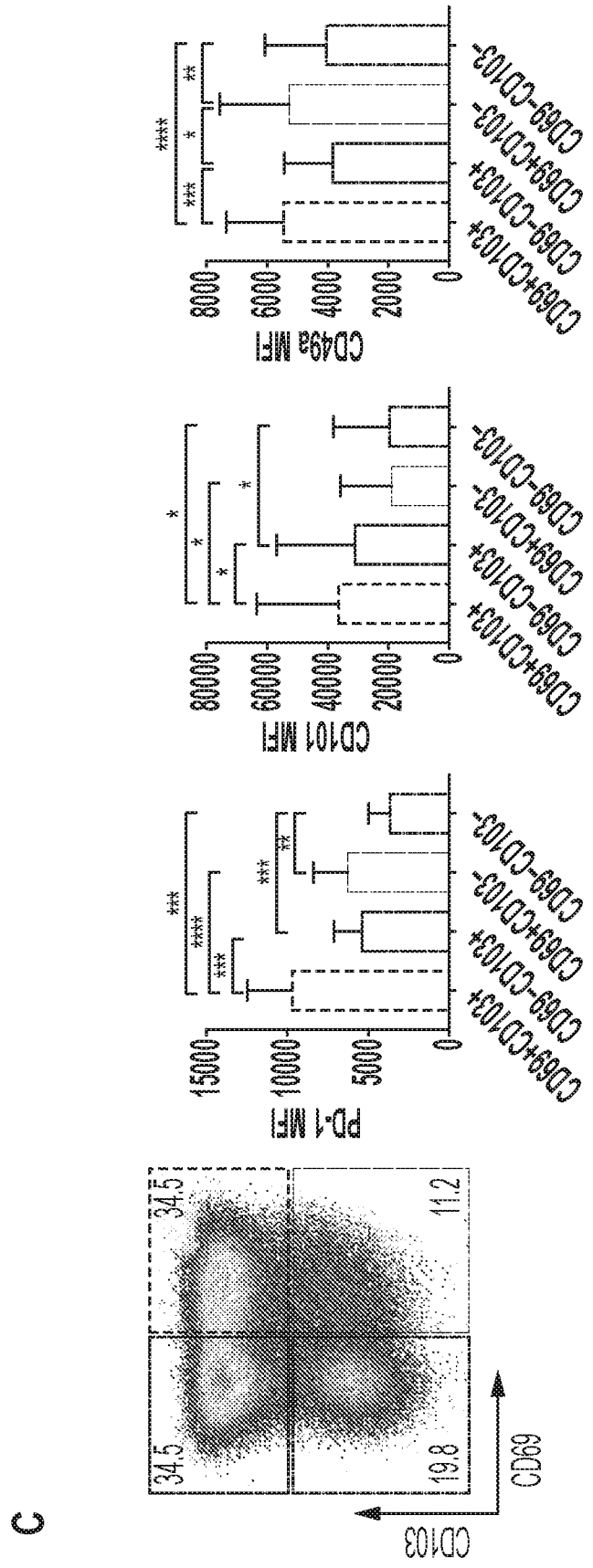

C
CD103
CD69
34.5
11.2
34.5
19.8
PD-1 MFI
15000
10000
5000
0
CD101 MFI
80000
60000
40000
20000
0
CD49a MFI
8000
6000
4000
2000
0
CD69+CD103+
CD69-CD103+
CD69+CD103-
CD69-CD103-

FIG. 3C

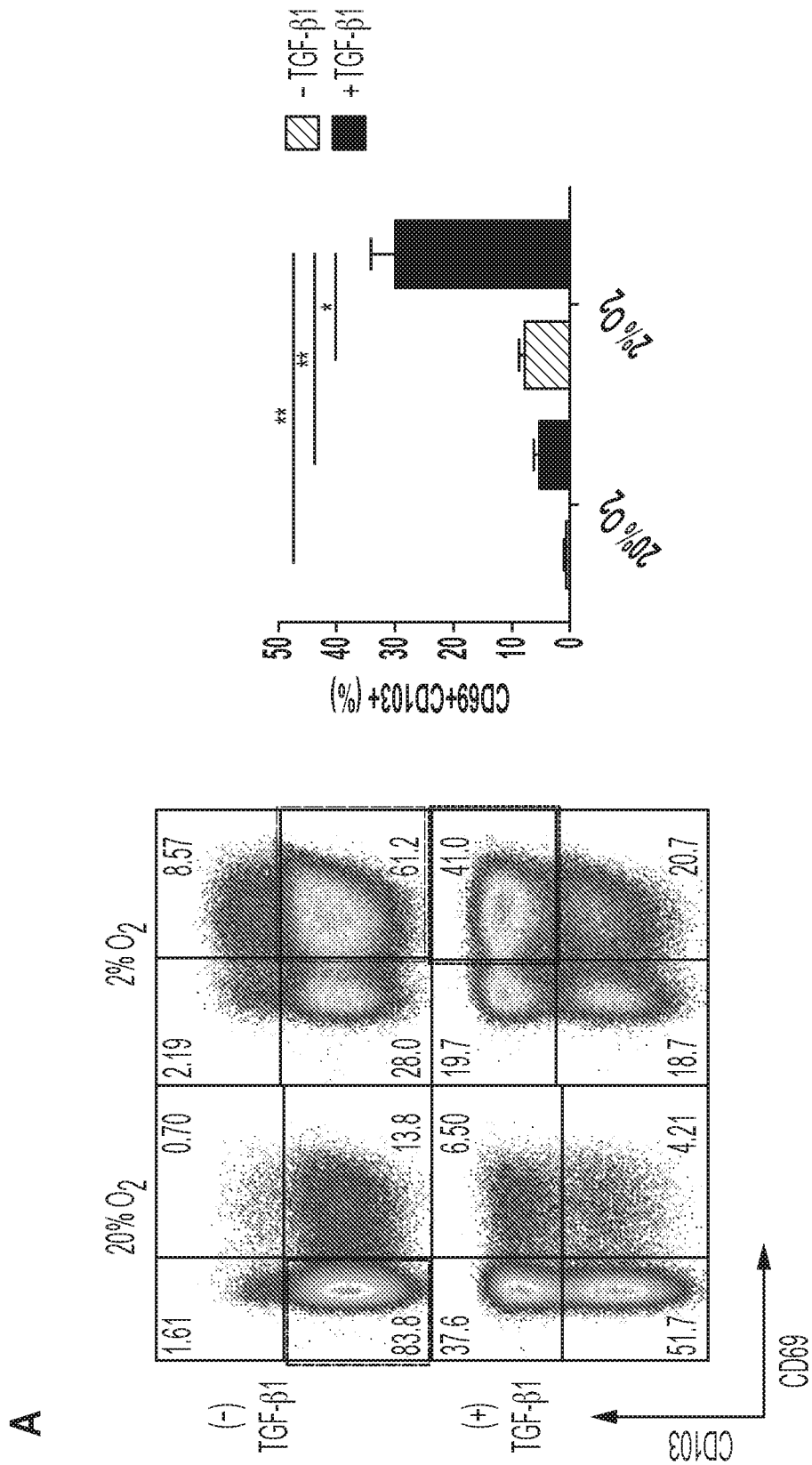

A
20% O2
2% O2
(-) TGF-β1
(+) TGF-β1
CD103
CD69
1.61
83.8
0.70
13.8
2.19
28.0
8.57
61.2
37.6
6.50
51.7
4.21
19.7
41.0
18.7
20.7
- TGF-β1
+ TGF-β1
CD69+CD103+ (%)
50
40
30
20
10
0
20% O2
2% O2
**
**
*

FIG. 4A

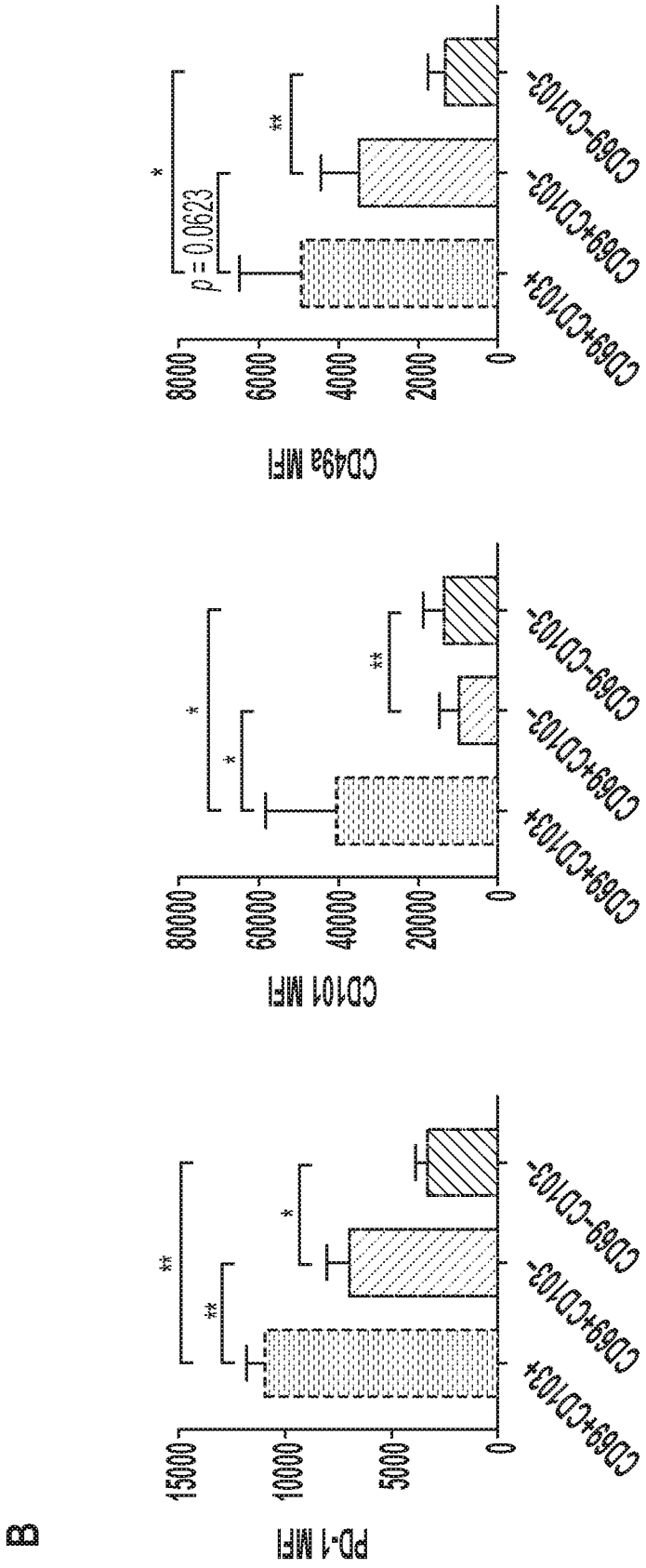

B
PD-1 MFI
15000
10000
5000
0
**
**
*
CD69-CD103-
CD69+CD103-
CD69+CD103+
CD101 MFI
80000
60000
40000
20000
0
**
*
*
CD69-CD103-
CD69+CD103-
CD69+CD103+
CD49a MFI
8000
6000
4000
2000
0
**
*
p = 0.0623
CD69-CD103-
CD69+CD103-
CD69+CD103+

FIG. 4B

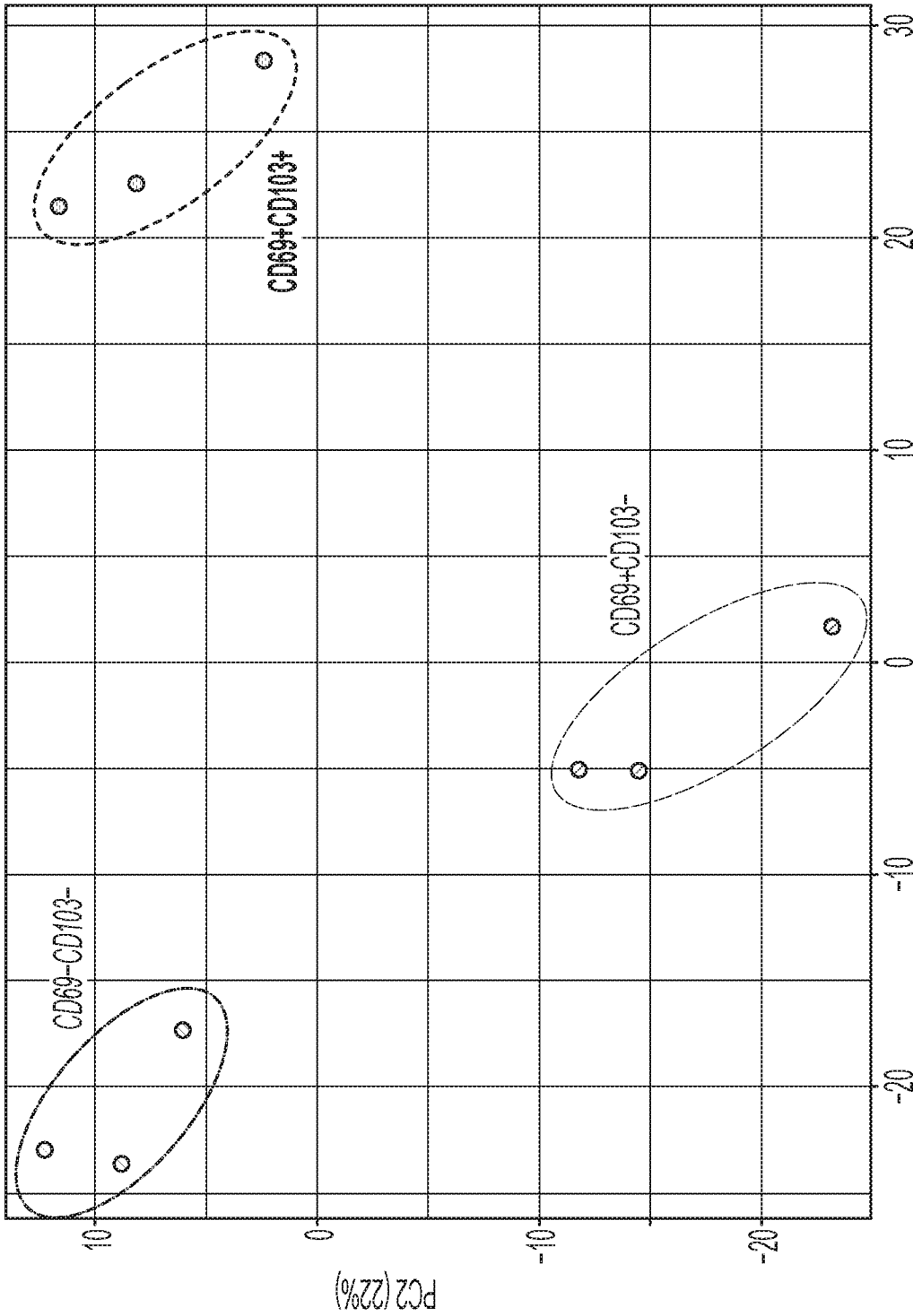

CD69+CD103+
CD69+CD103-
CD69-CD103-
PC1 (51%)
PC2 (22%)
30
20
10
0
-10
-20
10
0
-10
-20

FIG. 5A

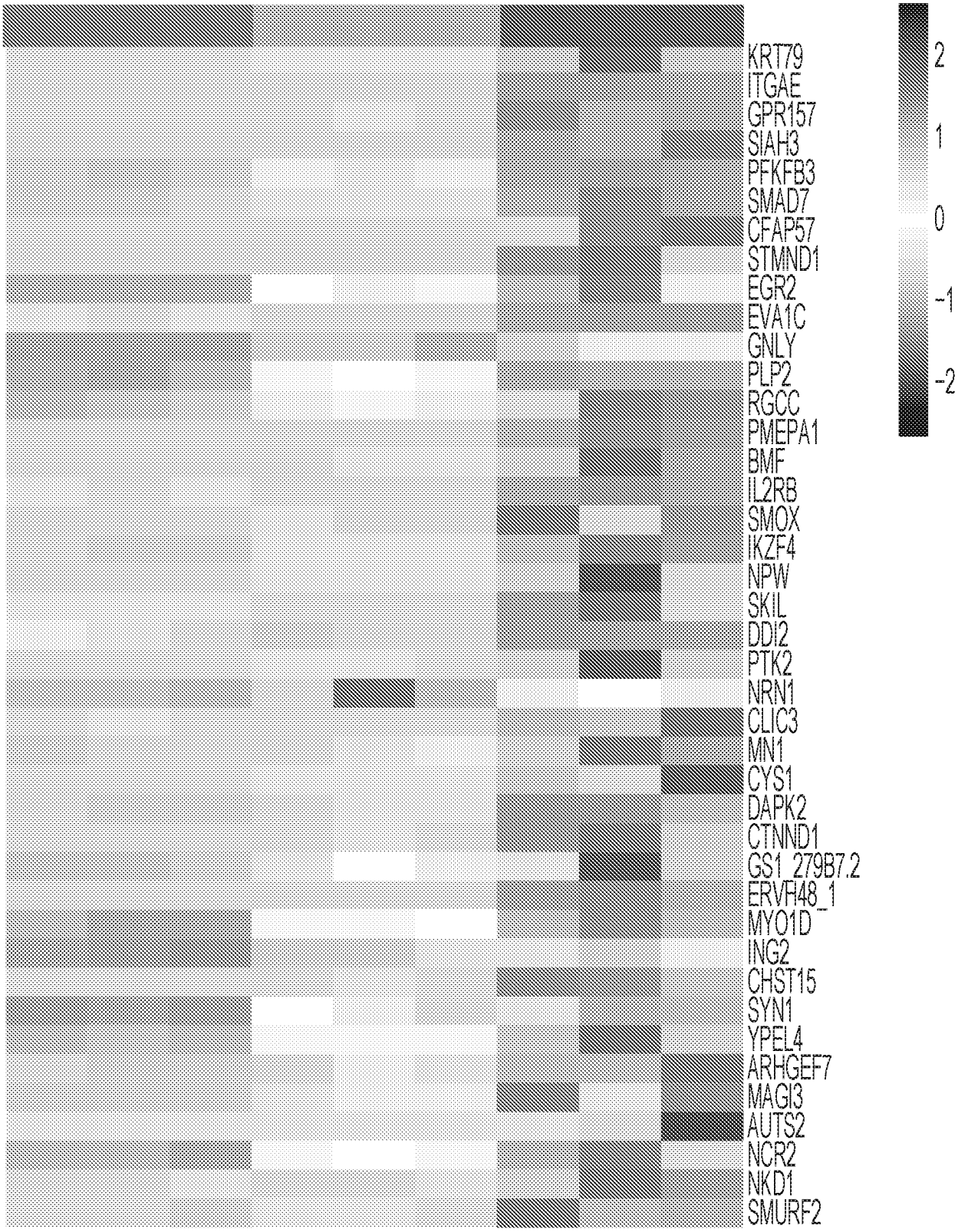

KRT79
ITGAE
GPR157
SIAH3
PFKFB3
SMAD7
CFAP57
STMND1
EGR2
EVA1C
GNLY
PLP2
RGCC
PMEPA1
BMF
IL2RB
SMOX
IKZF4
NPW
SKIL
DDI2
PTK2
NRN1
CLIC3
MN1
CYS1
DAPK2
CTNND1
GS1_279B7.2
ERVH48_1
MYO1D
ING2
CHST15
SYN1
YPEL4
ARHGEF7
MAGI3
AUTS2
NCR2
NKD1
SMURF2
2
1
0
-1
-2

FIG. 5B

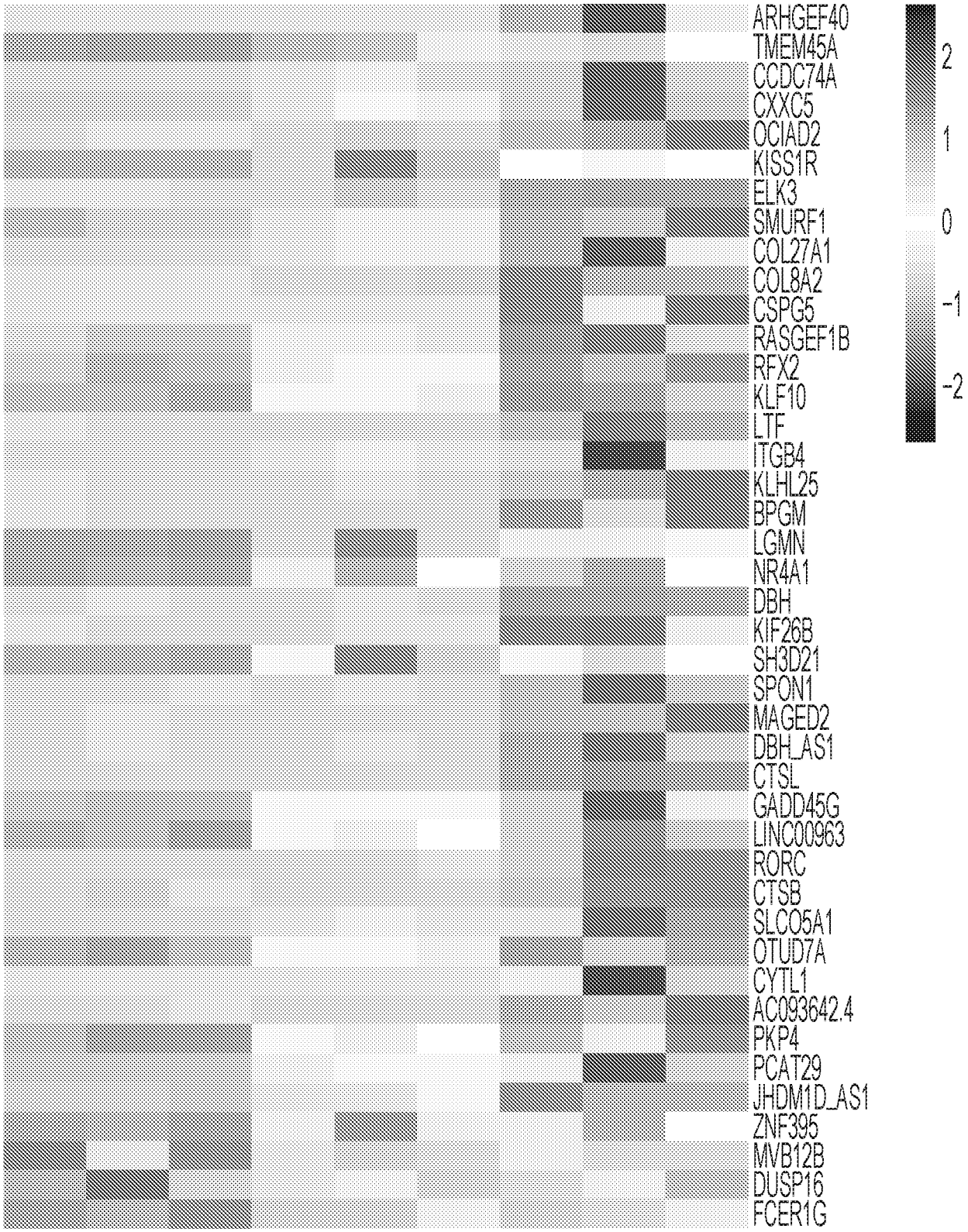


FIG. 5B continued

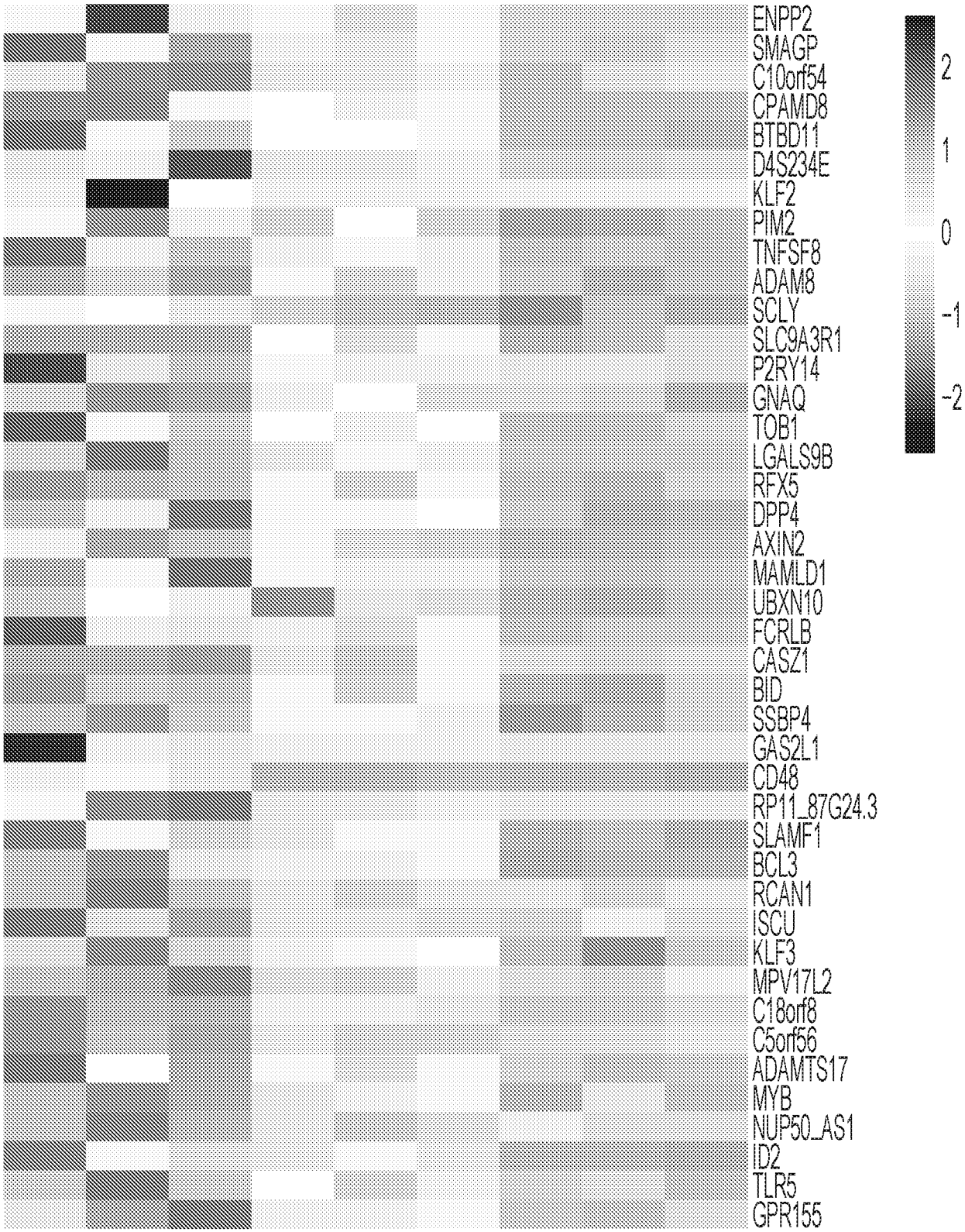


FIG. 5B continued

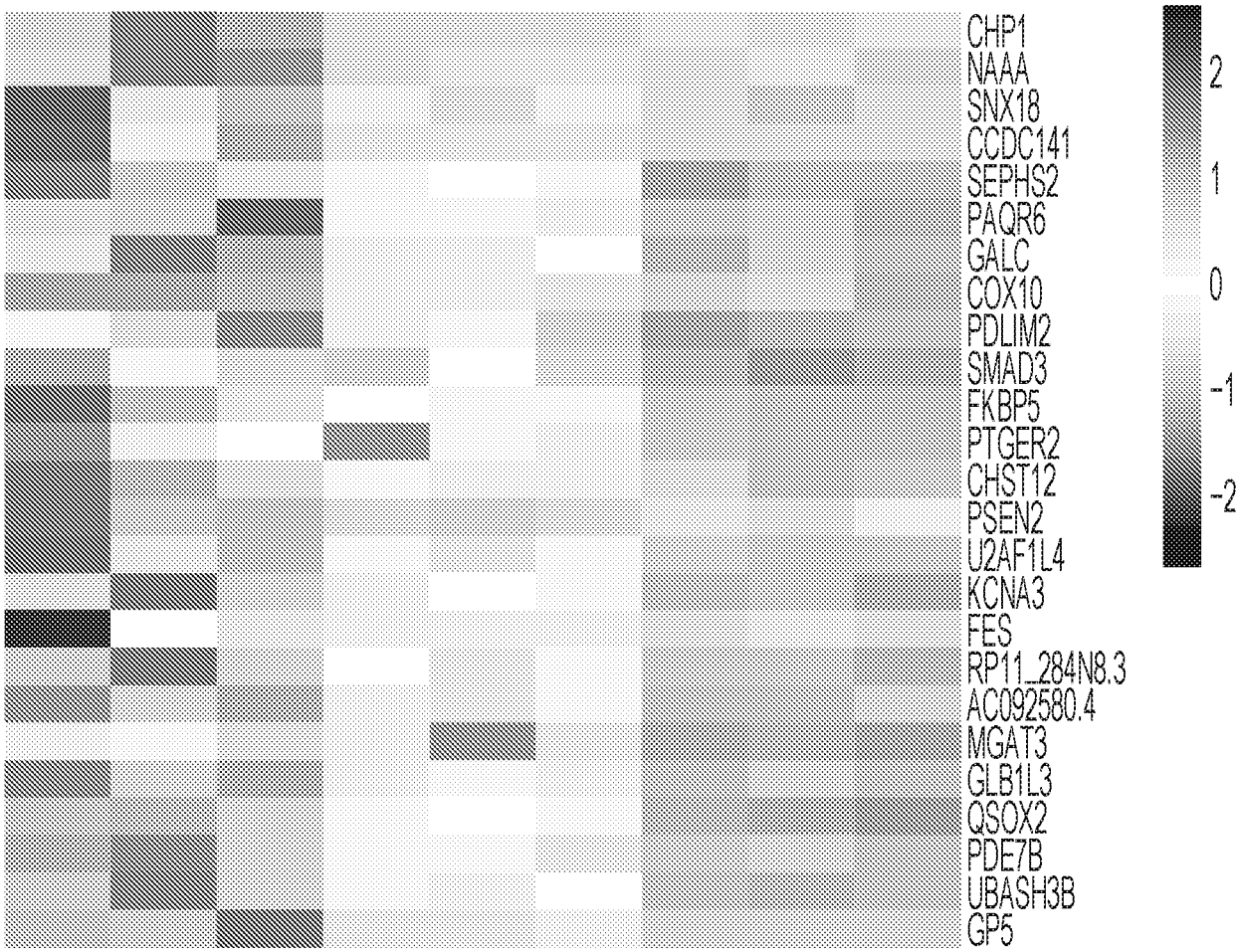


FIG. 5B continued

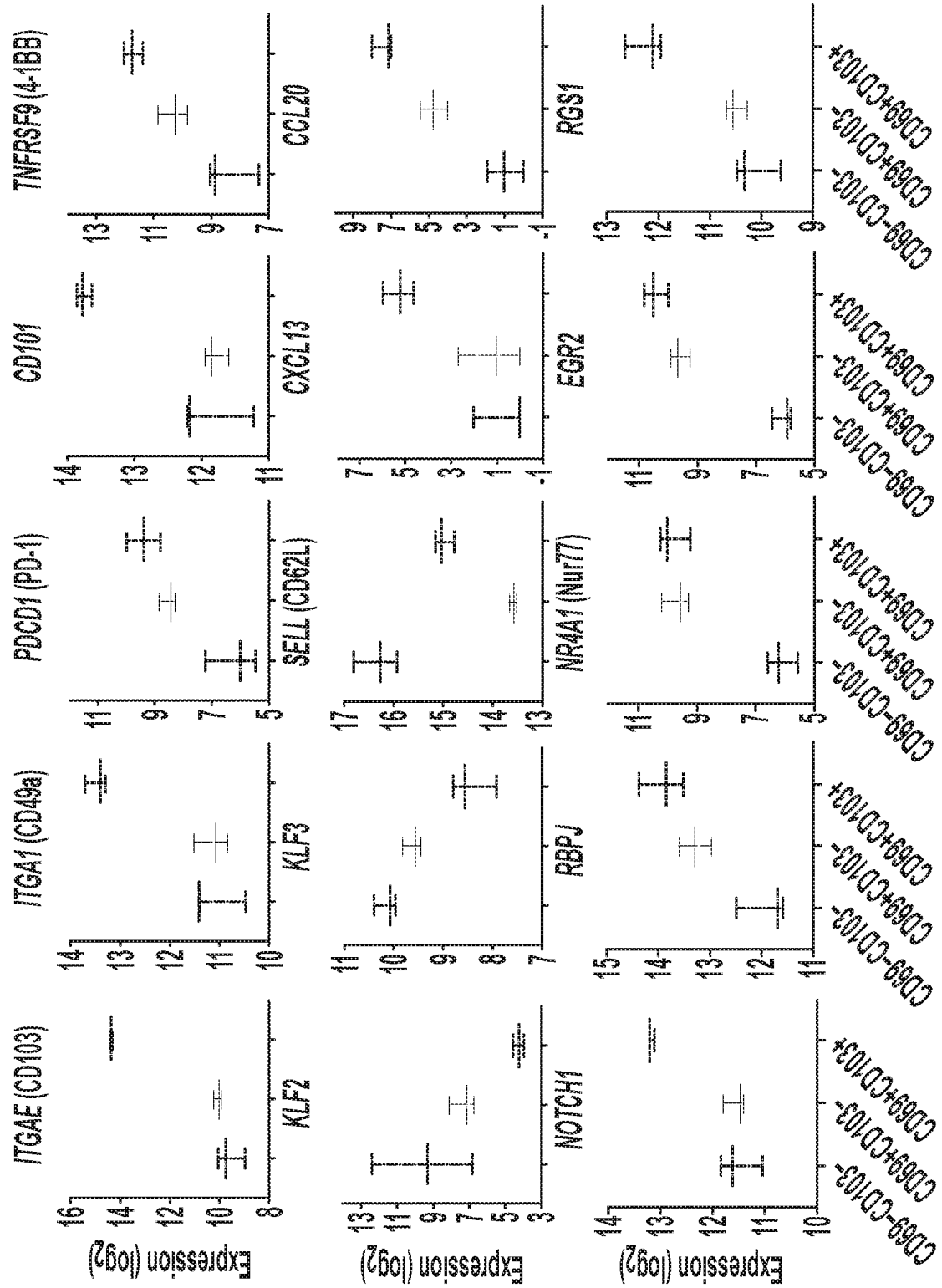

ITGAE (CD103)
ITGA1 (CD49a)
PDCD1 (PD-1)
CD101
TNFRSF9 (4-1BB)
KLF2
KLF3
SELL (CD62L)
CXCL13
CCL20
NOTCH1
RBPJ
NR4A1 (Nur77)
EGR2
RGS1
Expression (log2)
CD69-CD103-
CD69+CD103-
CD69+CD103+

FIG. 5C

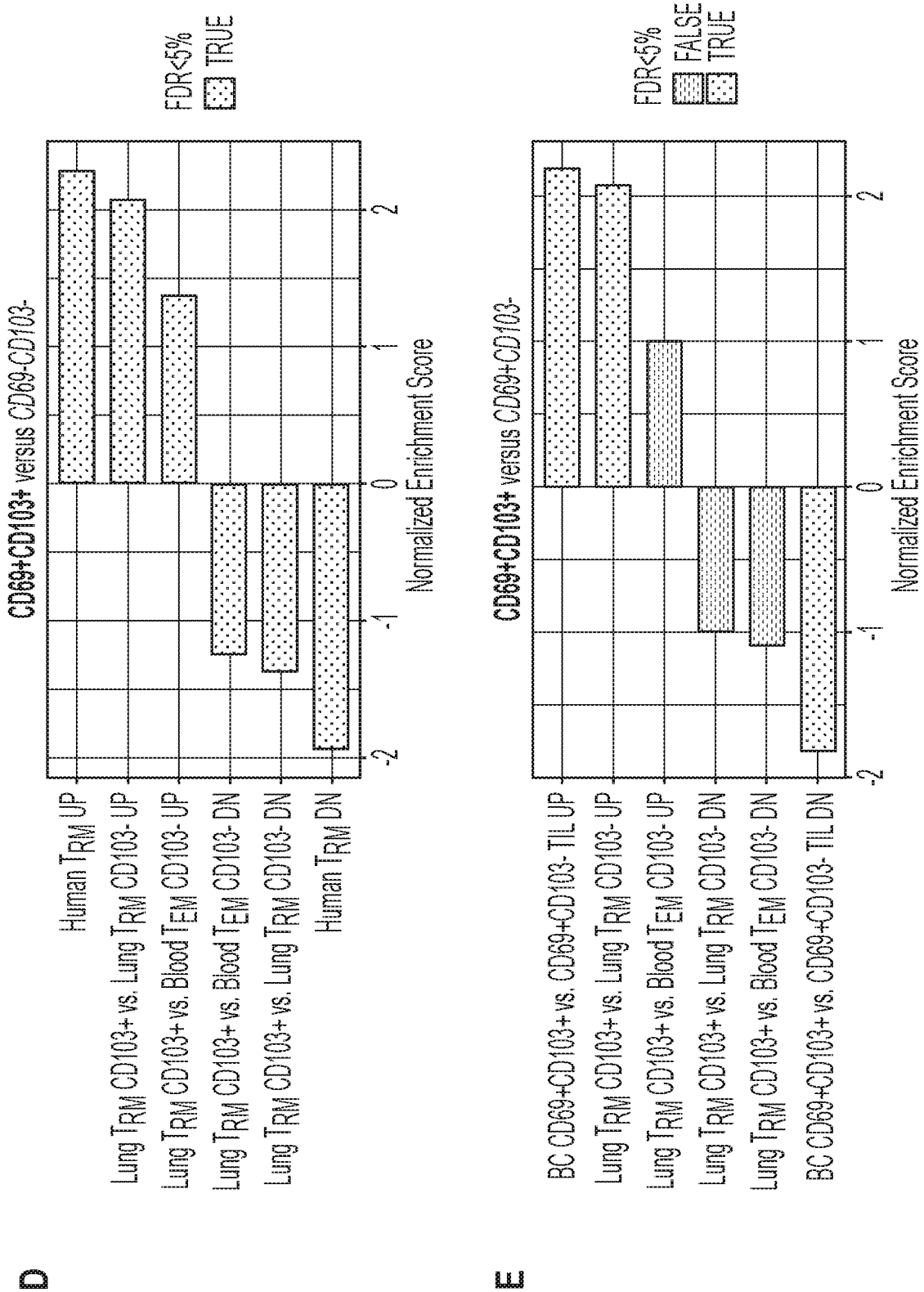

D
CD69+CD103+ versus CD69-CD103-
FDR<5%
TRUE
Human TRM UP
Lung TRM CD103+ vs. Lung TRM CD103- UP
Lung TRM CD103+ vs. Blood TEM CD103- UP
Lung TRM CD103+ vs. Blood TEM CD103- DN
Lung TRM CD103+ vs. Lung TRM CD103- DN
Human TRM DN
-2
-1
0
1
2
Normalized Enrichment Score
E
CD69+CD103+ versus CD69+CD103-
FDR<5%
FALSE
TRUE
BC CD69+CD103+ vs. CD69+CD103- TIL UP
Lung TRM CD103+ vs. Lung TRM CD103- UP
Lung TRM CD103+ vs. Blood TEM CD103- UP
Lung TRM CD103+ vs. Lung TRM CD103- DN
Lung TRM CD103+ vs. Blood TEM CD103- DN
BC CD69+CD103+ vs. CD69+CD103- TIL DN
-2
-1
0
1
2
Normalized Enrichment Score

FIG. 5D-E

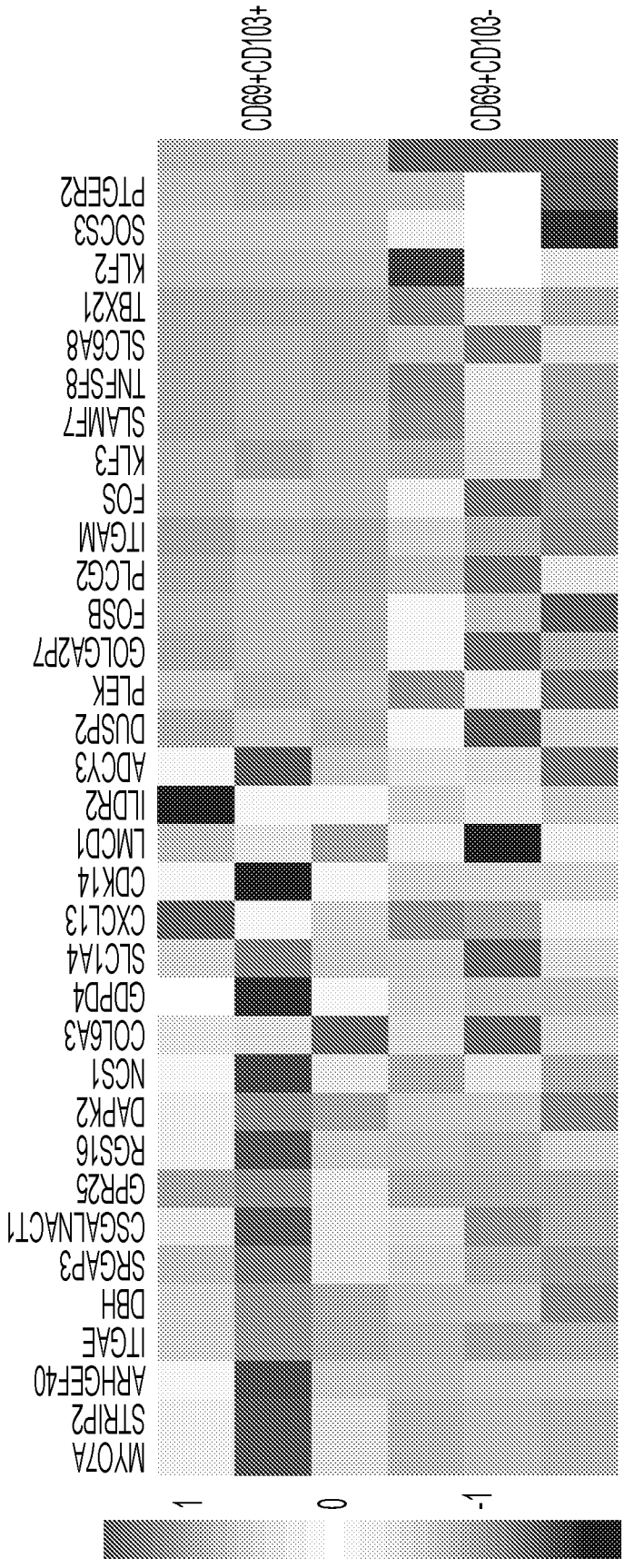

CD69+CD103+
CD69+CD103-
PTGER2
SOCS3
KLF2
TBX21
SLC6A8
TNFSF8
SLAMF7
KLF3
FOS
ITGAM
PLCG2
FOSB
GOLGA2P7
PLEK
DUSP2
ADCY3
ILDR2
LMCD1
CDK14
CXCL13
SLC1A4
GDPD4
COL6A3
NCS1
DAPK2
RGS16
GPR25
CSGALNACT1
SRGAP3
DBH
ITGAE
ARHGEF40
STRIP2
MY07A
1
0
-1

FIG. 5F

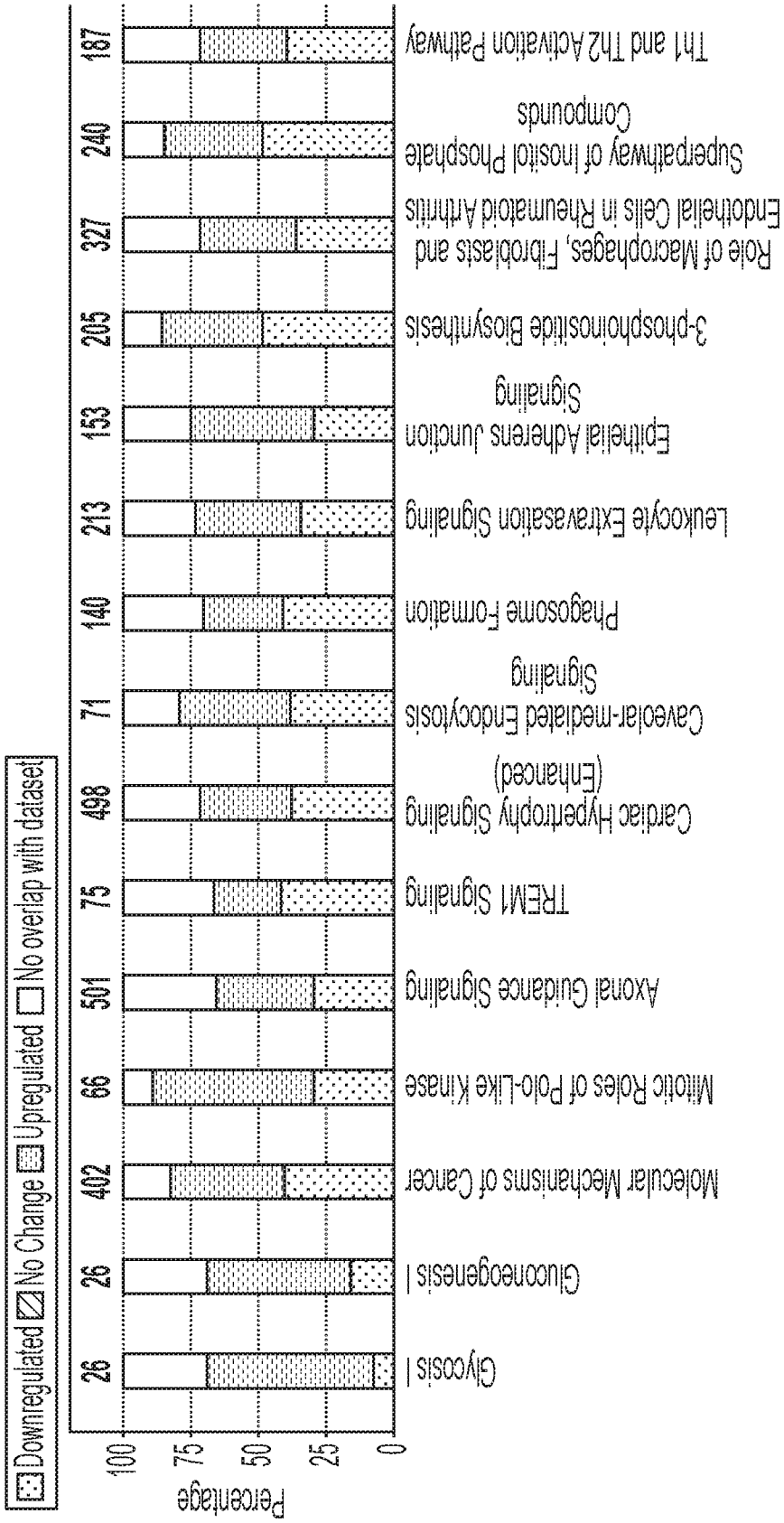

Downregulated
No Change
Upregulated
No overlap with dataset
Percentage
100
75
50
25
0
Glycosis I
Gluconeogenesis I
Molecular Mechanisms of Cancer
Mitotic Roles of Polo-Like Kinase
Axonal Guidance Signaling
TREM1 Signaling
Cardiac Hypertrophy Signaling (Enhanced)
Caveolar-mediated Endocytosis Signaling
Phagosome Formation
Leukocyte Extravasation Signaling
Epithelial Adherens Junction Signaling
3-phosphoinositide Biosynthesis
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis
Superpathway of Inositol Phosphate Compounds
Th1 and Th2 Activation Pathway
26
26
402
66
501
75
498
71
140
213
153
205
327
240
187

FIG. 6A

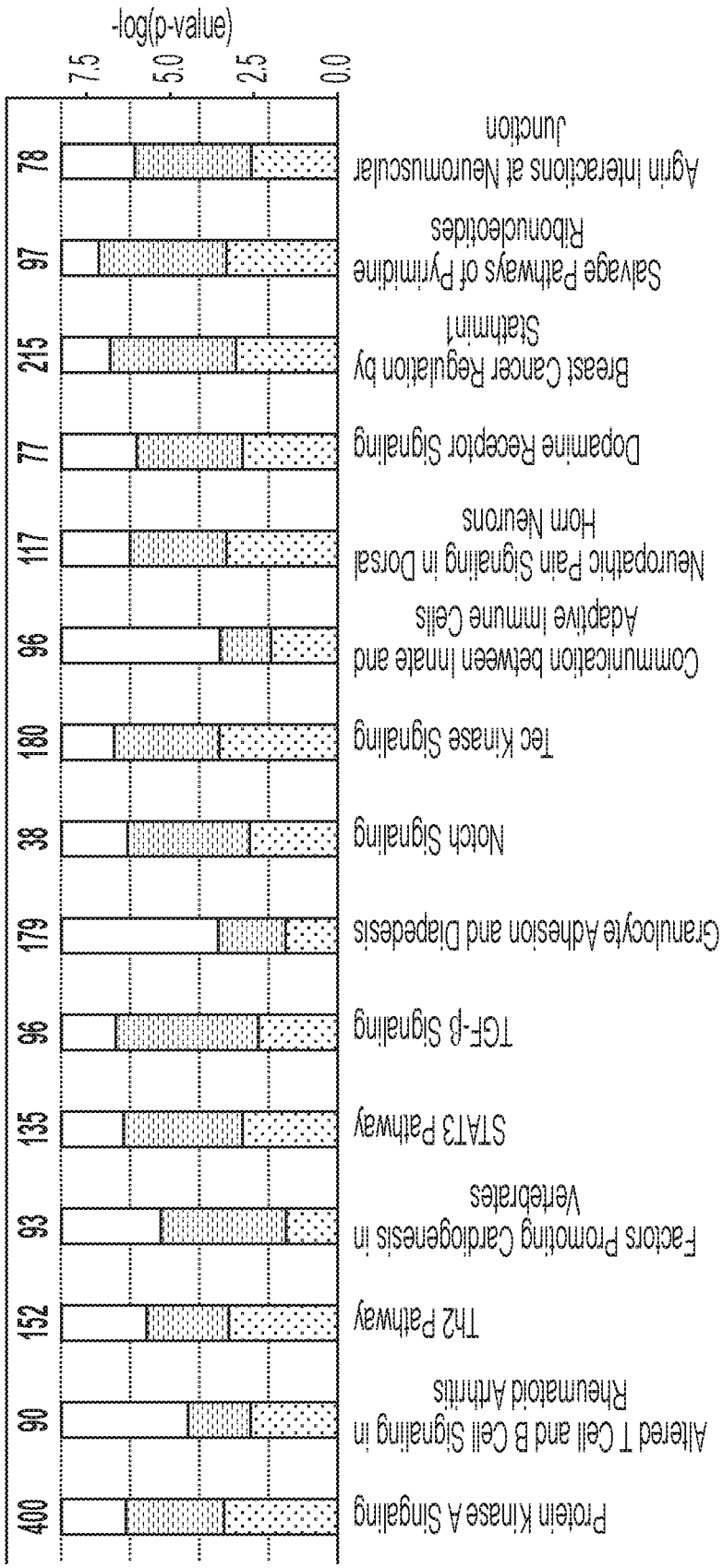


FIG. 6A continued

	Ingenuity Canonical Pathway	-log(*P*-value)		
		CD69+CD103+ vs CD69-CD103-	CD69+CD103+ vs CD69+CD103-	Kumar et al. Lung CD8+CD69+ vs CD8+CD69-
Cellular Migration	Axonal Guidance Signaling	4.21	7.74	3.55
	Ephrin A Signaling	2.11	3.57	1.51
	Leukocyte Extravasation Signaling	3.32	5.27	N/A
	Granulocyte Adhesion and Diapedesis	2.7	5.95	2.62
Focal Adhesion	Epithelial Adherens Junction Signaling	3.26	1.75	N/A
	Integrin Signaling	2.23	4.29	N/A
	Paxillin Signaling	2.06	5.87	1.5
Inositol Phosphate Signaling	3-phosphoinositide Biosynthesis	3.23	1.91	2.2
	Superpathway of Inositol Phosphate Compounds	3.14	2.01	1.76
	D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis	2.11	0.633	N/A
	D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis	2.11	0.633	N/A
T-cell Activation and Differentiation	Th1 and Th2 Activation Pathway	3.13	10.9	3.58
	Th2 Pathway	2.91	9.28	3.15
	Notch Signaling	2.5	1.38	1.45

FIG. 6B

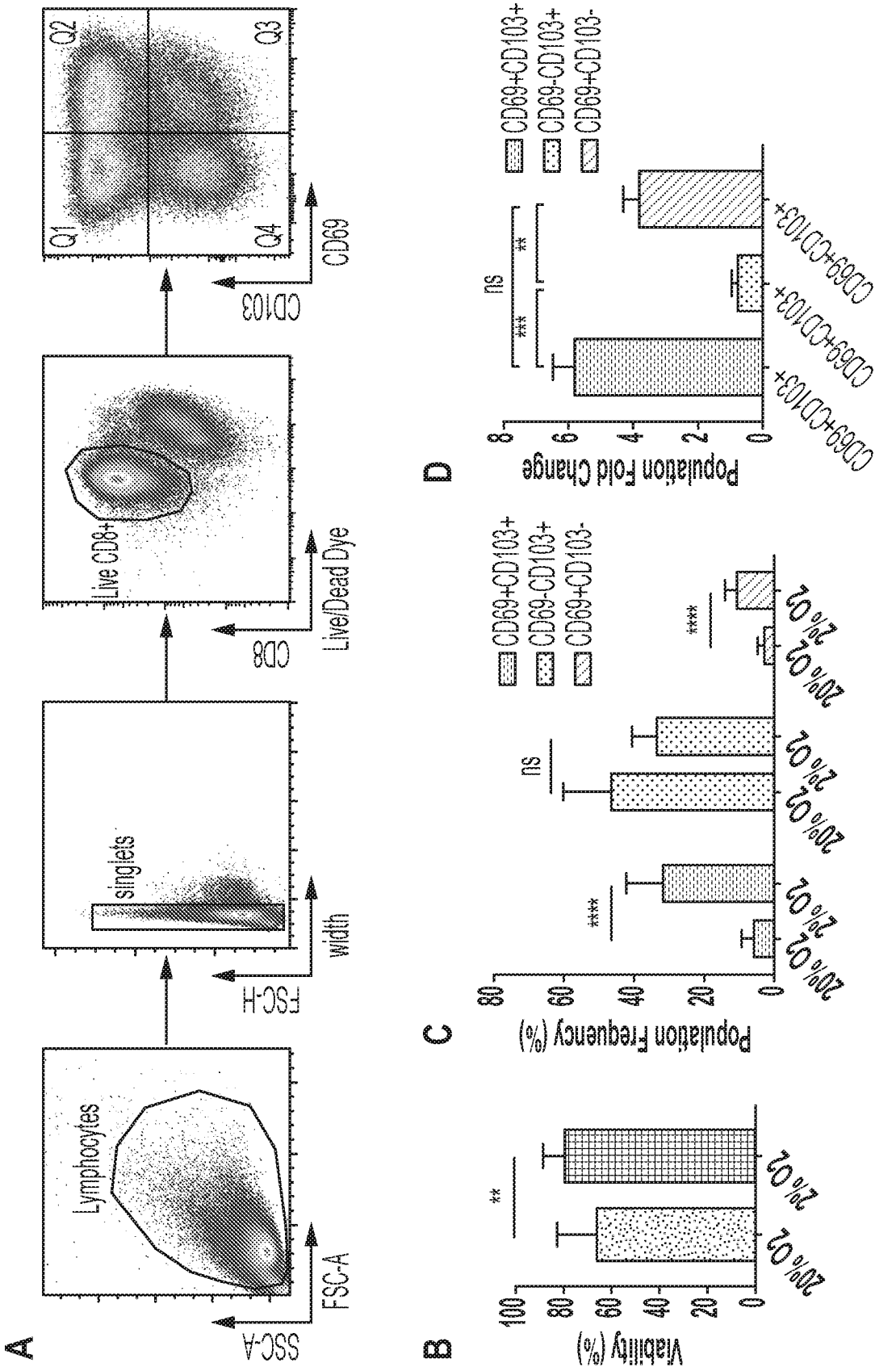

A
Lymphocytes
SSC-A
FSC-A
singlets
FSC-H
width
Live CD8+
CD8
Live/Dead Dye
Q1
Q2
Q3
Q4
CD103
CD69
B
Viability (%)
**
20%
2%
C
Population Frequency (%)
CD69+CD103+
CD69-CD103+
CD69+CD103-
ns
****
D
Population Fold Change
CD69+CD103+
CD69+CD103+
CD69+CD103+
ns
**
***

FIG. 7A-D

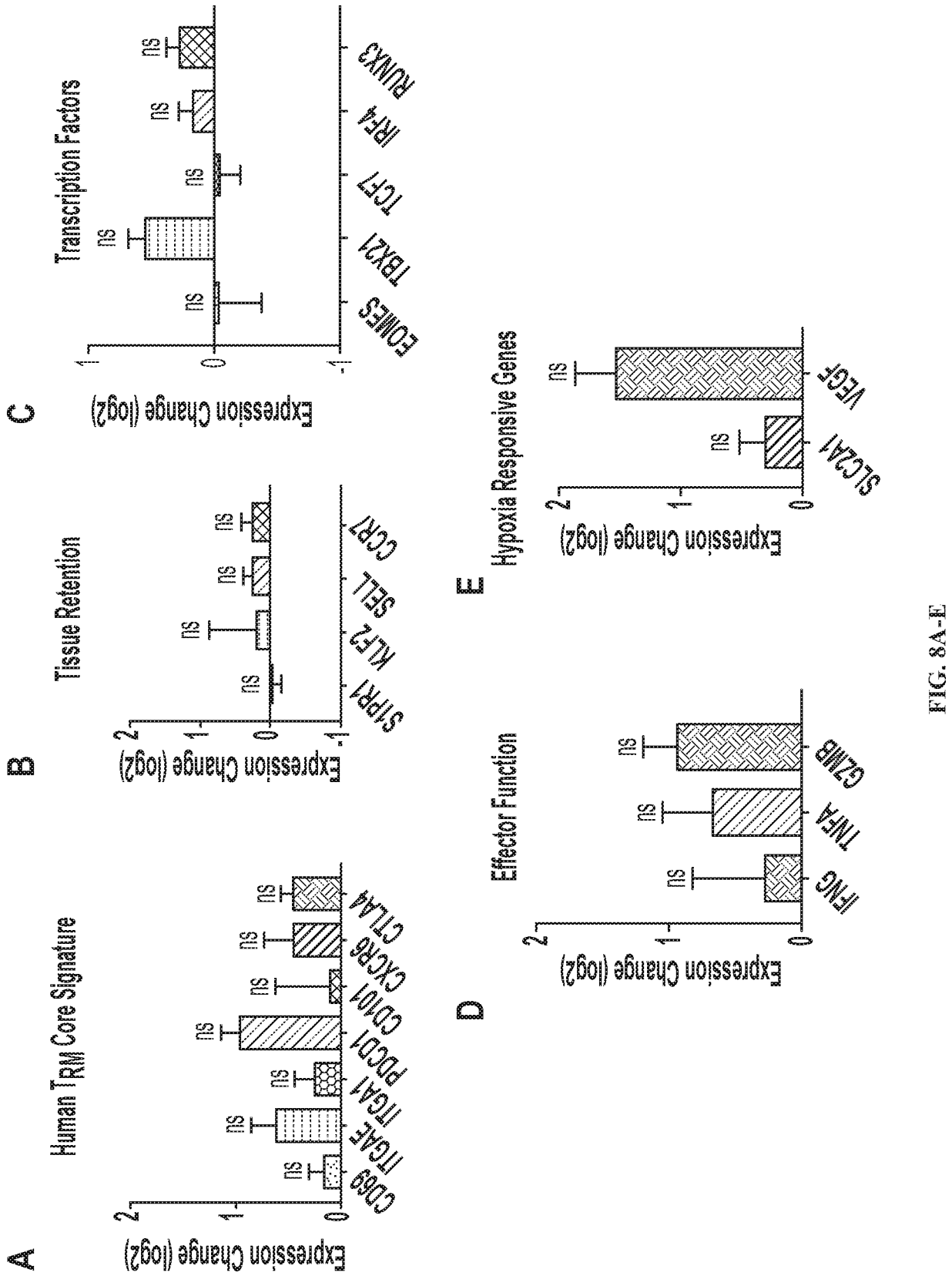

A
Human TRM Core Signature
Expression Change (log2)
CD69
ITGAE
ITGA1
PDCD1
CD101
CXCR6
CTLA4
B
Tissue Retention
S1PR1
KLF2
SELL
CCR7
C
Transcription Factors
EOMES
TBX21
TCF7
IRF4
RUNX3
D
Effector Function
IFNG
TNFA
GZMB
E
Hypoxia Responsive Genes
SLC2A1
VEGF
ns

FIG. 8A-E

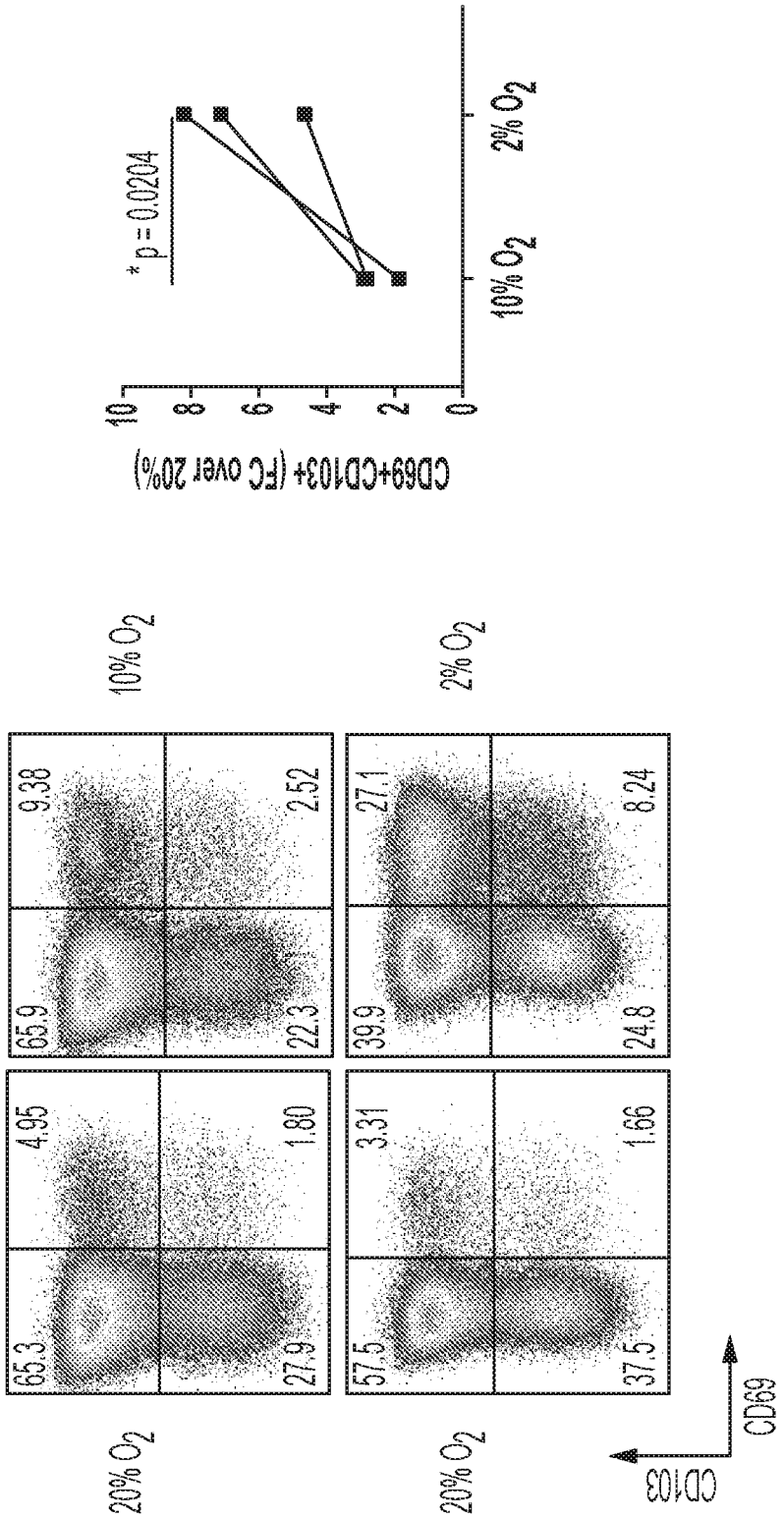

CD69+CD103+ (FC over 20%)
10
8
6
4
2
0
* p = 0.0204
10% O2
2% O2
10% O2
2% O2
65.3
4.95
9.38
27.9
1.80
2.52
57.5
3.31
27.1
37.5
1.66
8.24
65.9
39.9
22.3
24.8
20% O2
20% O2
CD103
CD69

FIG. 8F

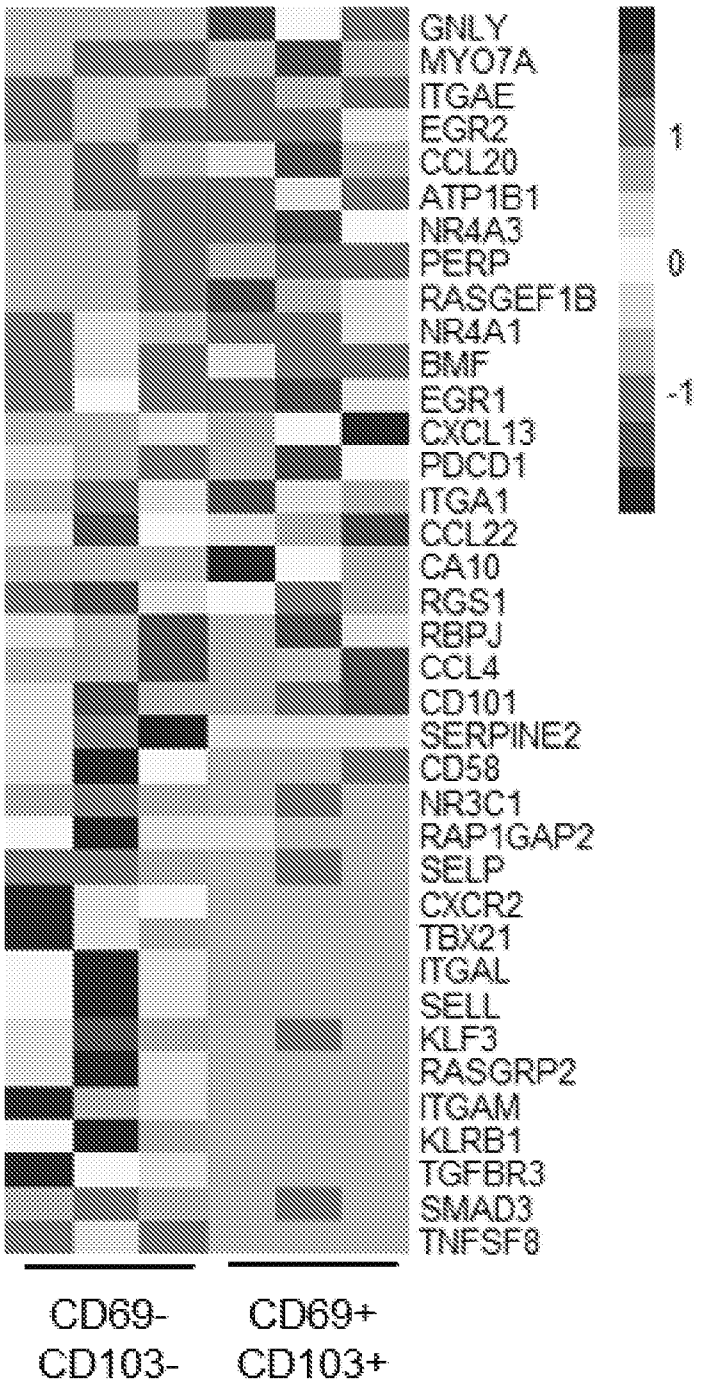

GNLY
MYO7A
ITGAE
EGR2
CCL20
ATP1B1
NR4A3
PERP
RASGEF1B
NR4A1
BMF
EGR1
CXCL13
PDCD1
ITGA1
CCL22
CA10
RGS1
RBPJ
CCL4
CD101
SERPINE2
CD58
NR3C1
RAP1GAP2
SELP
CXCR2
TBX21
ITGAL
SELL
KLF3
RASGRP2
ITGAM
KLRB1
TGFBR3
SMAD3
TNFSF8
1
0
-1
CD69-
CD103-
CD69+
CD103+

FIG. 9A

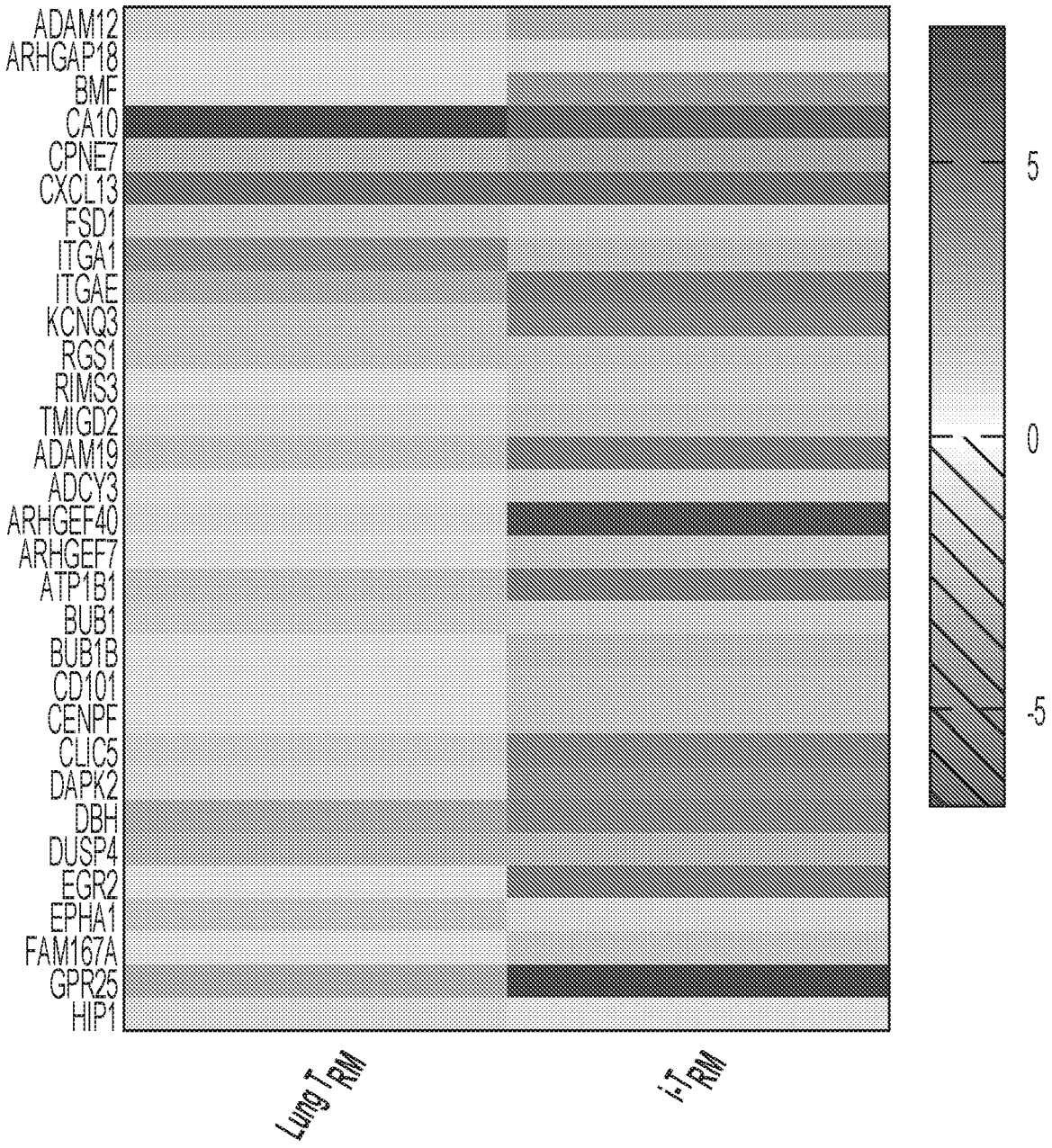

ADAM12
ARHGAP18
BMF
CA10
CPNE7
CXCL13
FSD1
ITGA1
ITGAE
KCNQ3
RGS1
RIMS3
TMIGD2
ADAM19
ADCY3
ARHGEF40
ARHGEF7
ATP1B1
BUB1
BUB1B
CD101
CENPF
CLIC5
DAPK2
DBH
DUSP4
EGR2
EPHA1
FAM167A
GPR25
HIP1
5
0
-5
Lung TRM
i-TRM

FIG. 9B

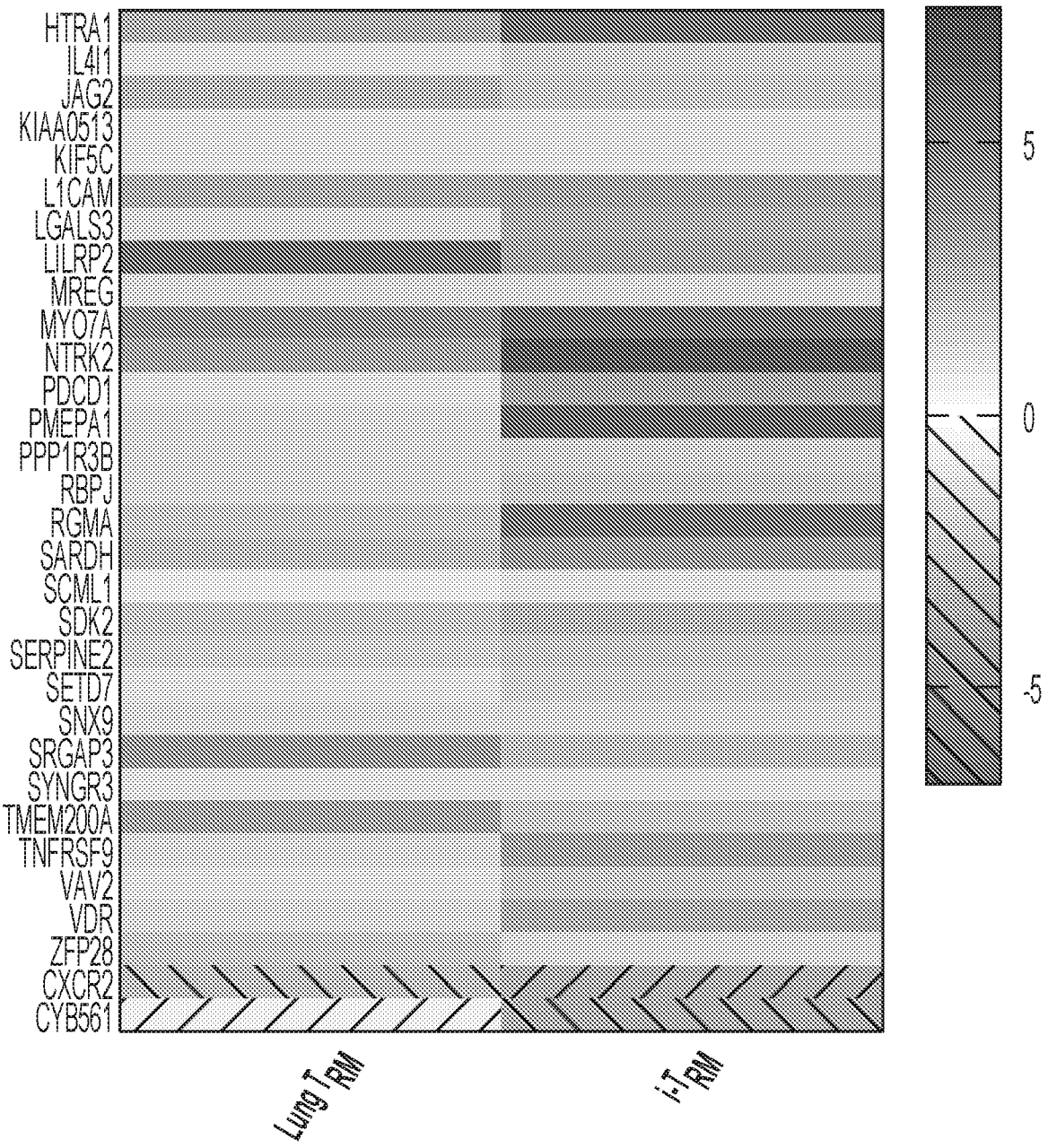


FIG. 9B continued

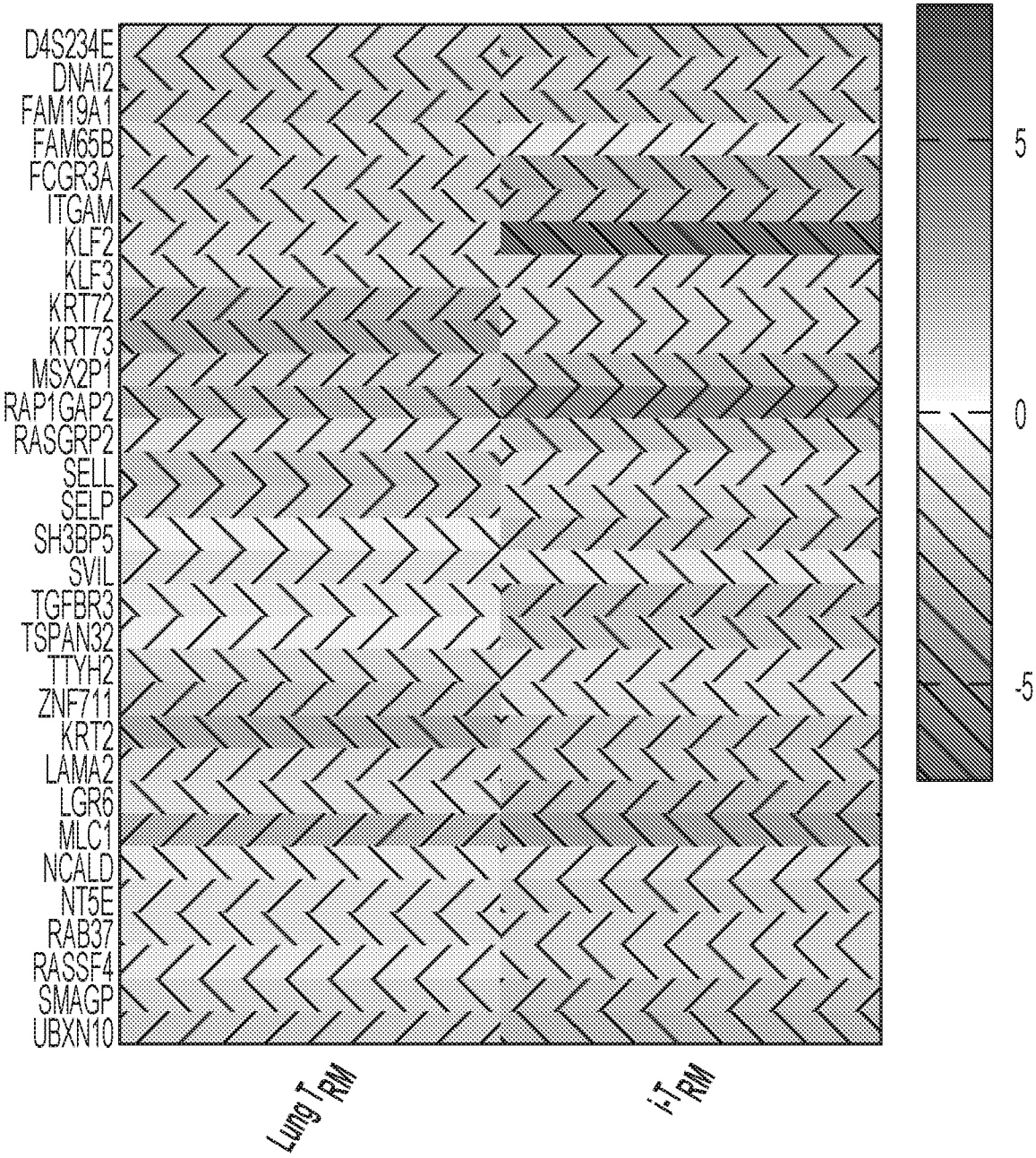


FIG. 9B continued

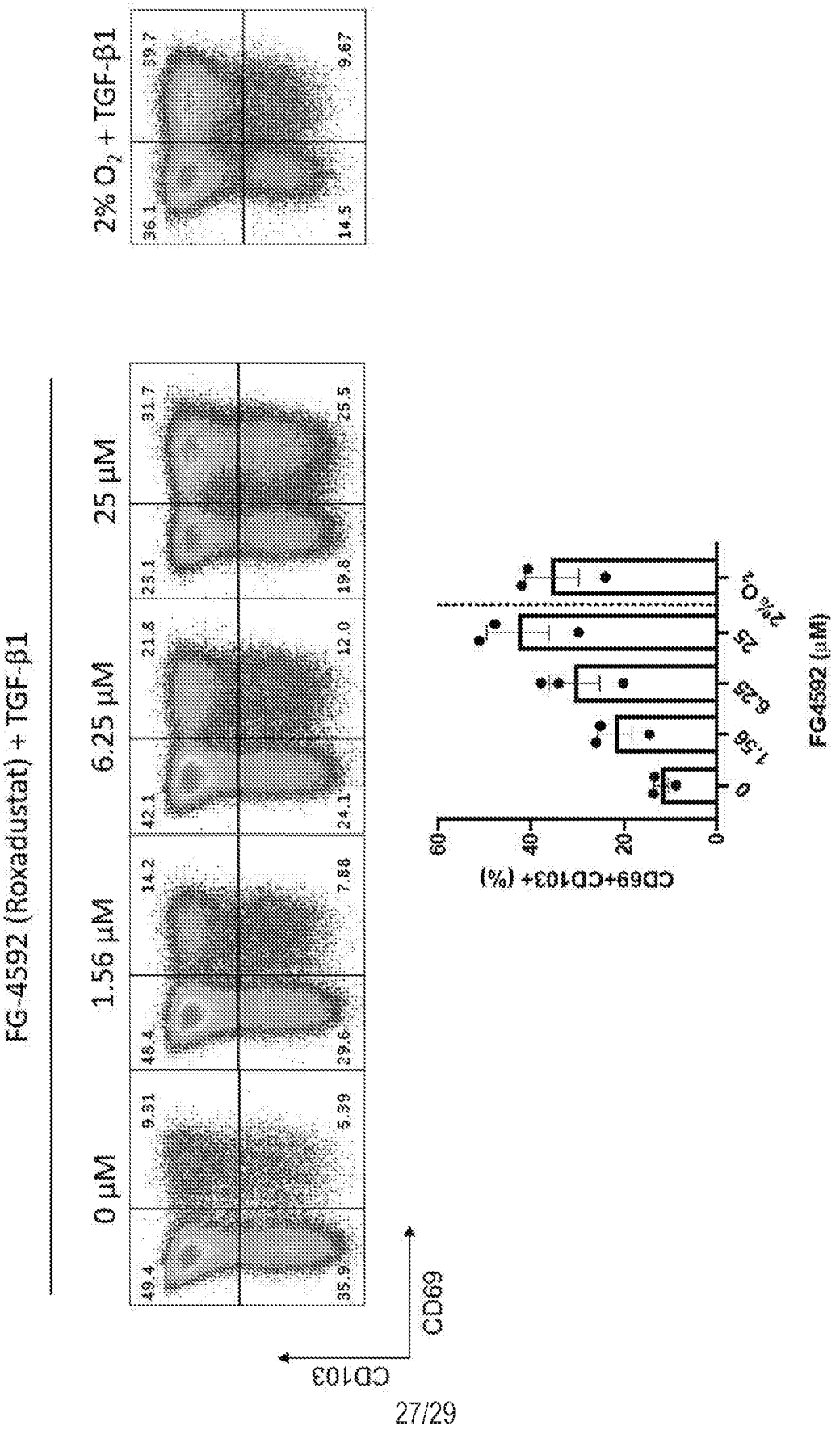
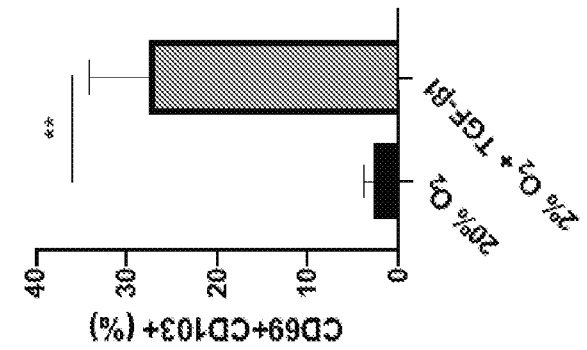

FG-4592 (Roxadustat) + TGF-β1
0 μM
1.56 μM
6.25 μM
25 μM
2% O2 + TGF-β1
49.4
9.91
48.4
14.2
42.1
21.8
23.1
31.7
36.1
39.7
35.9
5.39
29.6
7.86
24.1
12.0
19.8
25.5
14.5
9.67
CD103
CD69
CD69+CD103+ (%)
60
40
20
0
0
1.56
6.25
25
2% O2
FG4592 (μM)

FIG. 10A


CD69+CD103+ (%)
40
30
20
10
0
**
20% O2
2% O2 + TGF-β1

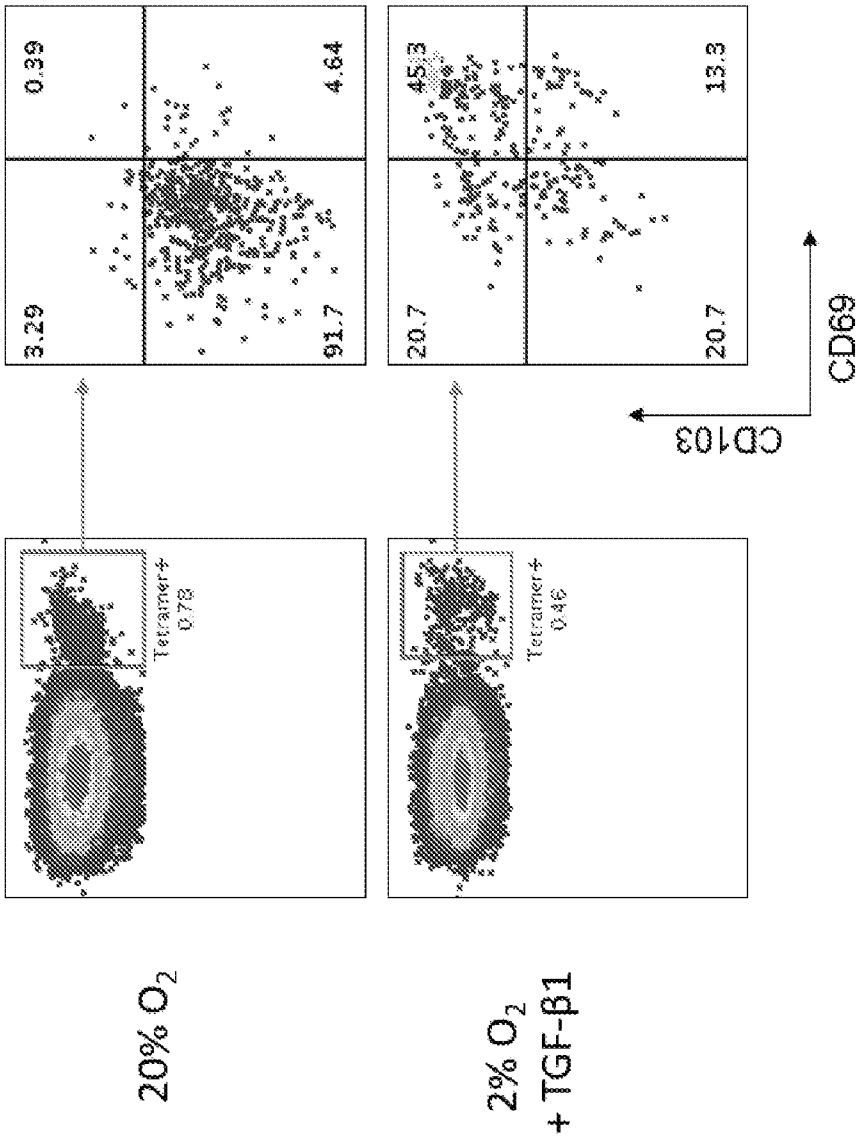

0.39
4.64
3.29
91.7
45.3
13.3
20.7
20.7
CD103
CD69
Tetramer+
0.78
Tetramer+
0.46
20% O2
2% O2
+ TGF-β1

FIG. 10B

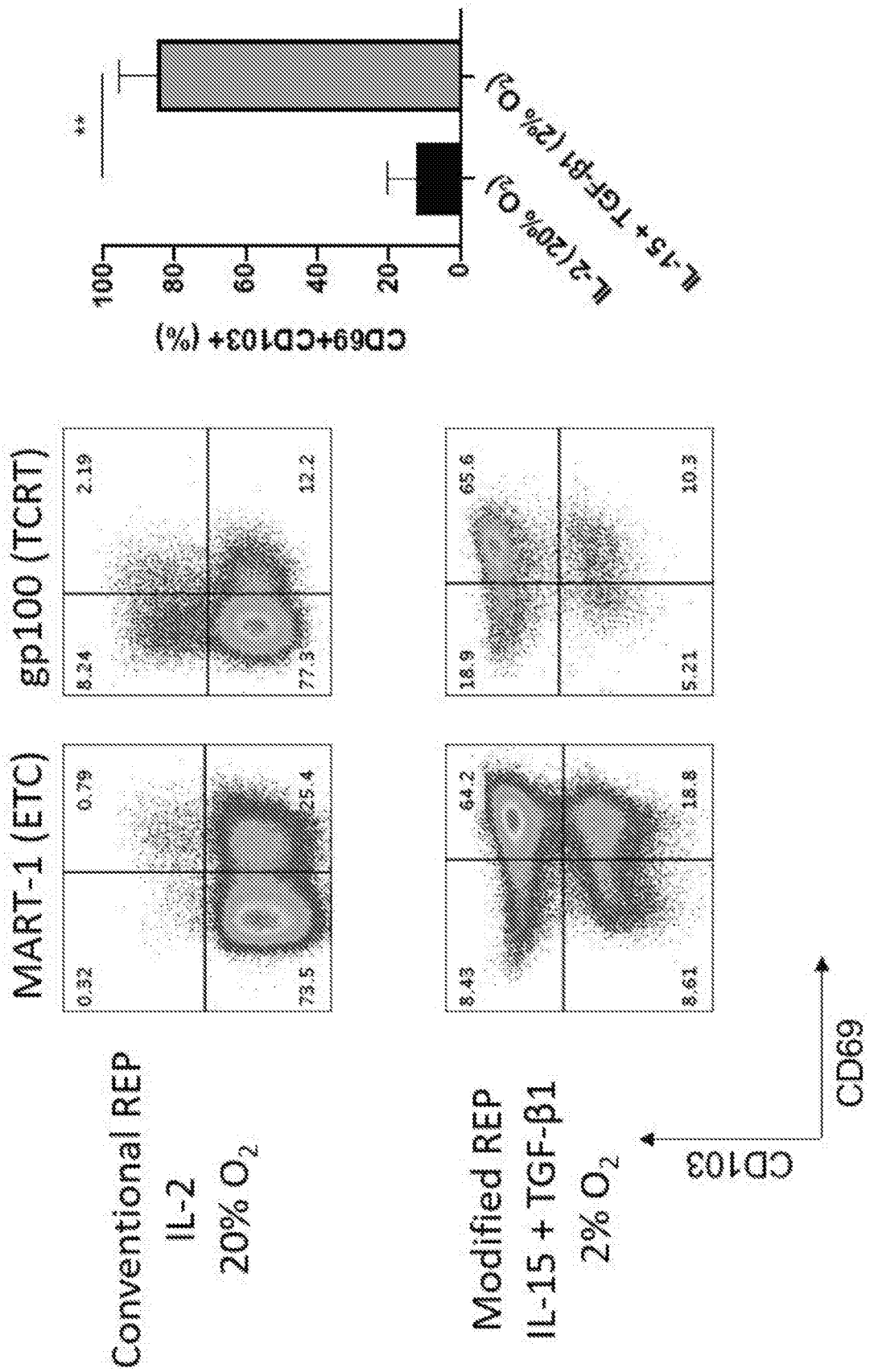

CD69+CD103+ (%)
100
80
60
40
20
0
**
IL-2 (20% O2)
IL-15+ TGF-β1 (2% O2)
gp100 (TCRT)
MART-1 (ETC)
0.32
0.79
73.5
25.4
8.24
2.19
77.3
12.2
8.43
64.2
8.61
18.8
18.9
65.6
5.21
10.3
Conventional REP
IL-2
20% O2
Modified REP
IL-15 + TGF-β1
2% O2
CD103
CD69

FIG. 10C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/57016

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 82, 94, 107, 109
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8, 11-17, 30-72 limited to the T cells of the starting population are CD8+ peripheral blood T cells

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/57016

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/17, G01N 33/50, C12N 5/0783, A61K 39/00 (2020.01)

CPC - A61K 35/17, A61K 39/0011, C12N 5/0636, G01N 33/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/079113 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 11 May 2017 (11.05.2017) Claim 1, para [0024], [0026], [0027], [0028], [0031], [0046], [0057], [0089], [0108], [0121], [0126], [0163]	1-6, 8, 11-15, 17, 30-34, 36-40, 44-48, 52-53, 61, 72 ----- 7, 16, 35, 41-43, 49-51, 54-60, 62-71
Y	US 5,962,318 A (ROONEY et al.) 05 october 1999 (05.10.1999) abstract	7
Y	US 2015/0299656 A1 (GATTINONI et al.) 22 October 2015 (22.10.2015) para [0086], Table 1	16
Y	US 2018/0117021 A1 (SHENYANG SUNSHINE PHARMACEUTICAL CO. LTD.) 03 May 2018 (03.05.2018) para [0009]	35
Y	US 2008/0131415 A1 (RIDDELL et al.) 05 June 2008 (05.06.2008) para [0029]	41-43, 49-51
Y	WO 2018/106972 A1 (LA JOLLA INSTITUTE FOR ALLERGY AND IMMUNOLOGY) 14 June 2018 (14.06.2018) para [0034], [0072], [0206], Fig. 4D, Fig. 7B	54-70

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2020	28 FEB 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/57016

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOPRAK et al. "Down-regulation of cell surface CXCR6 expression during T cell activation is predominantly mediated by calcineurin" Cellular Immunology, May 2003, Vol 223, No 1, pp 1-12; abstract	71

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 19/57016

Continuation of:
Box No. III. Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Groups I+: Claims 1-80, drawn to an in vitro method for producing tissue resident memory-like T cells (Trm-like T cells). The method will be searched to the extent that the T cells of the starting population are CD8+ peripheral blood T cells (see instant claim 2). It is believed that claims 1-8, 11-17, 30-72 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass CD8+ peripheral blood T cells. Additional T cells of the starting population will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected T cells of the starting population. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be wherein the T cells of the starting population comprising engineered T cells (see instant claim 18) (Claims 1, 8, 11-28, 30-80). Another exemplary election would be wherein the T cells of the starting population comprising tumor infiltrating lymphocytes (see instant claims 9 and 29) (Claims 1, 8-17, 29-72).

Groups II: Claims 81, 83-93, 95-106, drawn to a Trm-like T cell, wherein the T cells has essentially no expression of CXCR6 protein, and a pharmaceutical composition thereof.

Groups III: Claims 108, 110-124, drawn to a method of treating an immune-related disorder in a subject.

The inventions listed as Groups I+, II and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Groups I+ and III include the special technical feature of a method which differs from the special technical feature of a composition, as disclosed by Group II.

Group I+ includes the special technical feature of a method comprising culturing the starting population of T cells in hypoxic conditions and transforming growth factor beta 1, not required by Group III.

Group III includes the special technical feature of a method comprising administering an effective amount of Trm-like T cells, not required by Groups I+.

Common Technical Features

The inventions of Groups I+, II and III share the technical feature of Trm-like T cells.
The inventions of Groups I+ and III share the technical feature of Trm-like T cells produced by the method of Claim 1.

However, these shared technical features do not represent a contribution over prior art in view of WO 2017/079113 A1 to The United States Of America, As Represented By The Secretary, Department Of Health And Human Services (hereinafter "HHS").

HHS teaches (instant claim 1) an in vitro method for producing tissue resident memory-like T cells (Trm-like T cells) comprising:
(a) obtaining a starting population of T cells (para [0027], For example, the T cells can be obtained from the mammal by a blood draw or a leukapheresis. In an embodiment of the invention, the method comprises isolating peripheral blood lymphocytes (PBL) or a peripheral blood mononuclear cells (PBMC) from a mammal.);
(b) culturing the starting population of T cells in hypoxic conditions or in the presence of a hypoxia-inducing agent to generate early effector cells (Claim 1, A method of producing an isolated population of T cells for adoptive cell therapy, the method comprising culturing isolated T cells having antigenic specificity for a cancer antigen in vitro in the presence of a prolyl hydroxylase domain-containing protein (Phd) inhibitor.); and
(c) further culturing the early effector cells in the presence of transforming growth factor beta 1 (TGF-B I) (para [0089], For human in vitro iTreg induction assays, naive CD4+CD45RA+CD45RO-CD62L+CCR7+ cells were FACS purified from three biologically independent healthy donor buffy coats and activated with plate-bound human anti-CD3 and soluble anti-CD28.....with human TGF-beta1 (5 ng per mL, R&D Systems).....Mouse and human cells were cultured under standard incubator conditions or 5% or 2% oxygen as indicated.....through the entire duration of culture. Where indicated, the PHD protein inhibitor DMQG....., Rapamycin....., or 2-deoxyglucose.....was added for the duration of in vitro culture.) to produce Trm-like T cells (para [0024], CD8+ T cells that have been cultured in the presence of one or both of (i) a Phd inhibitor and (ii) a low-oxygen atmosphere.; [0089], naive CD4+CD45RA+CD45RO-CD62L+CCR7+ cells were FACS purified.; Note, A Trm-like T cells comprises CD45RA+CCR7+CD8+, see instant claim 4).

The inventions of Groups II and III share the technical feature of Trm-like T cells with essentially no expression of CXCR6 protein.

However, these shared technical features do not represent a contribution over prior art in view of the article "Down-regulation of cell surface CXCR6 expression during T cell activation is predominantly mediated by calcineurin" by Koprak et al. (hereinafter "Koprak").

Koprak teaches (instant claim 81) a Trm-like T cell, wherein the T cell has essentially no expression of CXCR6 protein (abstract, CXCR6,the receptor for the membrane-anchored chemokine, CXCL16, is expressed on a subset of CCR5-bearing memory T cells, and may play a role in recruiting these cells to sites of inflammation. Here, we set out to determine the effect of T cell activation on CXCR6 expression. Highly purified human peripheral blood T cells were cultured for 7?8 days in presence of IL-2 (400 U/ml) to enhance CXCR6 expression. Overnight stimulation with anti-CD3 mAb+ anti-D28 mAb, which resulted in CD69 induction and cytokine (IL-2 and IFN-gamma) production, reduced cell surface expression of CXCR6 by 85% and that of CCR5 by 76%n.....CCR7,whose expression was low on CXCR6+ T cells, was little affected by any of these modes of activation.) (Note, Trm-like T cells comprising CCR7 and CD69 (see instant Specification Claim 4 and 54.).

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Continuation of:
Box No. III. Observations where unity of invention is lacking

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+, II and III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.