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(71) Applicant: **SUNNYBROOK RESEARCH INSTITUTE** [CA/CA]; 2075 Bayview Avenue, Toronto, Ontario M4N 3M5 (CA).

(72) Inventor: **ZUNIGA-PFLUCKER, Juan Carlos**; 2075 Bayview Avenue, M7-619, Toronto, Ontario M4N 3M5 (CA).

(74) Agent: **BERESKIN & PARR LLP/S.E.N.C.R.L., S.R.L.**; Scotia Plaza, 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

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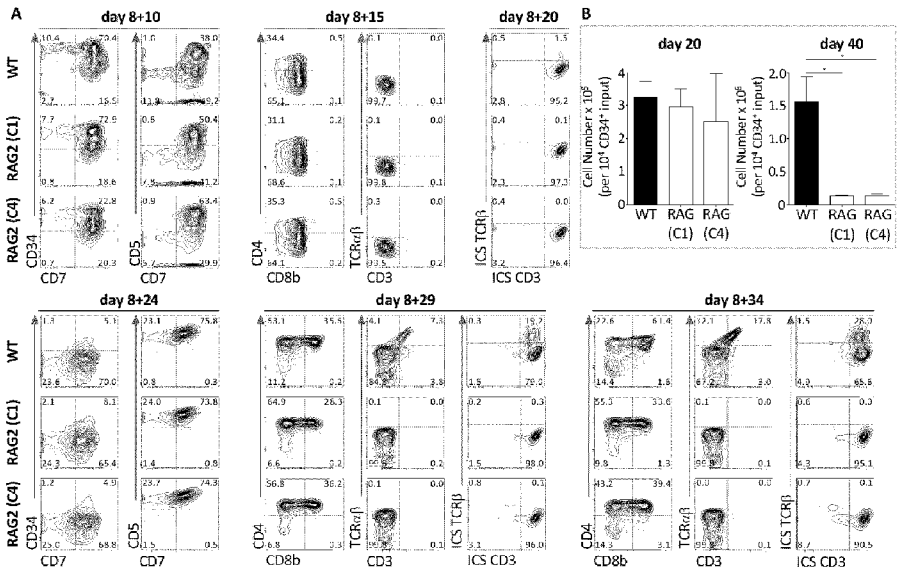


Fig. 2

(57) Abstract: A method of generating stem cells that are unable to undergo T cell receptor (TCR) or B cell receptor (BCR) gene rearrangements is provided. In particular, methods, compositions and kits for use in generating cells of the T cell lineage or B cell lineage comprising an unrearranged TCR gene locus or BCR gene locus, respectively, are 5 provided. In one embodiment, the cells are further engineered to express a TCR, bCR or CAR conferring specificity to an antigen of interest. Cells, compositions, kits and uses thereof are also provided.

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Title: STEM CELLS COMPRISING AN UNREARRANGED T CELL RECEPTOR (TCR) GENE LOCUS AND METHODS OF USE THEREOF

Cross-Reference to Related Application

[0001] This application claims the benefit of priority to United States Provisional Application No. 63/178,990 filed April 23, 2021, the contents of which are incorporated herein by reference in their entirety.

Field

[0002] The application relates to stem cells that are unable to undergo T cell receptor (TCR) or B cell receptor (BCR) gene rearrangements. In particular, the application relates to methods, compositions and kits for use in generating cells of the T cell lineage or B cell lineage comprising an unrearranged TCR gene locus or BCR gene locus, respectively.

Background

[0003] The ability of T cells to specifically recognize antigens is accomplished through the expression of specific T cell receptors encoded by the uniquely rearranged genomic loci of the TCRα and β chains. T cell development is predicated on the successful rearrangement of the T cell receptor (TCR) gene loci, which encode for antigen-specific receptors.

[0004] The V(D)J Recombination Activating Genes (RAG) 1 and 2 are two essential DNA processing enzymes required for the rearrangement of the B cell receptor and T cell receptor gene loci (Schatz at al., 1989; Oettinger et al, 1990). RAG1/2 initiate V(D)J recombination by forming a complex that first recognizes and binds to recombination signal sequences (RSS) found adjacent to each V, D, and J gene segment. Upon formation of a synapse with another RSS, the RAG complex induces a double-strand DNA break, which is repaired by non-homologous end-joining process (Jones and Gellert, 2004; Smith et al, 2019). This ultimately results in the imperfect joining of different V, D, and J gene segments to potentially generate millions of different antigen receptors from a few hundred V(D)J segments.

Summary

[0005] The inventors differentiated human pluripotent stem cells with a CRISPR/Cas9-directed deletion of the *RAG2* gene (RAG2-KO) and showed that human RAG2-deficient developing T cells progress up to the CD4+CD8+ double positive stage.

The inventors also showed that expression of a rearranged TCRβ chain promotes cell survival and/or proliferation of developing human T-cells at the double positive stage.

[0006] Accordingly, the disclosure provides a method of generating stem or progenitor cells unable to undergo T cell receptor (TCR) gene rearrangements (TCR), the method comprising:

(a) culturing a sample comprising stem cells or progenitor cells,

wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells.

[0007] In one embodiment, the method further comprises (b) isolating cells of the T cell lineage.

[0008] In another embodiment, the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

[0009] In another embodiment, the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

[0010] In another embodiment, the stem cells are pluripotent stem cells. Optionally, the pluripotent stem cells are embryonic stem cells or induced pluripotent stem cells (iPSCs).

[0011] In another embodiment, the stem cells or progenitor cells are human cells.

[0012] In another embodiment, the cells of the T cell lineage are progenitor T (proT) cells, optionally CD45+CD34+CD7+ progenitor T (proT) cells.

[0013] In another embodiment, the cells of the T cell lineage are CD4+CD8+ double positive cells or CD4+CD8+CD3+ double positive cells.

[0014] In another embodiment, the cells of the T cell lineage are CD8+CD3+ single positive cells or CD4+CD3+ single positive cells.

[0015] In another embodiment, the method further comprises engineering the stem cells or progenitor cells or the cells of the T cell lineage to comprise at least one of

a nucleic acid encoding a T cell receptor (TCR), a TCRβ chain and a chimeric antigen receptor (CAR).

[0016] In another embodiment, the stem cells or progenitor cells or the cells of the T cell lineage express the at least one of a T cell receptor (TCR), a TCRβ chain and a chimeric antigen receptor (CAR).

[0017] In another embodiment, the method further comprises engineering the stem cells or progenitor cells or the cells of the T cell lineage to comprise a nucleic acid encoding a TCRβ chain. In a further embodiment, the stem cells or progenitor cells or the cells of the T cell lineage that comprise a nucleic acid encoding a TCRβ chain do not comprise a nucleic acid that encodes a TCRβ chain or a chimeric antigen receptor (CAR).

[0018] In another embodiment, the stem cells or progenitor cells or the cells of the T cell lineage express a TCRβ chain.

[0019] In another embodiment, the stem cells or progenitor cells or the cells of the T cell lineage comprise a nucleic acid encoding a TCRβ chain and a nucleic acid encoding a CAR.

[0020] In another embodiment, the TCR or CAR confers specificity to an antigen, optionally a tumor-associated antigen, viral antigen or self antigen.

[0021] The disclosure also provides a cell of the T cell lineage, wherein the cell is generated by a method described herein.

[0022] In one embodiment, the cell of the T cell lineage is a CD4+CD8+ double positive cell or a CD4+CD8+CD3+ double positive cell.

[0023] In another embodiment, the cell is a CD45+CD34+CD7+ progenitor T cell, CD8+CD3+ single positive cell or CD4+CD3+ single positive cell.

[0024] The disclosure also provides a stem or progenitor cell, wherein expression of at least one gene or protein required for V(D)J recombination in the stem cell or progenitor cell is reduced or eliminated compared to a wild-type stem cell or progenitor cell.

[0025] In one embodiment, the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

[0026] In another embodiment, the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent

protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

[0027] In another embodiment, the stem or progenitor cell further comprises at least one of a nucleic acid encoding a T cell receptor (TCR), a TCRβ chain and a chimeric antigen receptor (CAR).

[0028] In another embodiment, the stem or progenitor cell further comprises a nucleic acid encoding a TCRβ chain. In a further embodiment, the stem or progenitor cell that comprises a nucleic acid encoding a TCRβ chain does not comprise a nucleic acid that encodes a TCRβ chain or a chimeric antigen receptor (CAR).

[0029] In another embodiment, the stem cell is a pluripotent stem cell, optionally an embryonic stem cell or induced pluripotent stem cell (iPSC).

[0030] In another embodiment, the stem cell or progenitor cell is a human cell.

[0031] The disclosure also provides a use of a stem or progenitor cell as described herein for generating cells of the T cell lineage.

[0032] The disclosure also provides a kit comprising (i) a stem or progenitor cell as described herein and (ii) instructions for use of the stem or progenitor cell as described herein for generating cells of the T cell lineage.

[0033] The disclosure further provides a method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells or progenitor cells to a subject in need thereof,

wherein the stem cells or progenitor cells are engineered to comprise at least one of a nucleic acid encoding a T cell receptor (TCR) and a chimeric antigen receptor (CAR) that confers specificity to an antigen.

[0034] The disclosure also provides a method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, and isolating cells of the T cell lineage wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells of the T cell lineage to a subject in need thereof,

wherein the stem cells or progenitor cells or the cells of the T cell lineage are engineered to comprise at least one of a nucleic acid encoding a T cell receptor (TCR) and a chimeric antigen receptor (CAR) that confers specificity to an antigen.

[0035] In one embodiment, the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

[0036] In another embodiment, the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

[0037] In another embodiment, the disease is cancer and the antigen is a tumor-associated antigen.

[0038] The disclosure also provides a method of generating stem or progenitor cells unable to undergo T cell receptor (BCR) gene rearrangements, the method comprising:

(a) culturing a sample comprising stem cells or progenitor cells,

wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells.

[0039] In one embodiment, the method further comprises (b) isolating cells of the B cell lineage.

[0040] In another embodiment, the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

[0041] In another embodiment, the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent

protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

[0042] In another embodiment, the stem cells are pluripotent stem cells, optionally embryonic stem cells or induced pluripotent stem cells (iPSCs).

[0043] In another embodiment, the stem cells or progenitor cells are human cells.

[0044] In another embodiment, the cells of the B cell lineage are CD20+ or CD19+ cells.

[0045] In another embodiment, the cells of the B cell lineage are Tumor-Infiltrating B Cells (TIBs).

[0046] In another embodiment, the method further comprises engineering the stem cells or progenitor cells or the cells of the B cell lineage to comprise at least one of a nucleic acid encoding a B cell receptor (BCR), a BCRβ chain and a chimeric antigen receptor (CAR).

[0047] In another embodiment, the stem cells or progenitor cells or the cells of the B cell lineage express the B cell receptor (BCR), the BCRβ chain or the chimeric antigen receptor (CAR).

[0048] In another embodiment, the BCR or CAR confers specificity to an antigen, optionally a tumor-associated antigen, viral antigen or self antigen.

[0049] The disclosure also provides a cell of the B cell lineage, wherein the cell is generated by a method as described herein.

[0050] In one embodiment, the cell of the B cell lineage is a CD20+ or CD19+ cell.

[0051] In another embodiment, the cell of the B cell lineage is a Tumor-Infiltrating B Cell (TIB).

[0052] The disclosure also provides a use of a stem or progenitor cell as described herein for generating cells of the B cell lineage.

[0053] The disclosure also provides a kit comprising (i) a stem or progenitor cell as described herein and (ii) instructions for use of the stem or progenitor cell as described herein for generating cells of the B cell lineage.

[0054] The disclosure also provides a method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the stem cells or progenitor cells to a subject in need thereof,

wherein the stem cells or progenitor cells are engineered to comprise a nucleic acid encoding a B cell receptor (BCR) that confers specificity to an antigen.

[0055] The disclosure also provides a method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, and isolating cells of the B cell lineage wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells of the B cell lineage to a subject in need thereof,

wherein the stem cells or progenitor cells or the cells of the B cell lineage are engineered to comprise a nucleic acid encoding a B cell receptor (BCR) that confers specificity to an antigen.

[0056] In one embodiment, the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

[0057] In another embodiment, the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

[0058] In another embodiment, the disease is cancer and the antigen is a tumor-associated antigen.

[0059] Other features and advantages of the present application 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 application are given by way of illustration only, since various changes and modifications within the spirit and scope of the application will become apparent to those skilled in the art from this detailed description.

Brief description of the drawings

[0060] Embodiments of the disclosure will now be described in relation to the drawings in which:

[0061] **Fig. 1 (A-E) shows generation and characterization of RAG2-KO hESC lines.** Fig. 1A shows Sanger Sequencing of genomic DNA of CRISPR/Cas9-targeted RAG2 of clones 1 and 4. The altered allele is depicted in chromatograph and sequence above it, while alignment of the altered and WT alleles are shown below. Fig. 1B shows an immunoblot for RAG2 protein expression of *in vitro*-derived T-lineage cells from RAG2-KO clones (1 and 4) and Control WT hESCs, or Control PBMCs obtained from a T-ALL patient. GAPDH serves as a loading control. Fig. 1C shows immunofluorescence of RAG2-KO hESC clones for pluripotency markers. The scale bar corresponds to 100 µm. Fig. 1D shows immunohistochemistry of RAG2-KO hESC clones against ectoderm (neural tissue), mesoderm (cartilage), and endoderm (glandular tissue) lineages of a teratoma formation assay. Fig. 1E shows $CD34^+$ hemogenic endothelial cell yield and enrichment after 8 days of embryoid body differentiation from Control WT, RAG2-KO-1, and RAG2-KO-4 hESCs. (n=3 of three independent experiments).

[0062] **Fig. 2 (A-B) shows T cell development from Control WT and RAG2-KO hESC lines.** Fig. 2A shows a representative flow cytometry analysis of Control WT and RAG2-KO (clones 1 and 4) hPSC-derived T-lineage cells from d8 EBs + 10-34d OP9-DL4-7FS co-cultures, as indicated. Cells were pre-gated for $DAPI^-CD45^+$. (n=5 of five independent experiments). Fig. 2B shows a cell numbers of Control WT and RAG2-KO (clones 1 and 4) hPSC-derived T-lineage cells per 10,000 input of $CD34^+$ cells after indicated number of days of culture on OP9-DL4-7FS. (n=3 of three independent experiments).

[0063] **Fig. 3 (A-B) shows forced expression of a rearranged TCRβ chain in RAG2-KO $CD4^+CD8^+$ DP cells results in cell expansion.** Fig. 3A shows a schematic of the experimental approach. Fig. 3B shows cell counts of RAG2-KO DPs (clones 1

and 4) retrovirally-transduced with empty vector (dTomato), TCR alpha chain (TRA 1383i), or TCR beta chain (TRB 1383i) per 10,000 input of $CD34^+$ cells. Transduced T-lineage cells ($dTomato^+$) were first sorted for $CD7^+CD5^+CD4^+CD8^+$ double positive cells and then re-cultured on OP9-DL4-7FS for 10 days. (n=3 of three independent experiments).

[0064] Fig. 4 (A-B) shows TCR gene expression and $CD4^+CD8^+$ signature gene expression. Fig. 5A shows a heatmap analysis of specific TCR genes expressed in Control WT DPs but absent in RAG2-KO1/4 DPs and TCRβ-transduced RAG2-KO1/4 DPs. Fig. 5B shows expression of $CD4^+CD8^+$ signature genes (as determined by thymus DP signature genes shown in **Table 3**) in RAG2-KO, TCRβ-transduced RAG2-KO, and umbilical cord blood (UCB) derived DPs. (n=2 for Control WT, RAG2-KO-1, RAG2-KO-4, and UCB, and n=1 for RAG2-KO-1 TCRβ-transduced and RAG2-KO-4 TCRβ-transduced of one independent experiment).

[0065] Fig. 5 (A-B) shows transcriptomic analysis of Control WT, RAG2-KO, and RAG2-KO TCRβ-transduced $CD4^+CD8^+$ DP cells. RNA-seq analysis of Control WT, RAG2-KO1/4, and $TCR\beta^+$ RAG2-KO1/4 $CD4^+CD8^+$ DPs. Shown are genes that are differentially highly expressed in Control WT DPs compared to RAG2-KO1/4 DPs (Fig. 5A) and differentially highly expressed in RAG2-KO1/4 DPs compared to Control WT DPs (Fig. 5B). (n=2 for Control WT, RAG2-KO-1, and RAG2-KO-4, and n=1 for RAG2-KO-1 TCRβ-transduced and RAG2-KO-4 TCRβ-transduced of one independent experiment).

[0066] Fig. 6 (A-B) shows an analysis of proliferation and differentiation genes and biological pathways. Fig. 6A is a heatmap analysis of differentially expressed genes in DPs from RAG2-KO1/4 (KO) and TCRβ-transduced RAG2-KO1/4 (KOTCRb) cells. Fig. 6B shows names of gene ontology biological pathways that involve genes that are differentially up-regulated in Control WT DPs compared to RAG2-KO DPs. Highlighted are biological pathways that are relevant to cell survival and/or proliferation. Within the group of biological pathways that are involved in specific aspects of leukocyte regulation, the specific genes involved are also indicated.

[0067] Fig. 7 (A-B) shows forced expression of TCRαβ and TCRγδ chains in RAG2-KO $CD4^+CD8^+$ DPs. Fig. 7A shows TCRαβ- and TCRγδ-transduced DPs displayed higher cell numbers after 4 and 10 days of culture compared to dTomato-transduced DPs. From left to right are dTomato, TCRαβ- and TCRγδ. Fig. 7B is a flow

cytometric analysis of TCR-transduced RAG2-KO DP cells showing expression of the corresponding αβ and γδ TCRs, respectively, on the cell surface of T-lineage cells.

Detailed Description

[0068] As described above, the inventors differentiated human pluripotent stem cells with a CRISPR/Cas9-directed deletion of the *RAG2* gene (RAG2-KO) and showed that human RAG2-deficient developing T cells progress up to the CD4+CD8+ stage. The inventors also showed that RAG2-KO CD4+CD8+ double positive cells can be engineered to express TCRαβ and TCRγδ chains and that expression of a rearranged TCRβ chain promotes cell survival and/or proliferation of developing human T-cells at the double positive stage.

I. Method for generating cells

[0069] Accordingly, the disclosure provides a method of generating stem or progenitor cells unable to undergo T cell receptor (TCR) gene rearrangements, the method comprising:

(a) culturing a sample comprising stem cells or progenitor cells,

wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells. In one embodiment, the method further comprises (b) isolating cells of the T cell lineage.

[0070] The disclosure also provides a method of generating a cell of the T cell lineage comprising an unrearranged T cell receptor (TCR) gene locus, the method comprising: (a) culturing a sample comprising stem cells or progenitor cells, wherein the expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem or progenitor cells.

[0071] The term “cells of the T cell lineage” refers to cells that show at least one phenotypic characteristic of a T cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for T lineage commitment on cells or a precursor or progenitor thereof, or a physiological, morphological, functional, or immunological feature specific for a T cell.

[0072] In one embodiment, the cells of the T cell lineage are human cells.

[0073] As used therein, the term "a cell" or "the cell" includes a plurality of cells.

[0074] As used therein, the term "isolated" means that a cell has been separated or purified from cellular or biological material found with the cells in their native environment. It thus distinguishes the cells from how they exist in nature.

[0075] The disclosure also provides a method of generating stem or progenitor cells unable to undergo B cell receptor (BCR) gene rearrangements, the method comprising:

(a) culturing a sample comprising stem cells or progenitor cells,

wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells. In one embodiment, the method further comprises (b) isolating cells of the B cell lineage.

[0076] The disclosure further provides a method of generating a cell of the B cell lineage comprising an unrearranged B cell receptor (BCR) gene locus, the method comprising: (a) culturing a sample comprising stem cells or progenitor cells, and (b) isolating cells of the B cell lineage, wherein the expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem or progenitor cells.

[0077] The term "cells of the B cell lineage" refers to cells that show at least one phenotypic characteristic of a B cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for B-lineage on cells or a precursor or progenitor thereof, or a physiological, morphological, functional, or immunological feature specific for a B cell. In another embodiment, the cells of the B cell lineage are CD20+ or CD19+ cells. In another embodiment, the cells of the B cell lineage are Tumor-Infiltrating B Cells (TIBs).

[0078] In one embodiment, the cells of the B cell lineage are human cells.

[0079] Cells of the T cell lineage may be (a) progenitor or precursor cells committed to the T cell lineage ("progenitor T cells" or "proT cells", as described herein); (b) CD7+ immature T cells; (c) cells that have undergone CD4 or CD8 lineage commitment (e.g. CD4+CD8^{lo}TCR^{int} cells); (d) characterized by TCR gene

rearrangement; (e) precursor thymocytes that are CD4+CD8+ double positive (DP); (f) CD4-CD8+ or CD4+CD8- and optionally TCR^{hi}; (g) CD3+CD90+; (h) single positive (SP) cells that are CD4-CD8+ or CD4+CD8- and TCR^{hi}; (i) TCR-$\alpha\beta^+$ and/or TCR-$\gamma\delta^+$; (j) characterized by expression of any of multiple Vβ chains (e.g. Vβ-3, -6, and 17a); or (k) mature and functional or activated T cells which may be characterized as $TCR/CD3^{hi}$, CD4-CD8+ or CD4+CD8-.

[0080] In one embodiment, a cell of the T cell lineage is a "progenitor T cell' or "proT cell". The term "progenitor T cell" or "proT cell" as used herein means a T cell that is capable of maturing into a mature T cell or lymphocyte. In one embodiment, the pro T cell is a CD45+CD34+CD7+ proT cell.

[0081] In another embodiment, the progenitor T cell is a human progenitor T cell. Phenotypes of human progenitor T cells include CD34+CD7+ and CD7+CD5+CD1a-.

[0082] The present inventors showed that human RAG2-deficient developing T cells progress up to the CD4+CD8+ double positive stage. Accordingly, in another embodiment, a cell of the T cell lineage is a CD4 and CD8 double positive (DP) cell characterized by a CD4+CD8+ or CD4+CD8+CD3+ phenotype.

[0083] In another embodiment, a cell of the T cell lineage is a CD4 or CD8 single positive (SP) cell characterized by a CD4-CD8+, CD4+CD8- or CD4-CD8+CD3+, CD4+CD8-CD3+ phenotype.

[0084] As used herein an "unrearranged T-cell receptor (TCR) gene locus" refers to a TCR gene locus which has not undergone rearrangement and remains in the germline configuration. Similarly, the term "unrearranged B-cell receptor (BCR) gene locus" refers to a BCR gene locus which has not undergone rearrangement and remains in the germline configuration.

[0085] The TCR gene locus can comprise an alpha (α) chain and a beta (β) chain (encoded for example by genes TRA and TRB, respectively), or a gamma and delta (γ/δ) chains (encoded for example by genes TRG and TRD, respectively). As will be understood by a person of skill in the art, during development of a T cell, rearrangement of segments of the genes encoding the TCR gene locus occurs to encode for antigen-specific receptors.

[0086] As used herein, the term "gene or protein required for V(D)J recombination" refers to a gene of protein that is required for correct rearrangement of the B- or T-cell receptor (BCR or TCR) gene loci. In one embodiment, the gene or protein

required for V(D)J recombination is a lymphocyte specific gene such as RAG1 or RAG2 or TdT.

[0087] In another embodiment, the gene or protein required for V(D)J recombination is a member of the non-homologous end joining (NHEJ) pathway for DNA repair.

[0088] In another embodiment, the gene or protein required for V(D)J recombination is Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1; also known as Cernunnos or XRCC4-like factor (XLF)), Paralog of XRCC4 and XLF (PAXX), or the DNA polymerases λ and μ.

[0089] As noted above, in one embodiment, the gene or protein required for V(D)J recombination is RAG1 or RAG2. The V(D)J Recombination Activating Genes (RAG) 1 and 2 encode two essential DNA processing enzymes required for the rearrangement of the B- and T-cell receptor (BCR or TCR) gene loci (Schatz at al, 1989; Oettinger et al, 1990). As described above, the inventors differentiated human pluripotent stem cells with a CRISPR/Cas9-directed deletion of the *RAG2* gene (RAG2-KO) and showed that human RAG2-deficient developing T-cells progress up to the CD4+CD8+ stage.

[0090] Accordingly, in the methods disclosed herein, a cell of the T cell lineage or B cell lineage may be generated by culturing a sample comprising stem cells or progenitor cells, wherein the expression and/or function of RAG1 and/or RAG2 in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells.

[0091] As used herein, RAG1 refers to V(D)J Recombination Activating Protein (RAG) 1, which is encoded by the *RAG1* gene. The term "RAG1" includes RAG1 from any species or source. The term also includes sequences that have been modified from any of the known published sequences of RAG1 proteins and genes. RAG1 or the *RAG1* gene may have any of the known published sequences for RAG1 which can be obtained from public sources such as GenBank. In one embodiment, RAG1 is human RAG1. Examples of human amino acid sequences for RAG1 include GenBank accession no. AAQ13571.1. Examples of human nucleic acid sequences for *RAG1* include include GenBank no. NM_001377277.1 (Gene ID: 5896). The aforementioned sequences are incorporated herein by reference.

[0092] RAG2 refers to V(D)J Recombination Activating Protein (RAG) 2, which is encoded by the *RAG2* gene. The term "RAG2" includes RAG2 from any species or source. The term also includes sequences that have been modified from any of the known published sequences of RAG2 proteins and *RAG2* genes. RAG2 or the *RAG2* gene may have any of the known published sequences for RAG2 which can be obtained from public sources such as GenBank. In one embodiment, RAG2 is human RAG2. Examples of the human amino acid sequences for RAG2 include GenBank accession no. AAH22397.1. Examples of human nucleic acid sequences for *RAG2* include GenBank accession no. NM_000536.4 (Gene ID: 5897). The aforementioned sequences are incorporated herein by reference.

[0093] As used herein, the term "RAG1 expression" includes both RAG1 protein expression and *RAG1* gene expression. Likewise, term "RAG2 expression" includes both RAG2 protein expression and *RAG2* gene expression.

[0094] As used herein, the term "RAG1 function" and "RAG2 function" refers to the biological activity of the RAG1 and RAG2, respectively. For example, cells lacking the biological activity of RAG1 and/or RAG2 enzymes are unable undergo rearrangement of the B cell receptor and T cell receptor (BCR or TCR) gene loci.

[0095] Endogenous expression and/or function of a gene or protein required for V(D)J recombination (for example endogenous expression and/or function of RAG1 and/or RAG2) can be reduced or eliminated using any of a number of methods known in the art.

[0096] Reduced or eliminated expression and/or function of a gene or protein required for V(D)J recombination may be accomplished by mutation of deletion of the corresponding gene sequence. Reduced or eliminated expression and/or function of RAG1 or RAG2 may be accomplished for example by mutation or deletion of *RAG1* or *RAG2* gene sequences. For example, *RAG1* or *RAG2* gene sequences may be mutated or deleted from the genome of pluripotent stem cells using, for example, gene editing methods. Thus, for example, approaches employing RNA/DNA guided endonucleases (e.g., Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/Cas9, Cpf1, and Argonaute), Transcription Activator-Like Effector (TALE)-nucleases, zinc finger nucleases (ZFN), or meganucleases can be adapted for use in embodiments of the present disclosure. In various examples, insertions or deletions are made by gene editing to cause a frame shift mutation, leading to gene knock out (i.e., lack of expression of a functional gene product).

[0097] Specific examples of protocols used in the present invention in the context of *RAG2* include transfection of an expression vector containing cassettes for GFP, Cas9 endonuclease, a CRISPR chimeric cDNA and a gRNA moiety that targets *RAG2*.

[0098] In another embodiment, a RAG1 and/or RAG2 inhibitor is used to reduce endogenous RAG1 and/or RAG2 expression and/or function. The term "inhibitor" refers to an agent that reduces, decreases or otherwise blocks expression or activity of its target and includes any substance that is capable of inhibiting the expression or activity of the target and includes, without limitation, small molecules, antisense oligonucleotide molecules (antisense nucleic acid molecules), siRNAs or shRNAs, aptamers, proteins, antibodies (and fragments thereof), gene editing agents and other substances directed at the target expression or activity.

[0099] Endogenous RAG1 and/or RAG2 expression and/or function can be reduced transiently or permanently (for example, by introducing gene mutations or deletions into the germline).

[00100] As used herein, the term "wildtype" refers to a cell which has normal (non-modified), endogenous expression levels of a gene or protein required for V(D)J recombination. In one embodiment, the term "wildtype" refers to a cell which has normal (non-modified), endogenous expression levels of RAG1 and/or RAG2 genes or proteins. The wildtype cell is optionally a stem or progenitor cell.

[00101] In one embodiment of the present disclosure, endogenous RAG1 expression and/or function is reduced by at least 5%, 10%, 25%, 50%, 75% or 100% compared to a wild-type cell. In another embodiment, the cell has no detectable endogenous RAG1 expression and/or function.

[00102] In another embodiment, endogenous RAG2 expression and/or function is reduced by at least 5%, 10%, 25%, 50%, 75% or 100% compared to a wild-type cell. In another embodiment, the cell has no detectable endogenous RAG2 expression and/or function.

[00103] In one embodiment, the stem or progenitor cell is a pluripotent stem cell. As used herein, the term "pluripotent stem cell" refers to any stem cell having the potential to differentiate into all cell types of a human or animal body, not including extra-embryonic tissues. These stem cells include both embryonic stem cells (ESCs) and induced pluripotent cells (iPSCs). Hence, the cells suitable for the method of the present

invention include stem cells selected from iPSCs and ESCs. In one embodiment, the pluripotent stem cells are human pluripotent stem cells (hPSCs) and they include human iPSCs (hiPSCs) and human ESCs (hESCs).

[00104] The term "embryonic stem cell" or "ESC" as used herein refers to undifferentiated embryonic stem cells that have the ability to integrate into and become part of the germ line of a developing embryo.

[00105] The term "induced pluripotent stem cell" or "iPSC" as used herein refers to cells derived from somatic cells, such as skin or blood cells that have been reprogrammed back into an embryonic-like pluripotent state. In one embodiment, iPSCs are derived from T cells with a known or unknown TCR specificity (for example, T cells bearing TCRs with specificity against cancer).

[00106] In one embodiment, the stem or progenitor cell is a hematopoietic stem or progenitor cell (HSPC). In another embodiment, the stem or progenitor cell is a $CD34^+$ hematopoietic precursor cell, optionally a $CD34^+$ hemogenic endothelial precursor cell that has been differentiated from an ESC or iPSC, or a $CD34^+$ pre-hematopoietic cell differentiated from an ESC or pluripotent stem cell (PSC). Various differentiation protocols for obtaining $CD34^+$ cells are known in the art. For therapeutic applications, the stem cells or progenitor cells used to generate the cells of the T cell lineage may be obtained from the patient to be treated.

[00107] Stem or progenitor cells may be obtained from any suitable source, including, without limitation, umbilical cord blood, embryos, embryonic tissue, fetal tissue, bone marrow and blood.

[00108] Typically, a sample containing stem or progenitor cells is first depleted of non-stem cells or mature cells. Negative and positive selection methods known in the art may be used for enrichment of the stem or progenitor cells. For example, cells can be sorted based on cell surface antigens using a fluorescence activated cell sorter, or magnetic beads which bind cells with certain cell surface antigens. Negative selection columns can be used to remove cells expressing lineage specific surface antigens.

[00109] In an embodiment, a sample containing stem or progenitor cells is separated into lineage-negative (Lin^-) and lineage position (Lin^+) fractions. The Lin^- fraction can be sorted for $CD34^+$ cells.

[00110] The progenitor cells or stem cells may be cultured under suitable conditions to generate cells of the T cell lineage or B cell lineage. Methods of culturing

progenitor cells or stem cells to generate cells of the T cell lineage or B cell lineage are known in the art.

[00111] For example, as described herein, human pluripotent stem cells may be induced to differentiate as embryoid bodies, then CD34+ cells may be isolated by magnetic-assisted cell-sorting and placed in co-culture with OP9-DL4 cells to induce their differentiation towards the T cell. Such a protocol is described in Kennedy et al. 2012.

[00112] In one embodiment, the cells are cultured in the presence of one or more Notch ligands conjugated to a suspension support for a sufficient time to form cells of the T cell lineage.

[00113] The progenitor cells or stem cells may be cultured on plates or in suspension in a bioreactor, optionally a closed or a closed, automated bioreactor. Various bioreactors are known in the art and can include batch, fed batch or continuous bioreactors. An example of a continuous bioreactor is a continuous stirred-tank reactor model.

[00114] Various concentrations of progenitor cells or stem cells in the culture are contemplated. For example, the concentration of progenitor cells or stem cells in the culture may be anywhere from 1 to millions of cells per ml of media.

[00115] One or more positive cytokines that promote commitment and differentiation of cells of the T cell lineage or B cell lineage may also be added to the culture. The cytokines may be human in origin, or may be derived from other species. The concentration of a cytokine in a culture is typically about 1-10ng/ml. The following are representative examples of cytokines that may be employed in the present application to promote commitment and differentiation of cells of the T cell lineage: all members of the Flt-3-ligand, and interleukin-7 (IL-7) and Stem Cell Factor. In one embodiment, the cytokines used herein are Flt-3-ligand and IL-7 and Stem Cell Factor. The cytokines may be used in combination with equal molar or greater amounts of a glycosaminoglycan such as heparin sulfate. The cytokines are commercially available or can be produced by recombinant DNA techniques and purified to various degrees. Some of the cytokines may be purified from culture media of cell lines by standard biochemical techniques.

[00116] One or more additional molecules, optionally conjugated to a suspension support, may also be added to the culture. In one embodiment, the additional molecule

is a molecule that promotes T cell development (for example, promotes commitment and differentiation of cells of T cell lineage), also referred to herein as a "T cell co-stimulatory molecule". In one example, the inventors have shown that microbead-conjugated DL4 and VCAM1 cultured with HSPCs accelerated differentiation to the T cell lineage. Thus, in one embodiment, the T cell co-stimulatory molecule is VCAM1. As used herein, the term "VCAM1" refers to Vascular cell adhesion protein 1 also known as vascular cell adhesion molecule 1 (VCAM1) or cluster of differentiation 106 (CD106), a protein that in humans is encoded by the *VCAM1* gene. The term "VCAM1" also includes a mutant or variant of a VCAM1. In another embodiment, the T cell co-stimulatory molecule is a cytokine or chemokine (Stem Cell Factor, IL-7, CCL25, or CXCR4), Major Histocompatibility Complex (MHC) class I or class II, or co-stimulatory (CD80, CD86) molecule. Optionally, the T cell co-stimulatory molecule comprises at least one protein tag. Various protein tags are known in the art and can be used for a number of purposes. In one embodiment, the T cell co-stimulatory molecule comprises an Fc tag (also known as an Fc-fusion protein).

[00117] The progenitor cells and stem cells may be cultured in culture media comprising conditioned media, non-conditioned media, or embryonic stem cell media. Examples of suitable conditioned media include IMDM, DMEM, or αMEM, conditioned with embryonic fibroblast cells (e.g. human embryonic fibroblast cells or mouse embryonic fibroblast cells), or equivalent media. Examples of suitable non-conditioned media include Iscove's Modified Dulbecco's Medium (IMDM), DMEM, or αMEM, or equivalent media. The culture media may comprise serum (e.g. bovine serum, fetal bovine serum, calf bovine serum, horse serum, human serum, or an artificial serum substitute) or it may be serum free.

[00118] In one embodiment, the culture conditions entail culturing the progenitor cells or stem cells for a sufficient period of time so that cells in the preparation form proT cells. In another embodiment, the culture conditions entail culturing the progenitor cells or stem cells for a sufficient period of time so that cells in the preparation form mature T cells, for example mature SP T cells. It will be appreciated that the cells may be maintained for the appropriate amount of time required to achieve the desired cellular composition. Optionally, the progenitor cells or stem cells are cultured for at least 6, 8, 10, 12, 14, 21, 28, 35 or 42 days.

[00119] In one embodiment, the method further comprises engineering the cells to comprise a nucleic acid encoding a T cell receptor (TCR) or a chimeric antigen

receptor (CAR). In another embodiment, the method further comprises engineering the cells to comprise a nucleic acid encoding a T cell receptor beta chain (TCRβ).

[00120] The cells may be engineered to comprise a nucleic acid encoding a TCR, CAR and/or TCRβ at any time during the differentiation process. For example, in one embodiment, the stem or progenitor cells comprise a nucleic acid encoding a TCR, CAR and/or TCRβ. In another embodiment, the cells of the T cell lineage comprise a nucleic acid encoding a TCR, CAR and/or TCRβ. In further embodiments, the stem or progenitor cells and/or the cells of the T cell lineage express a TCR, CAR and/or TCRβ.

[00121] In one embodiment, the stem or progenitor cells or the cells of the T cell lineage comprise a nucleic acid encoding a TCRβ alone. In other words, the stem or progenitor cells or the cells of the T cell lineage comprise a nucleic acid encoding a TCRβ and do not comprise a nucleic acid encoding a TCR or CAR.

[00122] In another embodiment, the stem or progenitor cells or the cells of the T cell lineage comprise a nucleic acid encoding a TCRβ and a nucleic acid encoding a CAR. In such an embodiment, the TCRβ directs differentiation while the CAR can provide therapeutic efficacy.

[00123] In another embodiment, the method further comprises engineering the cells of the B-cell lineage to express a B cell receptor (BCR), B cell receptor beta chain (BCRβ) and/or CAR. The cells may be engineered to comprise a nucleic acid encoding a BCR, BCRβ and/or CAR at any time during the differentiation process. For example, in one embodiment, the stem or progenitor cells comprise a nucleic acid encoding a BCR, BCRβ and/or CAR. In another embodiment, the cells of the B cell lineage comprise a nucleic acid encoding a BCR, BCRβ and/or CAR. In further embodiments, the stem or progenitor cells and/or the cells of the B cell lineage express a BCR, BCRβ and/or CAR.

[00124] The cells may be engineered by any method known in the art to comprise a nucleic acid encoding a TCR, TCRβ, CAR, BCR or BCRβ. For example, the cells may be transformed with viral or non-viral vectors carrying a TCR, TCRβ, CAR, BCR or BCRβ. Examples of viral vectors include, but are not limited to, retroviruses (including lentivirus), adenoviruses and adeno-associated viruses. Examples of non-viral vectors include, but are not limited to, nude DNA, minicircle DNA vectors, liposomes, polymerizers and molecular conjugates.

[00125] The TCR, BCR or CAR optionally confers specificity to an antigen such as a tumour associated antigen, a viral antigen or a self-antigen.

[00126] A "tumor associated antigen" is an antigen produced by tumor cells. Examples of tumor associated antigens include, but are not limited to, alphafetoprotein (AFP), arcinoembryonic antigen (CEA), CA-125, MUC-1, Epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), WT1 and NYESO1.

[00127] A "viral antigen" is an antigen encoded by a viral genome. Examples of viral antigens include, but are not limited to EBV, CMV, HIV and SARS viral antigens.

[00128] A "self-antigen" is an antigen that is produced by the subject. A self-antigen can be a tumor associated antigens. A T cell expressing a TCR or CAR conferring specificity to a self-antigen could be used for example to make regulatory T cells that block self-reactive T cells.

II. Pluripotent stem cells

[00129] As described herein, the present inventors generated RAG2-deficient (RAG2-KO) human pluripotent stem cell (hPSC) lines.

[00130] Accordingly, the disclosure also provides an isolated stem or progenitor cell, wherein expression of at least one gene or protein required for V(D)J recombination, optionally RAG1 and/or RAG2, in the stem cell or progenitor cell is reduced or eliminated compared to a wildtype stem cell or progenitor cell. Optionally, the stem cell or progenitor cell is human cell.

[00131] In one embodiment, the stem cell is a pluripotent stem cell.

[00132] In another embodiment, the pluripotent stem cell is an embryonic stem cell or induced pluripotent stem cell (iPSC).

[00133] Reduced or eliminated expression and/or function of RAG1 or RAG2 may be accomplished by any one of a number of methods known in the art.

[00134] In one embodiment, the stem cell or progenitor cell comprises a mutated or deleted *RAG1* and/or *RAG2* gene sequence. For example, insertions or deletions in the *RAG1* and/or *RAG2* gene sequences may cause a frame shift mutation, leading to gene knock out (i.e., lack of expression of a functional gene product).

[00135] In one embodiment of the present disclosure, endogenous RAG1 expression and/or function is reduced in the stem cell or progenitor cell by at least 5%, 10%, 25%, 50%, 75% or 100% compared to a wild-type cell. In another embodiment, the cell has no detectable endogenous RAG1 expression and/or function.

[00136] In another embodiment, endogenous RAG2 expression and/or function is reduced in the stem cell or progenitor cell by at least 5%, 10%, 25%, 50%, 75% or 100% compared to a wild-type cell. In another embodiment, the cell has no detectable endogenous RAG2 expression and/or function.

[00137] In addition, in one embodiment, the stem cell or progenitor cell further comprises a nucleic acid encoding a TCR, CAR, TCRβ, BCR or BCRβ. In another embodiment, stem cell or progenitor cell expresses a TCR, CAR, TCRβ, BCR or BCRβ.

[00138] In a further embodiment, the stem or progenitor cell comprises a nucleic acid encoding a TCRβ and does not comprise a nucleic acid encoding a TCR or CAR.

IV. Cells of the T cell or B cell lineage

[00139] The disclosure further provides cells of the T cell lineage or B cell lineage generated by the methods, systems and kits described herein, or mitotic or differentiated cells that are progeny of the cells.

[00140] In one embodiment, the disclosure provides a "progenitor T cell' or "proT cell" generated by the methods described herein. In another embodiment, the progenitor T cell is a human progenitor T cell, for example a human progenitor T cell characterized by CD34+CD7+, CD7+CD5+CD1a- or CD45+CD34+CD7+.

[00141] The disclosure also provides a double positive (DP) T-cell characterized by CD4+CD8+ or CD4+CD8+CD3+. The disclosure further provides a cell of the T-cell lineage that is a single positive (SP) cell characterized by CD4-CD8+, CD4+CD8- or CD8+CD3+, CD4+CD3+.

[00142] In one embodiment, the cells of the T cell lineage further comprise a nucleic acid encoding a TCR, CAR or TCRβ. In another embodiment, the cells of the T cell lineage express a TCR, CAR or TCRβ.

[00143] In a further embodiment, the cells of the T cell lineage comprise a nucleic acid encoding a TCRβ and does not comprise a nucleic acid encoding a TCR or CAR.

[00144] In another embodiment, a cell of the T cell lineage generated by the methods described herein (for example, a progenitor T cell or a mature T cell) is engineered with a T cell receptor (TCR) or a chimeric antigen receptor (CAR) that confers specificity to an antigen.

[00145] The antigen is optionally a tumor associated antigen (TAA), viral antigen or self-antigen. T-cells engineered to express a TCR or a CAR to confer specificity to a TAA can be useful for treating conditions such as cancer.

[00146] Likewise, in another embodiment, the cells of the B cell lineage further comprise a nucleic acid encoding a CAR, TCRβ, BCR or BCRβ. In another embodiment, the cells of the B cell lineage express a CAR, BCR or BCRβ.

[00147] In another embodiment, a cell of the B cell lineage generated by the methods described herein is engineered with a B cell receptor (BCR) or a chimeric antigen receptor (CAR) that confers specificity to an antigen.

[00148] The antigen is optionally a tumor associated antigen (TAA), viral antigen or self-antigen. T-cells engineered to express a BCR or a CAR to confer specificity to a TAA can be useful for treating conditions such as cancer.

[00149] In another aspect, the present disclosure provides a pharmaceutical composition comprising isolated stem or progenitor cells or cells of the T cell lineage or B cell lineage generated by the methods described herein and a pharmaceutically acceptable diluent or carrier.

[00150] Suitable diluents and carriers are described, for example, in Remington's Pharmaceutical Sciences. On this basis, the compositions include, albeit not exclusively, solutions of the proT cells in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with physiological fluids.

[00151] Pharmaceutical compositions include, without limitation, lyophilized powders or aqueous or non-aqueous sterile injectable solutions or suspensions, which may further contain antioxidants, buffers, bacteriostats and solutes that render the compositions substantially compatible with the tissues or the blood of an intended recipient. Other components that may be present in such compositions include water, surfactants (such as Tween™), alcohols, polyols, glycerin and vegetable oils, for example. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, tablets, or concentrated solutions or suspensions. The composition may be supplied, for example but not by way of limitation, as a lyophilized powder which is reconstituted with sterile water or saline prior to administration to the patient.

[00152] Pharmaceutical compositions also include cyropreservative solutions. In one embodiment, cells of the T cell lineage generated by the methods described herein are cryopreserved in appropriate media, for example pharmaceutically acceptable or GMP-grade media and optionally formulated for administration to a subject in need thereof.

[00153] Suitable pharmaceutically acceptable carriers include essentially chemically inert and nontoxic compositions that do not interfere with the effectiveness of the biological activity of the pharmaceutical composition. Examples of suitable pharmaceutical carriers include, but are not limited to, water, saline solutions, glycerol solutions, ethanol, N-(1(2,3-dioleyloxy)propyl)N,N,N-trimethylammonium chloride (DOTMA), diolesylphosphotidyl-ethanolamine (DOPE), and liposomes. Such compositions should contain a therapeutically effective amount of the compound, together with a suitable amount of carrier so as to provide the form for direct administration to the patient.

[00154] The compositions can be administered for example, by parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol or oral administration. For parenteral administration, solutions of the pro-T cells described herein can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations.

[00155] Preferably the cells of the T cell lineage or B cell lineage are present in an amount effective for treating a disease state in a subject need thereof. In one embodiment the cell of the T cell lineage is present in an amount effective to enhance hematopoietic progenitor cell engraftment in a subject in need thereof. Optionally, the composition further comprises cells of the T cell lineage, or tissue for transplantation. In one embodiment the tissue comprises a thymus. In another embodiment the tissue comprises an organ.

III. Kits

[00156] Stem or progenitor cell as described herein may be prepared and packaged in kits for use in generating cells of the T cell lineage or cells of the B cell lineage.

[00157] Accordingly, also provided herein is a kit for producing cells of the T cell lineage or cells of the B cell lineage comprising a stem or progenitor cell, wherein the expression of RAG1 and/or RAG2 in the stem cell or progenitor cell is reduced or eliminated compared to wildtype stem cell or progenitor cells.

[00158] In one embodiment, the kit further comprises culture media for culturing a sample comprising stem cells or progenitor cells for producing cells of the T cell lineage or cells of the B cell lineage. Examples of culture media include conditioned media, non-conditioned media, or embryonic stem cell media. The culture media may comprise serum (e.g. bovine serum, fetal bovine serum, calf bovine serum, horse serum, human serum, or an artificial serum substitute) or it may be serum free.

[00159] In another embodiment, the kit further comprises one or more additional molecules. In one embodiment, the additional molecule is a molecule that promotes T cell development (for example, promotes commitment and differentiation of cells of T cell lineage), also referred to herein as a “T cell co-stimulatory molecule”. In another embodiment, the T cell co-stimulatory molecule is VCAM1.

[00160] The media optionally includes one or more cytokines that promote commitment and differentiation of cells of the T cell lineage or cells of the B cell lineage. The cytokines may be human in origin, or may be derived from other species. The concentration of a cytokine in a culture is typically about 1-10ng/ml. The following are representative examples of cytokines that may be employed in the present application: all members of the Flt-3-ligand, and interleukin-7 (IL-7) and Stem Cell Factor. In one embodiment, the cytokines used herein are Flt-3-ligand and IL-7 and Stem Cell Factor. The cytokines may be used in combination with equal molar or greater amounts of a glycosaminoglycan such as heparin sulfate. The cytokines are commercially available or can be produced by recombinant DNA techniques and purified to various degrees. Some of the cytokines may be purified from culture media of cell lines by standard biochemical techniques.

[00161] In one embodiment, the kit comprises one or more containers for the within-described reagents.

[00162] Printed instructions providing guidance in the use of the reagent(s) may also be included in the kit, in various embodiments. The term "instructions" or "instructions for use" typically includes a tangible expression describing the cells, culturing time periods, temperature, media conditions, and the like. For example, in one embodiment, the instructions describe a method comprising culturing a sample comprising stem cells or progenitor cells.

V. Therapeutic Applications

[00163] T cells and B cells engineered to recognize specific antigens have wide ranging therapeutic applications.

[00164] Accordingly, the disclosure provides method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells or progenitor cells to a subject in need thereof,

[00165] wherein the stem cells or progenitor cells are engineered to comprise at least one of a nucleotide sequence encoding a T cell receptor (TCR), a chimeric antigen receptor (CAR) or a B cell receptor (BCR) that confers specificity to an antigen.

[00166] The disclosure also provides a method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, wherein the expression of RAG1 and/or RAG2 in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells of the T cell lineage to a subject in need thereof,

wherein the cells of the T cell lineage are engineered with a T cell receptor (TCR) or a chimeric antigen receptor (CAR) to confer specificity to an antigen.

[00167] The disclosure also provides a use of cells of the T cell lineage, generated by the methods described herein for treating a disease or condition in a

subject, wherein the cells of the T cell lineage are engineered with a T cell receptor (TCR) or a chimeric antigen receptor (CAR) that confer specificity to an antigen. The disclosure further provides a use of cells of the T cell lineage, generated by the methods described herein for preparation of a medicament for treating a disease or condition in a subject, wherein the cells of the T cell lineage are engineered with a T cell receptor (TCR) or a chimeric antigen receptor (CAR) that confers specificity to an antigen.

[00168] The disclosure also provides cells of the T cell lineage, generated by the methods described herein for use in treating a disease or condition in a subject, wherein the cells of the T cell lineage are engineered with a T cell receptor (TCR) or a chimeric antigen receptor (CAR) that confers specificity to an antigen.

[00169] The disclosure also provides a method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, and isolating cells of the B cell lineage, wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells of the B cell lineage to a subject in need thereof,

[00170] wherein the cells of the B cell lineage are engineered with at least one B cell receptor (TCR) to confer specificity to an antigen.

[00171] The disclosure also provides a use of cells of the B cell lineage, generated by the methods described herein for treating a disease or condition in a subject, wherein the cells of the B cell lineage are engineered with a B cell receptor (BCR) or CAR that confers specificity to an antigen. The disclosure further provides a use of cells of the B cell lineage, generated by the methods described herein for preparation of a medicament for treating a disease or condition in a subject, wherein the cells of the B cell lineage are engineered with a B cell receptor (BCR) or CAR that confers specificity to an antigen.

[00172] The disclosure also provides cells of the B cell lineage, generated by the methods described herein for use in treating a disease or condition in a subject, wherein the cells of the B cell lineage are engineered with a B cell receptor (BCR) or CAR that confers specificity to an antigen.

[00173] The antigen is optionally a tumor associated antigen (TAA), a viral antigen or a self-antigen. In one embodiment, the antigen is a TAA and the disease is cancer.

[00174] As used herein, the phrase "effective amount" or "therapeutically effective amount" means an amount effective, at dosages and for periods of time necessary to achieve the desired result. Effective amounts may vary according to factors such as the disease state, age, sex, weight of the subject. The amount of a given cell preparation that will correspond to such an amount will vary depending upon various factors. Such as the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. An “effective amount” will preferably be an amount effective for the cell of the T cell lineage to engraft the subject being treated.

[00175] The term "treating" or “treatment” as used herein and as is well understood in the art, means an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. "Treating" and "treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treating" and "treatment" as used herein also includes prophylactic treatment.

[00176] The term “subject” as used herein means any member of the animal kingdom and is preferably a human.

[00177] The following non-limiting examples are illustrative of the present application:

EXAMPLES

Example 1

Materials and Methods:

[00178] **hESC Maintenance**. Human ESCs (H1; WiCell Research Institute, Madison, WI) were maintained and expanded on plates coated with Matrigel (Corning,

NY, USA) in TeSR-E8 medium (STEMCELL Technologies, Vancouver, Canada). Cells were passaged by non-enzymatic dissociation using 0.5 mM EDTA.

[00179] Generation of RAG2-KO hESCs. The pD1321-GFP expression vector, containing cassettes for GFP, Cas9 endonuclease, a CRISPR chimeric cDNA, and the gRNA moiety designed to target RAG2 [GGTTATGCTTTACATCCAGA (SEQ ID NO: 1)], was custom synthesized (DNA2.0). After transfection with Lipofectamine 3000 (Life Technologies, Carlsbad, CA), GFP^+ hESCs were sorted using flow cytometry. Individual clones were picked, expanded, and aliquots were collected for purification of genomic DNA using the Genomic DNA kit (Invitrogen). Mutations (indels) were validated by sequencing products of PCR amplification of the regions flanking the targeting sites. The RAG2 KO clone 1 exhibited a 1bp deletion in one allele and 16bp deletion in the other allele, while clone 4 exhibited a 11bp deletion in one allele and 23bp deletion in the other allele.

[00180] Western Blot Analysis. Briefly, cellular lysates were prepared by incubating the cells in lysis buffer (50 mM Tris-HCl, pH7.5, 150 mM NaCl, 0.5% NP-40, 2 mM EDTA) containing protease inhibitor cocktail (Roche) for 20 min at 4°C, followed by centrifugation at 14000×g for 15 min at 4°C. Proteins were separated by SDS-PAGE, transferred onto PVDF membrane (Millipore, Louis, MO) and probed with anti-RAG2 antibody (Abcam – Ab95955; 1:1000 dilution) overnight at 4°C followed by incubation with secondary antibody. The anti-RAG2 antibody used was a rabbit polyclonal made against a recombinant fragment corresponding to amino acids 271-519 of human RAG2 (Abcam), which are well beyond the gRNA targeting site. Immunoreactive bands were visualized using western blotting Luminol reagent (Thermo). PBMCs from a T-ALL patient were used as positive control (Bories et al, 1991).

[00181] Immunostaining. Cells were fixed in 4% paraformaldehyde, and permeabilization and blocking were performed in 5% NGS (Abcam, Cambridge, USA) and 1% Triton X-100 (Sigma-Aldrich, Louis, MO) in PBS for 30 min. Cells were stained and analyzed as previously described (Li et al, 2017).

[00182] Teratoma Formation. All animal studies were approved by Ethics Committee of Experimental Research of Peking University and were in accordance with the International Animal Care and Use Committee Guidelines. 6-8 week-old non-obese diabetic (NOD)/SCID mice were injected subcutaneously with 1×10^7 hESCs resuspended in DMED-F12 with 50% Matrigel to allow teratoma formation for 8 weeks. Histological analysis was performed as previously described (Li et al, 2017) .

[00183] hPSC Differentiation and CD34$^+$ Isolation. hPSCs, H1 embryonic stem cells (ESC) were differentiated as previously described (Kennedy et al, 2012). After 8 days of differentiation, embryoid bodies were harvested and dissociated into single-cells using Collagenase type IV and trypsin-EDTA as previously described (Kennedy et al, 2012), and positively selected using a MACS column (Miltenyi Biotec) with anti-CD34 PE-conjugated antibody (BD Biosciences) and anti-PE microbeads. The cell yield and purity of the positive selection was assessed pre- and post- MACS by flow cytometry.

[00184] OP9-DL4-7FS Co-Culture and Differentiation. OP9-DL4 cells expressing hIL-7, hFLT3-L, and hSCF (7FS) were generated and grown in α-MEM containing 10-20% FBS (Gibco), 1% Pen-Strep (Life Technologies), and phospho-ascorbic acid (Sigma-Aldrich) at 37°C, 5% CO_2. For co-cultures, OP9-DL4-7FS cells were plated in 6-well plates the previous day. MACS-purified CD34$^+$ cells were counted and seeded at a density of 5-20 x10^4 cells per 6-well plate, and differentiated by co-culture with OP9-DL4-7FS cells in OP9 medium at 37°C, 5% CO_2, and cell were passaged and co-cultured with fresh OP9-DL4-7FS cells every ~5 days.

[00185] Retroviral Transduction. PG13 cell lines stably expressing empty vector dTomato, TCRα-dTomato, or TCRβ-dTomato were grown towards 70% confluency, at which point the media was switched to α-MEM with 15% FBS and 1% Pen-Strep to condition the supernatant for 48 h before transducing OP9-DL4-7FS/T-cell cultures. On D8+24 of OP9-DL4-7FS/T-cell cultures, cells were transduced with corresponding PG13 supernatants once a day with 1 µl/ml polybrene and centrifuged at 2000xg for 90 min at RT for 4 d (2-3x10^6 cells per 2 ml of supe). Cells were rested for 3 d and then sorted as dTomato$^+$ CD7$^+$CD5$^+$CD4$^+$CD8$^+$ DPs. Sorted cells were placed in OP9-DL4-7FS co-cultures for up to 10 days.

[00186] Flow Cytometry. Cells were stained for 30 min on ice with the following mouse anti-human antibodies: CD3-BrilliantViolet421, CD4-AlexaFluor700, CD8β-PE, CD31-FITC, CD34-PE, CD45RA-PE/CF594, TCRgd-FITC (BD Biosciences), CD5-PE/Cy7, CD7-AlexaFluor700, CD45-APC/eFluor780, TCRab-APC (eBiosciences), CD8α-PE/Dazzle, CD38-BrilliantViolet421 (BioLegend). Cells were resuspended in flow cytometry buffer containing DAPI, data was collected using LSR Fortessa flow cytometer (BD Biosciences) and analyzed using FlowJo version 9.7.6. For intracellular staining, cells were fixed and permeabilized using Fixation/Permeabilization kit with GolgiPlug™ (BD Biosciences) as per manufacturer's instructions.

[00187] RNA-Seq Analysis of Control and RAG2-KO *in vitro* Derived $CD4^+CD8^+$ cells. Cells were collected at co-culture day 24-28 and stained with fluorochrome-labelled antibodies to CD45, CD7 (eBioscience), CD5, CD4, CD8 (BD Biosciences), DAPI, and sorted into $CD4^+CD8+$ populations using FACSVantage Diva or FACSAria cell sorters (BD Biosciences). Total RNA was extracted from the sorted cell populations using TRIzol. Purified RNA was subjected to RNA sequencing using Illumina Novaseq 6000. Library preparation was done using Illumina TruSeq Strandard Total RNA Sample Preparation kit. Sequencing was done using 100-cycle paired read protocol and multiplexing to obtain ~40 million reads/sample. Samples were aligned to GRCh38 using HISAT2. Read counts were calculated using HTSeq. Differential gene expression analysis was done using edgeR package in the R platform.

[00188] Statistical Analysis. The data and error bars are presented as mean ± standard error of mean. To determine statistical significance, a one-way ANOVA (comparing three means) was performed using Prism version 6. Statistical significance was determined as $*P<0.05$ and $**P<0.01$.

[00189] Data Availability. Raw and processed RNA-Seq data are available from the Gene Expression Omnibus under accession number GSE164276 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE164276).

Results and Discussion

[00190] Generation and characterization of RAG2-KO hPSC lines. To evaluate the role of RAG2 in human T-cell development, CRISPR-Cas9 gene editing was used to target exon 3 of the *RAG2* gene (**Fig. 1A)**. hPSCs were transfected with a plasmid encoding the *RAG2*-targeting guide RNA, the Cas9 enzyme, and green fluorescent protein (GFP). Transfected GFP^+ hPSCs were single-cell sorted and cultured. After expanding individual clones, two clones were identified that contained unique insertion-deletions with bi-allelic mutations (KO-1 and KO-4) (**Fig. 1A**). To evaluate the impact of the *RAG2* mutations on protein expression, Western blot analysis was performed, which confirmed the absence of detectable RAG2 protein in both KO-1 and -4 derived T-lineage cells (**Fig. 1B**).

[00191] To assess whether RAG2-KO hPSCs maintained pluripotency, the expression of key markers and teratoma formation was evaluated. Immunofluorescence staining showed that RAG2-KO hPSCs expressed OCT4, NANOG, SOX2, and SSEA-4 (**Fig. 1C**). To functionally test pluripotency, RAG2-KO hPSCs were injected into immunodeficient mice, and histological analysis revealed RAG2-KO teratoma formation

with all three germ layers (**Fig. 1D**), indicating that RAG2-KO hPSCs retained key features of pluripotency. To determine the capacity of RAG2-KO hESCs to generate hematopoietic progenitors, CD34 expression was analyzed after 8 days of embryoid-body differentiation cultures (Kennedy et al, 2012). Control WT, RAG2-KO-1 and -4 hPSCs gave rise to similar frequencies of hemogenic endothelial $CD34^+$ cells, which could be further enriched by magnetic-assisted cell sorting (MACS) (**Fig. 1E**).

[00192] T-cell development from RAG2-KO hPSCs. $CD34^+$ hemogenic endothelial cells were MACS-enriched and cultured with OP9-DL4 cells, expressing human IL-7, FLT3-ligand and stem cell factor (7FS), to induce T-cell differentiation. After 10 days of culture, Control WT, RAG2-KO-1 and -4 cells proceeded along the T-cell lineage, as marked by expression of both CD7 and CD5 (**Fig. 2A**). All three groups reached the $CD4^+$ intermediate single positive (ISP) stage by day 15 and displayed intracellular CD3 expression by day 20 (**Fig. 2A**). After 24 days of culture, the majority of cells from Control WT, RAG2-KO-1 and -4 groups were $CD7^+CD5^+$ (**Fig. 2A**). Of note, by 29-34 days of culture, Control WT, RAG2-KO-1 and -4 cells all reached the $CD4^+CD8^+$ DP stage (**Fig. 2A**). However, as expected, only Control WT cells displayed intracellular TCRβ expression, and cell surface CD3/TCRβ expression (**Fig. 2A**). These results indicate that RAG2-deficient human T-cell progenitors can differentiate up to the $CD4^+CD8^+$ DP stage.

[00193] To determine cell survival and expansion, total cellularity was quantified from Control WT, RAG2-KO-1 and -4 developing T-cells. After 20 days of culture, all three samples showed similar cell numbers (**Fig. 2B**). However, after 40 days of culture, Control WT T-lineage cells further increased their survival and expansion as opposed to RAG2-KO-1 and -4 T-lineage cells, with approximately 10-fold greater cellularity by this time-point (**Fig. 2B**).

[00194] Forced expression of a TCRβ chain in RAG2-KO $CD4^+CD8^+$ DPs. RAG2-KO hPSC-derived $CD34^+$ cells were cultured on OP9-DL4-7FS cells for 24 days, and DP cells were retrovirally-transduced with an empty vector (dTomato), a rearranged TCRα chain (TRA 1383i), or a rearranged TCRβ chain (TRB 1383i) (**Fig. 3A**). Transduced RAG-KO cells were sorted for $CD7^+CD5^+CD4^+CD8^+$ DP cells, and placed back on OP9-DL4-7FS cells for an additional 10 days to assess for cell survival and expansion (**Fig. 3A**). Both dTomato- and TCRα-transduced DPs showed similar cell numbers after 10 days of culture (**Fig. 3B**). However, TCRβ-transduced DPs displayed significantly higher cell numbers after 10 days of culture compared to dTomato- and

TCRα-transduced DPs (**Fig. 3B**). Without being bound by theory, this suggests that, in contrast to a TCRα chain, expression of a rearranged TCRβ chain promotes cell survival and/or proliferation of developing human T-cells at the DP stage.

[00195] Transcriptomic analysis of Control WT, RAG2-KO, and RAG2-KO TCRβ-transduced $CD4^+CD8^+$ DP cells. Control WT, RAG2-KO control-transduced, and RAG2-KO TCRβ-transduced DP cells were sorted for RNA sequencing (RNA-Seq) analysis. Control WT DP cells expressed a large set of TCRα, TCRβ, TCRγ, and TCRδ genes that were absent in RAG2-KO control-transduced and RAG-KO TCRβ-transduced DP cells, with the notable exception of some TCR genes, including the 1383i TCRβ used in the transduction and a few TCRα genes, likely the result of germline transcripts induced by the β-selection signals, as seen in mice (Villey et al, 1997) (**Table 1**, and **Fig. 4A**). Furthermore, the expression of genes from RAG2-KO control- and TCRβ-transduced DP cells were compared with that of umbilical cord-blood (UCB)-hematopoietic stem cell-derived DP cells for a set of DP-associated signature genes (Casero et al, 2015) (**Table 1**). This analysis revealed that TCRβ-transduced RAG2-KO DP cells gained the expression of a suite of genes that were present in the UCB-derived DP cells, including *RORC*, which is induced following β-selection in mice (He 2000) (**Fig. 4B**).

[00196] Differentially expressed gene analysis was performed to determine genes that were up-regulated in Control WT compared to RAG2-KO (KO-1 and KO-4) DP cells, and vice versa. Genes significantly up-regulated in Control WT compared to both RAG2-KO-1 and RAG2-KO-4 include *CCDC152*, *GPR183*, *IL32*, and *MAL* (**Table 1** and **Fig. 5A**). Genes significantly up-regulated ($p<0.05$) in Control WT compared to RAG2-KO-1 or -4 included *ADAMTS17*, *IL1RL1*, *PLXNA2*, and *TEAD1*, or *CTSW*, *IKBKG*, *IL21R*, and *IL4R,* respectively (**Table 1** and **Fig. 5A**). Of note, many of these genes (such as *TEAD1*, *IKBKG*, and *IL32*) were also highly expressed in TCRβ-transduced RAG2-KO DP cells similar to Control WT DP cells (**Fig. 5A**). Interestingly, the expression of a subset of genes (such as *MAL*) were not rescued with forced expression of a TCRβ chain (**Fig. 5A**). Genes significantly up-regulated in both RAG2-KO-1 and RAG2-KO-4 compared to Control WT include *HBG1*, *HBG2*, *LOC100240735*, and *LOC339975* (**Table 1** and **Fig. 5B**). Genes significantly up-regulated in RAG2-KO-1 or -4 compared to Control WT include *CCDC8*, *CPA4*, *EPHA4*, and *ID1*, or *ANKRD1*,

CMTM8, *MET*, and *RHOU*, respectively (**Table 1** and **Fig. 5B**). Of note, many of these genes (such as *ID1*, *RHOU*, and *HBG1*) also showed low expression in TCRβ-transduced RAG2-KO DPs similar to Control WT DP cells (**Fig. 5B**). Interestingly, the expression of a subset of genes (such as *LOC100240735*) were not reduced with forced expression of a TCRβ chain (**Fig. 5B**). Phylogenetic tree analysis again showed that TCRβ-transduced RAG2-KO DP cells were more similar to Control WT DP cells than to control-transduced RAG2-KO DP cells (**Fig. 5**).

[00197] Analysis of cell cycle regulators, survival and differentiation genes, which are known to be involved in mouse β-selection (Lefebvre et al, 2005; Klein et al, 2019; Sicinska et al, 2003), revealed a set of genes, such as *RORC*, *CD27*, *ERG* and *CCDN3*, that are also regulated following TCRβ expression in RAG2-KO DPs (**Fig. 6A**). A gene ontology analysis to determine biological pathways that involve genes up-regulated in Control WT compared to RAG2-KO DP cells revealed a genetic program involved in "negative regulation of intrinsic apoptotic signaling pathway" (**Fig. 6B**). Furthermore, a gene set pathway pertaining to leukocyte regulation, included genes involved in "positive regulation of lymphocyte activation" and "regulation of lymphocyte proliferation" (**Fig. 6B**). Thus, these data provide additional evidence that RAG2-dependent TCRβ expression in developing human T-cells supports their survival and/or proliferation.

Summary

[00198] RAG2-KO hPSCs were generated to assess the role of TCRβ during human T-cell development. In contrast to the effects seen with *Rag1*- or *Rag2*-deficient mice, which show a complete lack of DP thymocytes, developing human T-cells were able to reach the $CD4^+CD8^+$ DP stage in the absence of RAG2 expression. Lack of RAG1/2 in mice results in a definitive block at the $CD44^-CD25^+$ DN3 stage, as it is well documented that RAG1/2-mediated TCRβ rearrangement controls the transition from the DN3 to the $CD44^-CD25^-$ DN4 and DP stages (von Boehmer et al, 1999; Michie and Zuniga-Pflucker, 2002). However, the precise developmental block in the absence of RAG1/2 expression or TCRβ rearrangement during human T-cell development was largely unresolved (Rothenberg and Taghon, 2005; Carrasco et al, 2002).

[00199] It was previously suggested that the requirement for TCRβ-induced survival/proliferation, or β-selection, occurs at the $CD4^+$ ISP stage, in which 5% of cells express cell-surface TCRβ protein (Blom et al, 1999). In another study, it was suggested

that β-selection instead happens later, at the $CD4^+CD8\alpha^+CD8\beta^-$ early double positive (EDP) stage in which 25% of cells express intracellular TCRβ protein (Carrasco et al, 1999). Thus, based on these two studies, it was proposed that β-selection begins at the $CD4^+$ ISP stage and continues until the EDP stage. Here, robust development was detected past the ISP and EDP to the DP stage, as CD4, CD8α, and CD8β expression was observed in RAG2-KO developing T-cells. While the requirement for the TCRβ chain in the phenotypic differentiation to the DP stage differs between mice and humans, the TCRβ chain similarly promotes cellular expansion (survival/proliferation) in both mouse and human developing T-cells, as enforced TCRβ expression rescued cellular expansion. Without being bound by theory, the requirement for TCRβ, and thus the preTCR, during, rather than prior to, the DP stage during human T-cell development may reflect differential expression of cell cycle and survival genes. In mice, expression of a preTCR in DN3 cells leads to expression of Bmi-1, which represses the expression of the cell cycle inhibitor Cdkn2a. Repression of Cdkn2a is required for preTCR-induced cell proliferation and the DN3-DP transition (Miyazaki et al, 2008). In summary, this study reveals the unexpected timing required for TCRβ-mediated β-selection in developing human T-cells.

Example 2

Forced expression of a TCRαβ and TCRγδ chains in RAG2-KO $CD4^+CD8^+$ DPs

[00200] RAG2-KO hPSC-derived $CD34^+$ cells were cultured on OP9-DL4-7FS cells for 21 days, and DP cells were retrovirally-transduced with an empty vector (dTomato), rearranged TCRαβ chains (1383i-TCR) (Roszkowski et al., 2003), or rearranged TCRγδ chains (3C2-TCR) (Benveniste et al., 2018) (**Fig. 7**). Transduced RAG-KO cells were sorted for $CD7^+CD5^+CD4^+CD8^+$ DP cells, and placed back on OP9-DL4-7FS cells for an additional 4 and 10 days to assess for cell survival and expansion (**Fig. 7A**). TCRαβ- and TCRγδ-transduced DPs displayed higher cell numbers after 4 and 10 days of culture compared to dTomato-transduced DPs (**Fig. 7A**), with TCRαβ-transduced cells showing a much higher fold expansion than TCRγδ-transduced cells, and akin to what was seen with TCRβ-transduced DP cells (Fig. 3). Without being bound by theory, this suggests that similar to a TCRβ chain alone (preTCR, pTα/TCRβ), expression of a rearranged TCRαβ or TCRγδ chains promote cell survival and/or proliferation of developing human T-cells at the DP stage. Additionally, flow cytometric

analysis of TCR-transduced RAG2-KO DP cells showed the expression of the corresponding αβ and γδ TCRs, respectively, on the cell surface of T-lineage cells (**Fig. 7B**). These results show that developing T cells obtained from RAG2-deficient PSCs differentiated *in vitro* can be transduced to effectively express a γδ or αβ TCR.

Example 3

[00201] CRISPR-Cas9 gene editing is used to target each of the following genes: Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1; also known as Cernunnos or XRCC4-like factor (XLF)), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

[00202] hPSCs are transfected with a plasmid encoding the gene targeting guide RNA, the Cas9 enzyme, and green fluorescent protein (GFP). Transfected GFP^+ hPSCs are single-cell sorted and cultured.

[00203] To assess whether various KO hPSCs maintained pluripotency, the expression of key markers and teratoma formation are evaluated. Immunofluorescence staining shows that the KO hPSCs express OCT4, NANOG, SOX2, and SSEA-4.

[00204] To functionally test pluripotency, the KO hPSCs are injected into immunodeficient mice, and histological analysis reveals KO teratoma formation with all three germ layers, indicating that KO hPSCs retain key features of pluripotency. To determine the capacity of KO hESCs to generate hematopoietic progenitors, CD34 expression is analyzed after 8 days of embryoid-body differentiation cultures (Kennedy et al, 2012). Control WT and KO hPSCs give rise to similar frequencies of hemogenic endothelial $CD34^+$ cells.

[00205] **T-cell development from KO hPSCs.** $CD34^+$ hemogenic endothelial cells are MACS-enriched and cultured with OP9-DL4 cells, expressing human IL-7, FLT3-ligand and stem cell factor (7FS), to induce T-cell differentiation. After 10 days of culture, Control WT and KO cells proceed along the T-cell lineage, as marked by expression of both CD7 and CD5. All three groups reach the $CD4^+$ intermediate single positive (ISP) stage by day 15 and display intracellular CD3 expression by day 20. After 24 days of culture, the majority of cells from Control WT and KO groups are $CD7^+CD5^+$ By 29-34 days of culture, Control WT and KO cells all reach the $CD4^+CD8^+$ DP stage.

[00206] To determine cell survival and expansion, total cellularity is quantified from Control WT, and KO developing T-cells. After 20 days of culture, all three samples show similar cell numbers.

[00207] **Forced expression of a TCRβ chain in KO $CD4^+CD8^+$ DPs.** KO hPSC-derived $CD34^+$ cells are cultured on OP9-DL4-7FS cells for 24 days, and DP cells are retrovirally-transduced with an empty vector (dTomato), a rearranged TCRα chain (TRA 1383i), or a rearranged TCRβ chain (TRB 1383i). Transduced KO cells are sorted for $CD7^+CD5^+CD4^+CD8^+$ DP cells, and placed back on OP9-DL4-7FS cells for an additional 10 days to assess for cell survival and expansion. Both dTomato- and TCRα-transduced DPs show similar cell numbers after 10 days of culture. However, TCRβ-transduced DPs display significantly higher cell numbers after 10 days of culture compared to dTomato- and TCRα-transduced DPs.

Example 4

Generation of KO SP T cells

Generation of KO SP T cells. KO hPSCs engineered with one or more of a rearranged TCRβchain (TRB 1383i), a CAR or a TCR are differentiated into CD34+ cells and subsequently CD4+CD8+ DP cells as above or in stromal cell-free conditions (see for example WO2019157597A1, the contents of which are incorporated by reference in their entirety). Generated CD4+CD8+ DP cells are further differentiated into CD4-CD8+ SP and/or CD8-CD4+ SP T cells. KO DP cells engineered with TCRβ successfully progress to SP T cells in comparison with KO-only cells.

Table 1. List of genes significantly up-regulated ($p<0.05$) in Control WT DPs compared to RAG2-KO-1 DPs, or vice versa, as indicated: (NA: non-assigned TCR gene names).

Up in Control					
ENSEMBL	SYMBOL	LogFC	Ave Exp	t Value	p Value
ENSG00000198865	CCDC152	8.517702	-1.2060943	8.196432	2.4619E-05
ENSG00000211750	<NA>	8.318697	-2.8399921	12.630925	1.0213E-06
ENSG00000211746	<NA>	7.923854	-2.6560982	8.493945	1.8718E-05
ENSG00000276953	<NA>	7.718605	-2.9993099	8.146927	2.5048E-05
ENSG00000275158	<NA>	7.319261	-3.0939512	12.344204	1.1756E-06
ENSG00000274752	<NA>	6.821362	-3.0215316	8.377361	1.9917E-05
ENSG00000278030	<NA>	6.732332	-3.0446835	7.923013	2.9525E-05
ENSG00000211706	<NA>	6.633362	-1.9248385	8.05775	2.611E-05
ENSG00000282499	<NA>	6.417086	-2.0223909	6.873888	7.8658E-05
ENSG00000276557	<NA>	6.368357	-3.3316934	10.397811	4.0823E-06
ENSG00000211693	<NA>	6.307571	-1.3708525	5.433344	0.00037427
ENSG00000275791	<NA>	6.191937	1.60630085	9.452939	8.1556E-06
ENSG00000275743	<NA>	6.189794	-2.5862869	6.486807	0.00011582
ENSG00000241657	<NA>	6.073368	-2.4291257	9.598497	7.2677E-06
ENSG00000211721	<NA>	6.02149	-0.7628611	9.334584	8.888E-06
ENSG00000211710	<NA>	5.894499	-1.981085	7.189231	5.6671E-05
ENSG00000211728	<NA>	5.821217	-2.5371219	5.304768	0.00042805
ENSG00000233306	<NA>	5.820969	-3.0621388	4.320709	0.00149548
ENSG00000211745	<NA>	5.516755	-0.1808708	9.413935	8.2219E-06
ENSG00000276819	<NA>	5.461533	-3.5593405	8.314578	2.0077E-05
ENSG00000230006	ANKRD36BP2	5.414753	3.27069758	4.145606	0.00186578
ENSG00000211821	<NA>	5.360107	-0.3451044	5.975287	0.00019464
ENSG00000283063	<NA>	5.088158	-2.876393	5.022423	0.00058681
ENSG00000211734	<NA>	5.056142	-0.468777	7.965634	2.6874E-05
ENSG00000237702	<NA>	5.02606	-2.6835032	4.99639	0.0006046
ENSG00000172005	MAL	4.956678	4.04116362	4.478536	0.00116925
ENSG00000211747	<NA>	4.852242	-0.0395991	9.746459	6.2572E-06
ENSG00000211698	<NA>	4.674811	-2.0689517	4.365069	0.00134415
ENSG00000211817	<NA>	4.569333	-2.7916217	4.999176	0.00059255
ENSG00000226660	<NA>	4.568507	-1.5216111	3.92235	0.00245463
ENSG00000164241	C5orf63	4.412744	-1.2728461	2.98561	0.00972973
ENSG00000140470	ADAMTS17	4.114259	-0.2748657	2.257093	0.03045552
ENSG00000211810	<NA>	4.016163	-2.6681461	3.632342	0.00362759
ENSG00000211713	<NA>	3.765733	-2.1670369	4.191198	0.00164333
ENSG00000165633	VSTM4	3.717044	-2.2032977	2.036388	0.04241983
ENSG00000211849	<NA>	3.620071	-1.1571862	2.76704	0.01311932
ENSG00000232869	<NA>	3.48762	-0.5815655	4.649635	0.00089024

ENSG00000210194	<NA>	3.460159	-0.9030918	2.211914	0.03135952
ENSG00000211716	<NA>	3.365599	-0.2876694	4.592905	0.00095486
ENSG00000237254	<NA>	3.290203	-0.466529	4.784741	0.00074607
ENSG00000230099	<NA>	3.288033	-0.8427471	2.640981	0.01571927
ENSG00000251578	<NA>	3.224662	-1.0269264	3.405065	0.00490892
ENSG00000211749	<NA>	3.209309	-0.6280164	3.53171	0.00407815
ENSG00000211714	<NA>	3.100552	0.63664893	3.52919	0.00408052
ENSG00000115602	IL1RL1	2.918121	-0.6201869	3.231124	0.00629336
ENSG00000211724	<NA>	2.917557	-0.9573169	2.920958	0.01004788
ENSG00000229769	<NA>	2.873665	-2.2358285	3.649591	0.00341216
ENSG00000076356	PLXNA2	2.779821	1.2204853	2.462547	0.02032877
ENSG00000248905	FMN1	2.680991	3.55553195	3.328335	0.0054196
ENSG00000211818	<NA>	2.678134	-1.178139	2.833739	0.01140162
ENSG00000211752	<NA>	2.621173	0.71807768	4.534972	0.00101587
ENSG00000187079	TEAD1	2.398258	-0.6408802	1.898367	0.04842699
ENSG00000169508	GPR183	2.395666	-0.0382727	1.901482	0.04818661
ENSG00000211804	<NA>	2.276024	0.17880719	3.010357	0.00865148
ENSG00000211836	<NA>	2.200166	-1.0795964	1.965313	0.04330296
ENSG00000211839	<NA>	2.166826	-0.5365795	2.052539	0.03778579
ENSG00000008517	IL32	1.846931	4.18320491	2.442861	0.02053788
ENSG00000211772	<NA>	1.539048	7.83477128	3.108563	0.00743986

Table 2. List of genes significantly up-regulated ($p<0.05$) in Control WT DPs compared to RAG2-KO-4 DPs, or vice versa, as indicated: (NA: non-assigned TCR gene names).

Up in Control					
ENSEMBL	SYMBOL	LogFC	Ave Exp	t Value	p Value
ENSG00000211750	<NA>	8.162062	-2.8399921	12.360598	1.1956E-06
ENSG00000211746	<NA>	7.767219	-2.6560982	8.30179	2.1936E-05
ENSG00000276953	<NA>	7.56197	-2.9993099	7.956993	2.9463E-05
ENSG00000211734	<NA>	7.208251	-0.468777	9.045181	1.162E-05
ENSG00000275158	<NA>	7.162626	-3.0939512	12.038229	1.4115E-06
ENSG00000034533	ASTE1	6.972768	0.67498444	2.250502	0.03632517
ENSG00000211728	<NA>	6.825546	-2.5371219	6.409828	0.00012845
ENSG00000211710	<NA>	6.51719	-1.981085	7.584541	3.9866E-05
ENSG00000233306	<NA>	6.456816	-3.0621388	4.890575	0.00072978
ENSG00000276557	<NA>	6.211721	-3.3316934	10.09443	5.0497E-06
ENSG00000283063	<NA>	6.092487	-2.876393	6.256271	0.00014724
ENSG00000275743	<NA>	6.033159	-2.5862869	6.291026	0.00014157
ENSG00000274752	<NA>	5.872245	-3.0215316	7.011514	6.7324E-05
ENSG00000278030	<NA>	5.783216	-3.0446835	6.61118	0.00010036
ENSG00000211706	<NA>	5.711686	-1.9248385	6.987882	6.8525E-05

ENSG00000275791	<NA>	5.601616	1.60630085	8.856185	1.2829E-05
ENSG00000282499	<NA>	5.589299	-2.0223909	5.964671	0.00019857
ENSG00000198865	CCDC152	5.586937	-1.2060943	6.027127	0.00018534
ENSG00000226660	<NA>	5.579945	-1.5216111	4.410879	0.00131195
ENSG00000129824	RPS4Y1	5.458735	5.87336909	2.187663	0.03724085
ENSG00000211747	<NA>	5.332693	-0.0395991	10.173377	4.6369E-06
ENSG00000211693	<NA>	5.316543	-1.3708525	4.887327	0.00070055
ENSG00000276819	<NA>	5.304898	-3.5593405	8.022671	2.5753E-05
ENSG00000211810	<NA>	5.276514	-2.6681461	5.123064	0.00052226
ENSG00000211821	<NA>	5.247636	-0.3451044	5.816677	0.00023135
ENSG00000237702	<NA>	4.869425	-2.6835032	4.802004	0.00076692
ENSG00000211745	<NA>	4.844219	-0.1808708	8.491467	1.6946E-05
ENSG00000211721	<NA>	4.78909	-0.7628611	8.034847	2.5073E-05
ENSG00000211716	<NA>	4.72396	-0.2876694	6.054901	0.00017449
ENSG00000211698	<NA>	4.545294	-2.0689517	4.243552	0.00157464
ENSG00000229769	<NA>	4.491292	-2.2358285	5.949991	0.00019436
ENSG00000232869	<NA>	4.451973	-0.5815655	5.683435	0.00026184
ENSG00000211713	<NA>	4.38113	-2.1670369	4.823196	0.00073347
ENSG00000116016	EPAS1	4.295392	-0.8807061	4.713222	0.00084097
ENSG00000251578	<NA>	4.178802	-1.0269264	4.274236	0.00148993
ENSG00000230099	<NA>	4.042578	-0.8427471	3.100613	0.00800344
ENSG00000076864	RAP1GAP	3.959056	-1.7469106	2.664417	0.01569386
ENSG00000172005	MAL	3.95788	4.04116362	3.625373	0.00365575
ENSG00000237254	<NA>	3.856401	-0.466529	5.495478	0.00031948
ENSG00000028137	TNFRSF1B	3.79933	-0.6752704	2.033321	0.04289521
ENSG00000145506	NKD2	3.757573	0.27551971	2.394666	0.02383015
ENSG00000277048	<NA>	3.741285	1.44983714	2.310452	0.02725463
ENSG00000211817	<NA>	3.647657	-2.7916217	3.832094	0.0026902
ENSG00000241657	<NA>	3.627882	-2.4291257	5.573106	0.00029035
ENSG00000211714	<NA>	3.616438	0.63664893	4.073116	0.00192154
ENSG00000008517	IL32	3.284801	4.18320491	5.796536	0.0002236
ENSG00000211771	<NA>	3.268893	-1.512696	2.044763	0.04050564
ENSG00000211749	<NA>	3.198221	-0.6280164	3.451613	0.0045814
ENSG00000177096	PHETA2	3.152482	0.13121737	3.825269	0.00267658
ENSG00000226174	TEX22	3.002381	-0.5562848	2.246348	0.0288737
ENSG00000169508	GPR183	2.99354	-0.0382727	2.515646	0.01887334
ENSG00000274452	<NA>	2.97966	-0.9626708	2.031624	0.04056901
ENSG00000211724	<NA>	2.902091	-0.9573169	2.850146	0.01119255
ENSG00000199753	SNORD104	2.896863	-0.0738509	2.107037	0.0358039
ENSG00000104870	FCGRT	2.881885	-0.2470579	3.283793	0.00581431
ENSG00000077238	IL4R	2.86209	0.37944373	2.434344	0.0213187

ENSG00000211818	<NA>	2.861742	-1.178139	3.042362	0.00834138
ENSG00000211752	<NA>	2.859241	0.71807768	5.020002	0.00055247
ENSG00000276180	HIST1H4I	2.848206	5.47377595	1.965865	0.04466518
ENSG00000149506	ZP1	2.788498	0.14468104	2.009078	0.04155506
ENSG00000240972	MIF	2.76273	0.26136195	2.784547	0.01232517
ENSG00000172543	CTSW	2.705115	0.14611252	3.315826	0.00552314
ENSG00000136840	ST6GALNAC4	2.702985	1.71210383	2.035465	0.03966518
ENSG00000184489	PTP4A3	2.649319	0.22426429	1.923271	0.04719826
ENSG00000032444	PNPLA6	2.618052	2.52046666	2.051902	0.03848134
ENSG00000160932	LY6E	2.611928	3.19636005	2.56894	0.01712299
ENSG00000166311	SMPD1	2.521156	0.20609228	2.363001	0.02353312
ENSG00000239791	<NA>	2.487449	-0.4539044	2.619593	0.01577698
ENSG00000185347	TEDC1	2.478509	1.29168656	1.892602	0.0490595
ENSG00000103522	IL21R	2.403267	0.84514527	3.501089	0.00419294
ENSG00000160972	PPP1R16A	2.334936	1.11911499	2.063406	0.0373436
ENSG00000128322	IGLL1	2.316112	2.83224971	2.871366	0.01068525
ENSG00000186010	NDUFA13	2.300787	-0.2558601	1.911583	0.04723936
ENSG00000211699	<NA>	2.295547	-0.6068566	2.04705	0.03825609
ENSG00000161642	ZNF385A	2.292218	-0.1031169	1.877838	0.04976267
ENSG00000101445	PPP1R16B	2.283182	3.29912799	2.629054	0.01548246
ENSG00000275714	HIST1H3A	2.250361	4.48007601	1.874151	0.04996283
ENSG00000271869	<NA>	2.242191	-0.527668	2.101436	0.03509275
ENSG00000121680	PEX16	2.241312	2.55954191	2.392867	0.02229991
ENSG00000175274	TP53I11	2.143382	1.31932639	2.058014	0.03744372
ENSG00000279693	<NA>	2.131329	2.43865387	2.582929	0.01658628
ENSG00000000938	FGR	2.105363	2.03569751	1.887195	0.04872219
ENSG00000108840	HDAC5	2.074299	2.24683163	1.890163	0.04845715
ENSG00000134569	LRP4	2.01582	3.88594501	1.921207	0.04612707
ENSG00000133454	MYO18B	1.993414	5.99665506	1.992781	0.04128694
ENSG00000236008	LINC01814	1.986033	-0.7381362	1.939974	0.04478419
ENSG00000269335	IKBKG	1.940037	1.51124153	1.924645	0.04581363
ENSG00000261971	<NA>	1.858333	0.8888184	2.276229	0.02658208
ENSG00000104689	TNFRSF10A	1.858278	2.90340726	2.140771	0.03278424
ENSG00000211772	<NA>	1.844483	7.83477128	4.823551	0.00070023
ENSG00000103056	SMPD3	1.832234	6.47443056	3.044848	0.00818832
ENSG00000211804	<NA>	1.774673	0.17880719	1.895796	0.04777756
ENSG00000211751	<NA>	1.600269	7.81676926	2.101263	0.03479669

Table 3. List of genes significantly up-regulated ($p<0.05$) in thymus DNs (CD34+CD7+CD1a+CD4-CD8-) compared to thymus DPs (CD4+CD8+), or vice versa from Casero et al., 2015 (18), as indicated: (NA: non-assigned TCR gene names).

Up in DN					
ENSA167:D207	SYMBOL	LogFC	Ave Exp	t Value	p Value
ENSG00000157554	ERG	9.18E+00	2.59E+00	7.91E+00	7.23E-05
ENSG00000124780	KCNK17	7.98E+00	1.20E+00	5.36E+00	7.17E-04
ENSG00000004809	SLC22A16	7.63E+00	2.19E-01	6.23E+00	2.93E-04
ENSG00000136531	SCN2A	7.62E+00	2.19E-01	6.30E+00	2.73E-04
ENSG00000169994	MYO7B	7.60E+00	2.65E+00	4.43E+00	2.02E-03
ENSG00000181104	F2R	7.47E+00	1.30E+00	5.42E+00	6.58E-04
ENSG00000228065	<NA>	7.47E+00	1.44E-01	6.14E+00	3.18E-04
ENSG00000149131	SERPING1	7.41E+00	1.05E-01	5.78E+00	4.53E-04
ENSG00000137868	STRA6	7.41E+00	9.05E-01	6.11E+00	3.27E-04
ENSG00000000971	CFH	7.40E+00	1.51E+00	5.13E+00	8.94E-04
ENSG00000132465	JCHAIN	7.30E+00	1.47E+00	4.21E+00	2.60E-03
ENSG00000163625	WDFY3	7.26E+00	8.12E-01	4.34E+00	2.23E-03
ENSG00000224739	<NA>	7.20E+00	-2.20E-02	4.16E+00	2.77E-03
ENSG00000246985	SOCS2-AS1	7.14E+00	-2.84E-02	5.58E+00	5.50E-04
ENSG00000115956	PLEK	7.04E+00	-6.62E-02	5.57E+00	5.52E-04
ENSG00000128322	IGLL1	6.92E+00	6.57E-01	5.12E+00	8.85E-04
ENSG00000116574	RHOU	6.85E+00	6.24E-01	5.55E+00	5.59E-04
ENSG00000174059	CD34	6.83E+00	2.34E+00	6.21E+00	2.91E-04
ENSG00000024422	EHD2	6.81E+00	-1.94E-01	5.23E+00	7.86E-04
ENSG00000154188	ANGPT1	6.80E+00	-2.05E-01	5.08E+00	9.24E-04
ENSG00000251463	<NA>	6.79E+00	-1.93E-01	5.44E+00	6.28E-04
ENSG00000081189	MEF2C	6.78E+00	1.39E+00	5.49E+00	5.92E-04
ENSG00000100320	RBFOX2	6.61E+00	1.80E+00	3.95E+00	3.50E-03
ENSG00000211688	<NA>	6.60E+00	-2.81E-01	5.11E+00	8.84E-04
ENSG00000113140	SPARC	6.57E+00	-3.00E-01	5.15E+00	8.46E-04
ENSG00000132464	ENAM	6.54E+00	-3.30E-01	5.05E+00	9.44E-04
ENSG00000183780	SLC35F3	6.54E+00	-3.30E-01	5.05E+00	9.44E-04
ENSG00000211825	<NA>	6.53E+00	-3.24E-01	5.18E+00	8.25E-04
ENSG00000196460	RFX8	6.50E+00	1.96E+00	3.80E+00	4.24E-03
ENSG00000155254	MARVELD1	6.43E+00	4.15E-01	5.12E+00	8.72E-04
ENSG00000279117	<NA>	6.42E+00	1.91E+00	5.17E+00	8.29E-04
ENSG00000100448	CTSG	6.34E+00	-3.95E-01	4.01E+00	3.22E-03
ENSG00000185215	TNFAIP2	6.28E+00	9.81E-01	3.04E+00	1.19E-02
ENSG00000137731	FXYD2	6.22E+00	1.72E+00	4.62E+00	1.52E-03
ENSG00000271270	TMCC1-AS1	6.19E+00	2.78E-01	4.15E+00	2.70E-03
ENSG00000211695	<NA>	6.19E+00	1.21E+00	3.87E+00	3.84E-03

ENSG00000211689	TARP	6.13E+00	2.75E+00	6.04E+00	3.34E-04
ENSG00000249100	<NA>	6.10E+00	2.54E-01	4.79E+00	1.24E-03
ENSG00000150457	LATS2	6.05E+00	-5.41E-01	3.91E+00	3.60E-03
ENSG00000231533	<NA>	6.04E+00	-5.62E-01	4.52E+00	1.69E-03
ENSG00000224373	<NA>	6.01E+00	-5.90E-01	4.62E+00	1.51E-03
ENSG00000171502	COL24A1	6.00E+00	1.00E+00	4.50E+00	1.75E-03
ENSG00000272711	<NA>	5.97E+00	-6.09E-01	4.58E+00	1.59E-03
ENSG00000141431	ASXL3	5.92E+00	-6.32E-01	4.53E+00	1.67E-03
ENSG00000120833	SOCS2	5.86E+00	2.62E+00	5.70E+00	4.60E-04
ENSG00000164100	NDST3	5.86E+00	4.64E+00	5.71E+00	4.56E-04
ENSG00000113269	RNF130	5.85E+00	1.26E+00	4.65E+00	1.45E-03
ENSG00000019144	PHLDB1	5.85E+00	7.25E-01	4.03E+00	3.06E-03
ENSG00000223631	<NA>	5.82E+00	-6.92E-01	4.28E+00	2.24E-03
ENSG00000211692	<NA>	5.81E+00	-7.00E-01	4.15E+00	2.64E-03
ENSG00000110080	ST3GAL4	5.78E+00	8.55E-02	4.41E+00	1.92E-03
ENSG00000088826	SMOX	5.76E+00	4.41E-01	4.16E+00	2.60E-03
ENSG00000053524	MCF2L2	5.76E+00	1.46E+00	3.30E+00	8.09E-03
ENSG00000124570	SERPINB6	5.75E+00	-7.13E-01	4.34E+00	2.08E-03
ENSG00000145113	MUC4	5.72E+00	8.65E-01	4.22E+00	2.41E-03
ENSG00000269113	TRABD2B	5.72E+00	-7.17E-01	4.08E+00	2.87E-03
ENSG00000054793	ATP9A	5.70E+00	2.82E+00	3.79E+00	4.15E-03
ENSG00000163106	HPGDS	5.68E+00	1.47E+00	4.28E+00	2.22E-03
ENSG00000166265	CYYR1	5.65E+00	-7.74E-01	4.16E+00	2.58E-03
ENSG00000260244	<NA>	5.64E+00	2.19E+00	3.37E+00	7.32E-03
ENSG00000105655	ISYNA1	5.64E+00	6.30E-01	4.11E+00	2.77E-03
ENSG00000182749	PAQR7	5.63E+00	-7.72E-01	4.23E+00	2.37E-03
ENSG00000176641	RNF152	5.60E+00	2.00E-02	3.61E+00	5.27E-03
ENSG00000131969	ABHD12B	5.60E+00	-7.59E-01	3.29E+00	8.10E-03
ENSG00000101194	SLC17A9	5.59E+00	-4.23E-03	4.27E+00	2.26E-03
ENSG00000166482	MFAP4	5.56E+00	4.34E+00	6.45E+00	2.20E-04
ENSG00000115297	TLX2	5.56E+00	-8.08E-01	4.15E+00	2.61E-03
ENSG00000148335	NTMT1	5.53E+00	-8.47E-01	3.82E+00	3.98E-03
ENSG00000259042	<NA>	5.51E+00	-8.55E-01	3.82E+00	3.95E-03
ENSG00000248870	<NA>	5.50E+00	3.10E-01	3.92E+00	3.46E-03
ENSG00000214711	CAPN14	5.48E+00	-6.95E-02	3.75E+00	4.35E-03
ENSG00000090376	IRAK3	5.47E+00	2.57E+00	5.63E+00	4.90E-04
ENSG00000229092	<NA>	5.46E+00	-8.67E-01	4.01E+00	3.10E-03
ENSG00000248105	<NA>	5.46E+00	-7.75E-02	3.77E+00	4.21E-03
ENSG00000128040	SPINK2	5.44E+00	-7.88E-02	4.12E+00	2.71E-03
ENSG00000010671	BTK	5.44E+00	-8.11E-02	4.11E+00	2.73E-03
ENSG00000211961	<NA>	5.43E+00	-7.40E-02	3.93E+00	3.44E-03

ENSG00000164627	KIF6	5.39E+00	6.70E-01	3.65E+00	4.92E-03
ENSG00000279404	<NA>	5.38E+00	-9.07E-01	3.93E+00	3.41E-03
ENSG00000253209	<NA>	5.38E+00	-8.73E-01	3.39E+00	7.01E-03
ENSG00000008441	NFIX	5.38E+00	-1.42E-01	3.14E+00	9.84E-03
ENSG00000170500	LONRF2	5.37E+00	1.23E+00	2.92E+00	1.35E-02
ENSG00000111321	LTBR	5.36E+00	-9.01E-01	3.89E+00	3.61E-03
ENSG00000123405	NFE2	5.36E+00	-8.83E-01	3.40E+00	6.86E-03
ENSG00000174099	MSRB3	5.34E+00	1.03E+00	4.20E+00	2.43E-03
ENSG00000240411	<NA>	5.34E+00	2.29E-01	3.77E+00	4.19E-03
ENSG00000124613	ZNF391	5.32E+00	-9.18E-01	3.78E+00	4.14E-03
ENSG00000137486	ARRB1	5.31E+00	2.45E+00	5.36E+00	6.41E-04
ENSG00000129473	BCL2L2	5.31E+00	1.35E+00	4.05E+00	2.91E-03
ENSG00000122592	HOXA7	5.30E+00	-9.61E-01	3.58E+00	5.39E-03
ENSG00000198300	PEG3	5.30E+00	-9.56E-01	3.75E+00	4.30E-03
ENSG00000107104	KANK1	5.30E+00	1.33E+00	3.31E+00	7.82E-03
ENSG00000250241	LOC101927359	5.29E+00	2.10E-01	3.46E+00	6.34E-03
ENSG00000106004	HOXA5	5.27E+00	-1.00E+00	2.90E+00	1.38E-02
ENSG00000198075	SULT1C4	5.26E+00	-9.81E-01	3.59E+00	5.33E-03
ENSG00000248309	MEF2C-AS1	5.25E+00	-1.79E-01	3.86E+00	3.74E-03
ENSG00000211694	<NA>	5.25E+00	1.60E+00	4.47E+00	1.75E-03
ENSG00000283118	<NA>	5.25E+00	-9.62E-01	3.83E+00	3.88E-03
ENSG00000221351	<NA>	5.25E+00	-9.69E-01	3.83E+00	3.86E-03
ENSG00000123836	PFKFB2	5.24E+00	4.68E+00	8.47E+00	4.08E-05
ENSG00000073792	IGF2BP2	5.20E+00	1.95E+00	4.78E+00	1.21E-03
ENSG00000211821	<NA>	5.19E+00	5.57E-01	2.80E+00	1.61E-02
ENSG00000174233	ADCY6	5.18E+00	-1.02E+00	3.38E+00	6.98E-03
ENSG00000134873	CLDN10	5.18E+00	-9.68E-01	3.17E+00	9.38E-03
ENSG00000175093	SPSB4	5.18E+00	-1.01E+00	3.74E+00	4.33E-03
ENSG00000137177	KIF13A	5.15E+00	-1.01E+00	3.72E+00	4.46E-03
ENSG00000248803	<NA>	5.13E+00	-1.04E+00	3.63E+00	4.96E-03
ENSG00000161133	USP41	5.12E+00	-1.03E+00	3.69E+00	4.60E-03
ENSG00000261251	<NA>	5.12E+00	-1.03E+00	3.69E+00	4.60E-03
ENSG00000276210	LINC00226	5.10E+00	-2.45E-01	3.77E+00	4.15E-03
ENSG00000132746	ALDH3B2	5.10E+00	1.41E+00	2.62E+00	2.08E-02
ENSG00000211968	<NA>	5.09E+00	-1.08E+00	3.00E+00	1.19E-02
ENSG00000061337	LZTS1	5.09E+00	-2.38E-01	3.27E+00	8.07E-03
ENSG00000262877	<NA>	5.08E+00	-2.67E-01	3.67E+00	4.74E-03
ENSG00000145362	ANK2	5.08E+00	-1.03E+00	3.35E+00	7.32E-03
ENSG00000100321	SYNGR1	5.07E+00	5.45E-01	3.45E+00	6.30E-03
ENSG00000039560	RAI14	5.06E+00	-1.02E+00	2.69E+00	1.87E-02
ENSG00000122025	FLT3	5.04E+00	1.46E+00	3.90E+00	3.51E-03

ENSG00000174348	PODN	5.03E+00	-1.11E+00	3.17E+00	9.31E-03
ENSG00000260188	<NA>	5.02E+00	7.05E-01	2.94E+00	1.30E-02
ENSG00000172828	CES3	5.01E+00	-1.08E+00	3.57E+00	5.35E-03
ENSG00000130649	CYP2E1	4.99E+00	4.70E-01	3.28E+00	8.01E-03
ENSG00000150681	RGS18	4.98E+00	4.47E-02	3.01E+00	1.17E-02
ENSG00000019485	PRDM11	4.98E+00	7.71E-01	2.86E+00	1.46E-02
ENSG00000276890	<NA>	4.96E+00	-1.13E+00	3.30E+00	7.79E-03
ENSG00000228203	RNF144A-AS1	4.95E+00	2.49E-01	2.33E+00	3.21E-02
ENSG00000104059	FAM189A1	4.94E+00	-1.09E+00	3.06E+00	1.09E-02
ENSG00000127507	ADGRE2	4.94E+00	4.09E+00	7.91E+00	6.16E-05
ENSG00000119411	BSPRY	4.94E+00	2.85E-01	3.44E+00	6.40E-03
ENSG00000104903	LYL1	4.93E+00	-1.12E+00	3.49E+00	5.92E-03
ENSG00000266794	<NA>	4.93E+00	-1.12E+00	3.47E+00	6.16E-03
ENSG00000147459	DOCK5	4.91E+00	4.63E+00	8.45E+00	4.08E-05
ENSG00000062370	ZNF112	4.91E+00	-3.47E-01	3.57E+00	5.37E-03
ENSG00000274482	<NA>	4.91E+00	3.08E+00	2.33E+00	3.18E-02
ENSG00000248735	<NA>	4.90E+00	-1.17E+00	3.19E+00	9.05E-03
ENSG00000282639	<NA>	4.89E+00	-3.50E-01	3.27E+00	8.03E-03
ENSG00000168671	UGT3A2	4.88E+00	3.52E+00	4.81E+00	1.15E-03
ENSG00000114315	HES1	4.88E+00	7.25E-01	2.73E+00	1.74E-02
ENSG00000188280	FAM230G	4.87E+00	-1.18E+00	3.19E+00	9.05E-03
ENSG00000100365	NCF4	4.85E+00	1.23E+00	2.48E+00	2.55E-02
ENSG00000275063	LOC102723407	4.85E+00	7.85E-01	3.77E+00	4.10E-03
ENSG00000145936	KCNMB1	4.85E+00	-1.17E+00	3.41E+00	6.59E-03
ENSG00000255642	<NA>	4.85E+00	-1.17E+00	3.41E+00	6.59E-03
ENSG00000119535	CSF3R	4.85E+00	4.36E-01	3.21E+00	8.73E-03
ENSG00000273894	<NA>	4.84E+00	-3.75E-01	3.24E+00	8.38E-03
ENSG00000281392	LINC00506	4.84E+00	1.87E+00	2.43E+00	2.74E-02
ENSG00000146094	DOK3	4.84E+00	7.87E-01	3.73E+00	4.33E-03
ENSG00000123843	C4BPB	4.83E+00	-1.22E+00	2.77E+00	1.65E-02
ENSG00000174776	WDR49	4.83E+00	1.03E+00	3.76E+00	4.15E-03
ENSG00000087253	LPCAT2	4.81E+00	1.09E+00	2.38E+00	2.94E-02
ENSG00000233968	LOC101928834	4.80E+00	-4.64E-02	2.88E+00	1.39E-02
ENSG00000164929	BAALC	4.78E+00	-3.97E-01	3.38E+00	6.84E-03
ENSG00000128309	MPST	4.78E+00	3.98E-01	3.12E+00	9.89E-03
ENSG00000211962	<NA>	4.78E+00	9.21E-01	3.12E+00	9.84E-03
ENSG00000165259	HDX	4.77E+00	-1.22E+00	3.25E+00	8.26E-03
ENSG00000153253	SCN3A	4.77E+00	5.02E+00	3.46E+00	6.14E-03
ENSG00000214796	<NA>	4.77E+00	-1.19E+00	3.19E+00	8.93E-03
ENSG00000273415	<NA>	4.77E+00	9.90E-01	2.31E+00	3.27E-02
ENSG00000196503	ARL9	4.74E+00	-1.21E+00	3.18E+00	9.01E-03

ENSG00000104154	SLC30A4	4.74E+00	1.74E-01	3.22E+00	8.52E-03
ENSG00000238057	ZEB2-AS1	4.72E+00	-1.23E+00	3.28E+00	7.84E-03
ENSG00000010438	PRSS3	4.71E+00	-1.22E+00	3.23E+00	8.43E-03
ENSG00000170558	CDH2	4.70E+00	2.23E+00	4.59E+00	1.47E-03
ENSG00000177103	DSCAML1	4.70E+00	-4.90E-01	2.52E+00	2.36E-02
ENSG00000163915	IGF2BP2-AS1	4.69E+00	-4.52E-01	3.35E+00	7.17E-03
ENSG00000274307	<NA>	4.68E+00	-1.23E+00	3.10E+00	1.02E-02
ENSG00000172830	SSH3	4.67E+00	-1.25E+00	3.20E+00	8.72E-03
ENSG00000102024	PLS3	4.67E+00	2.17E+00	4.16E+00	2.49E-03
ENSG00000238275	<NA>	4.66E+00	-1.22E+00	2.76E+00	1.65E-02
ENSG00000107968	MAP3K8	4.65E+00	-4.75E-01	3.30E+00	7.61E-03
ENSG00000274840	<NA>	4.65E+00	3.38E-01	2.87E+00	1.40E-02
ENSG00000205978	NYNRIN	4.65E+00	9.46E-01	3.67E+00	4.64E-03
ENSG00000281327	LINC01338	4.65E+00	6.11E-01	2.96E+00	1.24E-02
ENSG00000211949	<NA>	4.64E+00	-9.91E-02	3.15E+00	9.37E-03
ENSG00000258909	<NA>	4.63E+00	-1.27E+00	3.19E+00	8.89E-03
ENSG00000206557	TRIM71	4.63E+00	-1.27E+00	3.17E+00	9.09E-03
ENSG00000143167	GPA33	4.63E+00	1.44E+00	2.20E+00	3.85E-02
ENSG00000150760	DOCK1	4.62E+00	2.63E+00	4.95E+00	9.75E-04
ENSG00000128510	CPA4	4.62E+00	-1.27E+00	3.13E+00	9.60E-03
ENSG00000184113	CLDN5	4.61E+00	-4.91E-01	3.24E+00	8.22E-03
ENSG00000166342	NETO1	4.60E+00	1.66E+00	3.87E+00	3.55E-03
ENSG00000282320	<NA>	4.59E+00	-5.11E-01	2.98E+00	1.20E-02
ENSG00000211966	<NA>	4.58E+00	-1.30E+00	3.14E+00	9.54E-03
ENSG00000130202	NECTIN2	4.58E+00	-1.33E+00	2.92E+00	1.29E-02
ENSG00000124813	RUNX2	4.58E+00	5.02E+00	6.54E+00	1.96E-04
ENSG00000272953	<NA>	4.58E+00	-1.32E+00	3.02E+00	1.13E-02
ENSG00000279652	<NA>	4.57E+00	-4.93E-01	2.76E+00	1.63E-02
ENSG00000048052	HDAC9	4.56E+00	2.30E+00	2.79E+00	1.57E-02
ENSG00000135525	MAP7	4.56E+00	2.62E+00	3.68E+00	4.55E-03
ENSG00000152580	IGSF10	4.56E+00	-1.32E+00	3.07E+00	1.05E-02
ENSG00000148143	ZNF462	4.56E+00	3.26E+00	5.55E+00	5.08E-04
ENSG00000204764	RANBP17	4.55E+00	-1.29E+00	2.90E+00	1.34E-02
ENSG00000171115	GIMAP8	4.54E+00	9.66E-01	3.28E+00	7.83E-03
ENSG00000169554	ZEB2	4.54E+00	3.92E+00	2.91E+00	1.31E-02
ENSG00000231793	<NA>	4.53E+00	-1.36E+00	2.80E+00	1.55E-02
ENSG00000258172	<NA>	4.53E+00	-1.32E+00	3.08E+00	1.02E-02
ENSG00000234456	MAGI2-AS3	4.53E+00	5.86E-01	3.06E+00	1.06E-02
ENSG00000234432	LOC100129484	4.53E+00	-5.49E-01	3.06E+00	1.06E-02
ENSG00000211958	<NA>	4.51E+00	1.18E+00	2.66E+00	1.88E-02
ENSG00000137726	FXYD6	4.50E+00	-1.32E+00	2.97E+00	1.21E-02

ENSG00000105967	TFEC	4.50E+00	-5.18E-01	2.48E+00	2.48E-02
ENSG00000231718	LOC400800	4.48E+00	3.99E-01	2.93E+00	1.27E-02
ENSG00000254112	<NA>	4.48E+00	-1.32E+00	2.79E+00	1.56E-02
ENSG00000270885	RASL10B	4.48E+00	-1.33E+00	2.89E+00	1.35E-02
ENSG00000237667	LINC01115	4.47E+00	-1.38E+00	2.89E+00	1.36E-02
ENSG00000265579	<NA>	4.46E+00	-1.31E+00	2.52E+00	2.34E-02
ENSG00000213997	<NA>	4.46E+00	-1.35E+00	2.95E+00	1.24E-02
ENSG00000251301	LINC02384	4.45E+00	3.02E-02	2.94E+00	1.26E-02
ENSG00000228014	<NA>	4.45E+00	-5.79E-01	3.09E+00	1.01E-02
ENSG00000139117	CPNE8	4.43E+00	-1.78E-01	2.41E+00	2.74E-02
ENSG00000267199	<NA>	4.43E+00	2.05E-01	2.57E+00	2.16E-02
ENSG00000196358	NTNG2	4.43E+00	1.43E+00	2.09E+00	4.46E-02
ENSG00000213973	ZNF99	4.42E+00	-1.37E+00	2.96E+00	1.22E-02
ENSG00000227885	<NA>	4.39E+00	-2.68E-01	2.42E+00	2.70E-02
ENSG00000165568	AKR1E2	4.39E+00	-2.88E-04	2.88E+00	1.35E-02
ENSG00000166166	TRMT61A	4.39E+00	3.02E-02	2.60E+00	2.07E-02
ENSG00000125384	PTGER2	4.38E+00	-3.80E-03	2.58E+00	2.11E-02
ENSG00000151692	RNF144A	4.37E+00	4.11E+00	4.66E+00	1.35E-03
ENSG00000107833	NPM3	4.37E+00	-1.41E+00	2.91E+00	1.31E-02
ENSG00000166866	MYO1A	4.36E+00	-1.40E+00	2.88E+00	1.35E-02
ENSG00000236244	<NA>	4.36E+00	-1.40E+00	2.88E+00	1.35E-02
ENSG00000225028	<NA>	4.36E+00	-1.39E+00	2.77E+00	1.60E-02
ENSG00000164066	INTU	4.36E+00	1.61E+00	3.39E+00	6.66E-03
ENSG00000255354	<NA>	4.36E+00	-1.42E+00	2.87E+00	1.37E-02
ENSG00000271857	<NA>	4.36E+00	-1.42E+00	2.87E+00	1.37E-02
ENSG00000248393	<NA>	4.35E+00	1.48E+00	3.20E+00	8.66E-03
ENSG00000162641	AKNAD1	4.34E+00	-1.44E+00	2.74E+00	1.66E-02
ENSG00000241597	<NA>	4.34E+00	-6.58E-01	2.48E+00	2.44E-02
ENSG00000188916	INSYN2	4.33E+00	-1.44E+00	2.81E+00	1.50E-02
ENSG00000135046	ANXA1	4.33E+00	4.49E+00	3.99E+00	3.01E-03
ENSG00000198720	ANKRD13B	4.32E+00	-2.71E-01	2.63E+00	1.94E-02
ENSG00000162755	KLHDC9	4.32E+00	-1.41E+00	2.75E+00	1.63E-02
ENSG00000253709	<NA>	4.32E+00	-1.41E+00	2.75E+00	1.63E-02
ENSG00000272525	<NA>	4.32E+00	-1.41E+00	2.75E+00	1.63E-02
ENSG00000249978	<NA>	4.32E+00	-6.86E-01	2.27E+00	3.35E-02
ENSG00000237819	LOC101927497	4.30E+00	3.61E+00	6.30E+00	2.40E-04
ENSG00000235438	LOC105370105	4.30E+00	-1.46E+00	2.72E+00	1.71E-02
ENSG00000248557	<NA>	4.30E+00	-1.46E+00	2.72E+00	1.71E-02
ENSG00000132359	RAP1GAP2	4.30E+00	3.20E+00	4.05E+00	2.79E-03
ENSG00000169413	RNASE6	4.29E+00	2.48E+00	3.47E+00	5.94E-03
ENSG00000180758	GPR157	4.29E+00	-6.55E-01	2.94E+00	1.24E-02

ENSG00000108405	P2RX1	4.28E+00	2.27E+00	3.92E+00	3.26E-03
ENSG00000225302	<NA>	4.27E+00	-6.70E-01	2.68E+00	1.80E-02
ENSG00000116396	KCNC4	4.27E+00	3.08E+00	2.94E+00	1.24E-02
ENSG00000197816	CCDC180	4.25E+00	-1.48E+00	2.69E+00	1.77E-02
ENSG00000198795	ZNF521	4.25E+00	-1.48E+00	2.69E+00	1.77E-02
ENSG00000108452	<NA>	4.25E+00	-1.46E+00	2.78E+00	1.55E-02
ENSG00000251322	SHANK3	4.25E+00	-1.47E+00	2.78E+00	1.57E-02
ENSG00000232166	<NA>	4.24E+00	-1.46E+00	2.76E+00	1.61E-02
ENSG00000143545	RAB13	4.24E+00	8.16E-01	2.97E+00	1.18E-02
ENSG00000145391	SETD7	4.22E+00	3.17E+00	5.08E+00	8.26E-04
ENSG00000163093	BBS5	4.22E+00	-1.46E+00	2.71E+00	1.74E-02
ENSG00000164237	CMBL	4.20E+00	-9.47E-02	2.73E+00	1.68E-02
ENSG00000253449	<NA>	4.19E+00	-1.47E+00	2.63E+00	1.95E-02
ENSG00000071967	CYBRD1	4.19E+00	2.70E+00	2.69E+00	1.77E-02
ENSG00000196758	LOC401040	4.18E+00	-1.50E+00	2.72E+00	1.70E-02
ENSG00000257060	LOC105370982	4.18E+00	4.08E-01	2.68E+00	1.80E-02
ENSG00000167705	RILP	4.18E+00	-6.84E-01	2.61E+00	1.98E-02
ENSG00000233829	<NA>	4.18E+00	-3.44E-01	2.78E+00	1.56E-02
ENSG00000139132	FGD4	4.17E+00	-1.49E+00	2.67E+00	1.82E-02
ENSG00000225742	LINC02036	4.17E+00	-1.49E+00	2.67E+00	1.82E-02
ENSG00000267369	<NA>	4.17E+00	-1.49E+00	2.67E+00	1.82E-02
ENSG00000215386	MIR99AHG	4.17E+00	-7.37E-01	2.63E+00	1.94E-02
ENSG00000136213	CHST12	4.16E+00	3.55E+00	5.00E+00	9.02E-04
ENSG00000075618	FSCN1	4.15E+00	1.42E+00	3.13E+00	9.41E-03
ENSG00000170456	DENND5B	4.12E+00	1.47E+00	3.01E+00	1.10E-02
ENSG00000245317	LOC100996419	4.11E+00	-1.54E+00	2.64E+00	1.91E-02
ENSG00000261997	<NA>	4.11E+00	-1.54E+00	2.64E+00	1.91E-02
ENSG00000271605	MILR1	4.11E+00	-1.54E+00	2.64E+00	1.91E-02
ENSG00000186603	HPDL	4.11E+00	-7.07E-01	2.34E+00	2.98E-02
ENSG00000153721	CNKSR3	4.11E+00	-1.53E+00	2.63E+00	1.94E-02
ENSG00000250155	LOC107986412	4.11E+00	-7.70E-01	2.37E+00	2.85E-02
ENSG00000197993	KEL	4.11E+00	-1.51E+00	2.51E+00	2.32E-02
ENSG00000041353	RAB27B	4.10E+00	3.83E-01	2.86E+00	1.37E-02
ENSG00000143776	CDC42BPA	4.10E+00	2.07E-01	2.58E+00	2.07E-02
ENSG00000213062	<NA>	4.10E+00	-7.31E-01	2.61E+00	2.00E-02
ENSG00000145287	PLAC8	4.09E+00	3.61E+00	3.91E+00	3.31E-03
ENSG00000269961	<NA>	4.09E+00	-1.56E+00	2.59E+00	2.05E-02
ENSG00000145217	SLC26A1	4.09E+00	-1.53E+00	2.57E+00	2.10E-02
ENSG00000225200	<NA>	4.09E+00	-1.53E+00	2.57E+00	2.10E-02
ENSG00000132744	ACY3	4.09E+00	-7.13E-01	2.12E+00	4.19E-02
ENSG00000267127	<NA>	4.09E+00	-7.65E-01	2.70E+00	1.73E-02

ENSG00000128833	MYO5C	4.08E+00	2.71E+00	4.08E+00	2.66E-03
ENSG00000211957	<NA>	4.07E+00	9.63E-01	2.38E+00	2.80E-02
ENSG00000140022	STON2	4.07E+00	-1.24E-01	2.31E+00	3.09E-02
ENSG00000235823	OLMALINC	4.07E+00	1.33E+00	2.31E+00	3.11E-02
ENSG00000215146	LOC441666	4.06E+00	-7.62E-01	2.68E+00	1.79E-02
ENSG00000256589	<NA>	4.06E+00	-1.54E+00	2.49E+00	2.38E-02
ENSG00000222724	<NA>	4.05E+00	-3.96E-01	2.63E+00	1.92E-02
ENSG00000102230	PCYT1B	4.05E+00	9.64E-01	2.42E+00	2.65E-02
ENSG00000183605	SFXN4	4.05E+00	8.92E-01	2.39E+00	2.74E-02
ENSG00000105383	CD33	4.05E+00	-1.56E+00	2.57E+00	2.10E-02
ENSG00000173705	SUSD5	4.05E+00	-1.56E+00	2.57E+00	2.10E-02
ENSG00000128815	WDFY4	4.04E+00	3.10E+00	3.68E+00	4.42E-03
ENSG00000243175	<NA>	4.04E+00	-7.92E-01	2.64E+00	1.91E-02
ENSG00000162377	COA7	4.03E+00	1.02E+00	2.34E+00	2.99E-02
ENSG00000124743	KLHL31	4.03E+00	-1.58E+00	2.54E+00	2.20E-02
ENSG00000167123	CERCAM	4.03E+00	-1.58E+00	2.54E+00	2.20E-02
ENSG00000070614	NDST1	4.03E+00	2.09E+00	3.62E+00	4.80E-03
ENSG00000250400	<NA>	4.02E+00	3.28E+00	4.05E+00	2.75E-03
ENSG00000103202	NME4	4.02E+00	1.91E+00	3.62E+00	4.80E-03
ENSG00000156140	ADAMTS3	4.02E+00	-7.85E-01	2.67E+00	1.81E-02
ENSG00000089041	P2RX7	4.02E+00	1.01E-01	2.11E+00	4.22E-02
ENSG00000154914	USP43	4.02E+00	-7.83E-01	2.65E+00	1.86E-02
ENSG00000236296	GUSBP5	4.01E+00	-7.98E-01	2.65E+00	1.87E-02
ENSG00000185010	F8	4.01E+00	-7.72E-01	2.52E+00	2.25E-02
ENSG00000227747	<NA>	4.01E+00	1.65E+00	3.05E+00	1.05E-02
ENSG00000234072	LOC105374363	4.00E+00	1.67E+00	2.12E+00	4.15E-02
ENSG00000116711	PLA2G4A	4.00E+00	-1.57E+00	2.46E+00	2.47E-02
ENSG00000232640	<NA>	4.00E+00	-1.57E+00	2.46E+00	2.47E-02
ENSG00000177640	CASC2	3.98E+00	-4.59E-01	2.44E+00	2.53E-02
ENSG00000114019	AMOTL2	3.97E+00	-1.60E+00	2.48E+00	2.39E-02
ENSG00000204682	CASC10	3.96E+00	-1.61E+00	2.48E+00	2.38E-02
ENSG00000230881	<NA>	3.96E+00	-1.61E+00	2.48E+00	2.38E-02
ENSG00000091262	ABCC6	3.96E+00	-1.41E-02	2.43E+00	2.57E-02
ENSG00000230091	TMEM254-AS1	3.96E+00	-8.05E-01	2.37E+00	2.81E-02
ENSG00000167034	NKX3-1	3.95E+00	-8.39E-01	2.54E+00	2.19E-02
ENSG00000267771	<NA>	3.95E+00	-8.39E-01	2.54E+00	2.19E-02
ENSG00000224400	<NA>	3.94E+00	-1.60E+00	2.42E+00	2.59E-02
ENSG00000072422	RHOBTB1	3.93E+00	1.65E+00	3.15E+00	8.99E-03
ENSG00000258476	<NA>	3.93E+00	-8.27E-01	2.57E+00	2.10E-02
ENSG00000229619	MBNL1-AS1	3.92E+00	4.88E+00	4.85E+00	1.05E-03
ENSG00000158089	GALNT14	3.92E+00	-2.02E-01	2.01E+00	4.89E-02

ENSG00000132846	ZBED3	3.92E+00	3.08E+00	4.99E+00	9.02E-04
ENSG00000038945	MSR1	3.91E+00	-4.64E-01	2.24E+00	3.44E-02
ENSG00000173401	GLIPR1L1	3.91E+00	-8.56E-01	2.51E+00	2.27E-02
ENSG00000172986	GXYLT2	3.90E+00	5.04E+00	7.83E+00	6.34E-05
ENSG00000260418	<NA>	3.90E+00	-1.64E+00	2.42E+00	2.61E-02
ENSG00000229056	HECW2-AS1	3.89E+00	7.71E-01	2.60E+00	1.98E-02
ENSG00000248125	<NA>	3.89E+00	-1.65E+00	2.42E+00	2.62E-02
ENSG00000267454	ZNF582-AS1	3.89E+00	-8.86E-01	2.16E+00	3.89E-02
ENSG00000263089	<NA>	3.88E+00	-8.11E-02	2.29E+00	3.19E-02
ENSG00000047597	XK	3.88E+00	-1.64E+00	2.38E+00	2.76E-02
ENSG00000198816	ZNF358	3.88E+00	-1.64E+00	2.38E+00	2.76E-02
ENSG00000223764	LINC02593	3.88E+00	-1.64E+00	2.38E+00	2.76E-02
ENSG00000253552	HOXA-AS2	3.88E+00	-1.64E+00	2.38E+00	2.76E-02
ENSG00000159674	SPON2	3.88E+00	1.99E+00	3.38E+00	6.55E-03
ENSG00000136383	ALPK3	3.87E+00	1.24E+00	3.15E+00	9.00E-03
ENSG00000042493	CAPG	3.87E+00	1.98E+00	3.65E+00	4.55E-03
ENSG00000162722	TRIM58	3.87E+00	-9.04E-01	2.19E+00	3.70E-02
ENSG00000278473	<NA>	3.86E+00	1.01E+00	2.30E+00	3.09E-02
ENSG00000229153	EPHA1-AS1	3.86E+00	1.04E-01	2.29E+00	3.18E-02
ENSG00000135436	FAM186B	3.85E+00	4.43E-01	2.23E+00	3.48E-02
ENSG00000162687	KCNT2	3.84E+00	-8.77E-01	2.30E+00	3.10E-02
ENSG00000229589	ACVR2B-AS1	3.84E+00	-8.83E-01	2.48E+00	2.37E-02
ENSG00000280003	<NA>	3.84E+00	-8.83E-01	2.48E+00	2.37E-02
ENSG00000257298	<NA>	3.83E+00	-2.80E-01	2.38E+00	2.77E-02
ENSG00000198478	SH3BGRL2	3.82E+00	-9.08E-01	2.36E+00	2.83E-02
ENSG00000253883	<NA>	3.82E+00	6.02E-01	2.03E+00	4.69E-02
ENSG00000238149	<NA>	3.81E+00	-9.39E-02	2.31E+00	3.05E-02
ENSG00000029153	ARNTL2	3.80E+00	1.46E+00	3.18E+00	8.59E-03
ENSG00000240666	<NA>	3.80E+00	-5.73E-01	2.00E+00	4.94E-02
ENSG00000079482	OPHN1	3.79E+00	-5.61E-01	2.19E+00	3.68E-02
ENSG00000198046	ZNF667	3.79E+00	2.84E+00	4.45E+00	1.67E-03
ENSG00000227191	<NA>	3.79E+00	3.84E+00	3.99E+00	2.94E-03
ENSG00000108176	DNAJC12	3.79E+00	-3.15E-01	2.20E+00	3.60E-02
ENSG00000019991	HGF	3.78E+00	1.54E+00	3.23E+00	7.97E-03
ENSG00000278989	<NA>	3.78E+00	-1.40E-01	2.27E+00	3.24E-02
ENSG00000197472	ZNF695	3.78E+00	-1.32E-01	2.21E+00	3.55E-02
ENSG00000227470	<NA>	3.76E+00	3.18E-02	2.27E+00	3.26E-02
ENSG00000134780	DAGLA	3.75E+00	-9.43E-01	2.32E+00	3.00E-02
ENSG00000237172	B3GNT9	3.73E+00	-3.28E-01	2.29E+00	3.13E-02
ENSG00000120594	PLXDC2	3.73E+00	9.84E-01	2.62E+00	1.93E-02
ENSG00000124343	XG	3.73E+00	1.66E-01	2.23E+00	3.43E-02

ENSG00000205464	ATP6AP1L	3.73E+00	5.00E+00	6.67E+00	1.68E-04
ENSG00000164251	F2RL1	3.73E+00	-1.72E+00	2.25E+00	3.33E-02
ENSG00000228598	MACC1-AS1	3.73E+00	-1.72E+00	2.25E+00	3.33E-02
ENSG00000231445	<NA>	3.73E+00	-1.72E+00	2.25E+00	3.33E-02
ENSG00000254046	<NA>	3.72E+00	2.79E-01	2.21E+00	3.52E-02
ENSG00000114737	CISH	3.72E+00	2.76E+00	3.42E+00	6.13E-03
ENSG00000137124	ALDH1B1	3.72E+00	-5.80E-01	2.09E+00	4.27E-02
ENSG00000226306	NPY6R	3.72E+00	-3.26E-01	2.29E+00	3.14E-02
ENSG00000268658	LINC00664	3.71E+00	1.94E-01	2.47E+00	2.37E-02
ENSG00000138378	STAT4	3.71E+00	3.24E+00	3.74E+00	4.02E-03
ENSG00000281657	<NA>	3.71E+00	3.18E+00	3.46E+00	5.87E-03
ENSG00000116649	SRM	3.71E+00	3.11E+00	3.23E+00	8.00E-03
ENSG00000211973	<NA>	3.70E+00	-5.55E-01	2.17E+00	3.75E-02
ENSG00000110237	ARHGEF17	3.69E+00	3.98E-01	2.43E+00	2.53E-02
ENSG00000170153	RNF150	3.68E+00	3.44E+00	4.84E+00	1.06E-03
ENSG00000272986	<NA>	3.68E+00	-5.97E-01	2.28E+00	3.15E-02
ENSG00000235244	DANT2	3.68E+00	8.78E-01	2.40E+00	2.64E-02
ENSG00000169957	ZNF768	3.67E+00	-3.42E-01	1.99E+00	4.94E-02
ENSG00000233306	<NA>	3.67E+00	4.50E-01	2.07E+00	4.36E-02
ENSG00000225234	<NA>	3.67E+00	-3.43E-01	2.21E+00	3.55E-02
ENSG00000227268	KLLN	3.67E+00	1.23E+00	2.77E+00	1.52E-02
ENSG00000116353	MECR	3.66E+00	1.43E+00	2.99E+00	1.12E-02
ENSG00000239704	CDRT4	3.66E+00	-2.99E-02	2.07E+00	4.40E-02
ENSG00000140932	CMTM2	3.65E+00	-2.82E-02	2.13E+00	3.99E-02
ENSG00000146373	RNF217	3.65E+00	-9.46E-01	2.00E+00	4.86E-02
ENSG00000146858	ZC3HAV1L	3.65E+00	2.85E+00	3.54E+00	5.21E-03
ENSG00000188404	SELL	3.64E+00	6.25E+00	4.61E+00	1.38E-03
ENSG00000251248	<NA>	3.63E+00	-9.77E-01	2.27E+00	3.22E-02
ENSG00000275805	<NA>	3.62E+00	-6.40E-01	2.13E+00	3.95E-02
ENSG00000021826	CPS1	3.62E+00	6.25E-01	2.61E+00	1.93E-02
ENSG00000167291	TBC1D16	3.62E+00	1.41E+00	2.13E+00	3.94E-02
ENSG00000110492	MDK	3.61E+00	1.31E+00	2.63E+00	1.88E-02
ENSG00000164116	GUCY1A1	3.61E+00	4.31E+00	5.73E+00	4.09E-04
ENSG00000105472	CLEC11A	3.61E+00	6.83E-01	2.05E+00	4.49E-02
ENSG00000276231	PIK3R6	3.61E+00	2.67E+00	2.64E+00	1.84E-02
ENSG00000175857	GAPT	3.59E+00	1.64E+00	2.06E+00	4.44E-02
ENSG00000196976	LAGE3	3.59E+00	-3.59E-02	2.10E+00	4.15E-02
ENSG00000177096	PHETA2	3.59E+00	-2.16E-01	2.13E+00	3.97E-02
ENSG00000254602	<NA>	3.59E+00	-1.02E+00	2.17E+00	3.73E-02
ENSG00000274561	<NA>	3.58E+00	1.57E+00	3.16E+00	8.80E-03
ENSG00000200114	<NA>	3.57E+00	-1.00E+00	2.04E+00	4.56E-02

ENSG00000144045	DQX1	3.56E+00	9.13E-02	2.03E+00	4.58E-02
ENSG00000152611	CAPSL	3.56E+00	6.90E-02	2.06E+00	4.41E-02
ENSG00000255084	LOC101928896	3.55E+00	-6.71E-01	2.10E+00	4.13E-02
ENSG00000162654	GBP4	3.55E+00	2.98E+00	2.37E+00	2.75E-02
ENSG00000272701	<NA>	3.54E+00	-1.04E+00	2.14E+00	3.90E-02
ENSG00000166821	PEX11A	3.53E+00	-4.26E-01	2.12E+00	4.03E-02
ENSG00000279415	<NA>	3.53E+00	-2.34E-01	2.23E+00	3.37E-02
ENSG00000155974	GRIP1	3.52E+00	4.55E-01	2.27E+00	3.19E-02
ENSG00000224429	<NA>	3.51E+00	1.57E+00	2.35E+00	2.83E-02
ENSG00000204740	MALRD1	3.51E+00	1.59E+00	3.10E+00	9.49E-03
ENSG00000280079	<NA>	3.50E+00	1.54E+00	3.00E+00	1.10E-02
ENSG00000173548	SNX33	3.49E+00	-4.50E-01	2.07E+00	4.30E-02
ENSG00000211938	<NA>	3.48E+00	-4.36E-01	2.05E+00	4.46E-02
ENSG00000226686	LINC01535	3.48E+00	-1.04E+00	2.07E+00	4.33E-02
ENSG00000230295	<NA>	3.46E+00	-1.07E+00	2.10E+00	4.11E-02
ENSG00000268119	LOC105372321	3.45E+00	2.48E+00	3.21E+00	8.05E-03
ENSG00000211950	<NA>	3.45E+00	4.54E+00	5.97E+00	3.20E-04
ENSG00000099256	PRTFDC1	3.44E+00	1.92E+00	3.15E+00	8.84E-03
ENSG00000262222	<NA>	3.43E+00	-1.07E+00	2.04E+00	4.52E-02
ENSG00000225172	<NA>	3.43E+00	1.77E+00	2.56E+00	2.06E-02
ENSG00000137752	CASP1	3.43E+00	1.31E+00	2.70E+00	1.68E-02
ENSG00000076258	FMO4	3.42E+00	6.89E-02	2.28E+00	3.10E-02
ENSG00000140479	PCSK6	3.41E+00	-1.11E+00	2.00E+00	4.77E-02
ENSG00000169313	P2RY12	3.41E+00	-1.11E+00	2.00E+00	4.77E-02
ENSG00000073536	NLE1	3.41E+00	2.11E+00	2.91E+00	1.23E-02
ENSG00000198947	DMD	3.40E+00	-4.93E-01	1.99E+00	4.84E-02
ENSG00000251002	<NA>	3.39E+00	3.73E+00	3.07E+00	9.83E-03
ENSG00000251669	FAM86EP	3.38E+00	-1.12E+00	2.01E+00	4.66E-02
ENSG00000148175	STOM	3.38E+00	1.48E+00	2.84E+00	1.37E-02
ENSG00000186314	PRELID2	3.37E+00	1.17E+00	2.62E+00	1.86E-02
ENSG00000117586	TNFSF4	3.36E+00	4.21E+00	4.48E+00	1.58E-03
ENSG00000280399	<NA>	3.34E+00	2.34E+00	2.63E+00	1.84E-02
ENSG00000140682	TGFB1I1	3.34E+00	-3.51E-01	2.02E+00	4.64E-02
ENSG00000126016	AMOT	3.33E+00	2.20E+00	3.02E+00	1.05E-02
ENSG00000227006	<NA>	3.31E+00	2.66E+00	2.01E+00	4.68E-02
ENSG00000128487	SPECC1	3.30E+00	3.12E+00	4.15E+00	2.37E-03
ENSG00000221926	TRIM16	3.30E+00	1.86E+00	2.60E+00	1.91E-02
ENSG00000173905	GOLIM4	3.29E+00	3.31E+00	3.41E+00	6.17E-03
ENSG00000189337	KAZN	3.28E+00	4.51E-01	2.23E+00	3.31E-02
ENSG00000125388	GRK4	3.27E+00	1.08E+00	2.57E+00	2.01E-02
ENSG00000250280	<NA>	3.27E+00	-3.76E-01	2.01E+00	4.64E-02

ENSG00000160360	GPSM1	3.25E+00	1.00E+00	2.53E+00	2.11E-02
ENSG00000152642	GPD1L	3.25E+00	2.63E+00	3.04E+00	1.02E-02
ENSG00000267023	<NA>	3.24E+00	2.09E+00	2.21E+00	3.39E-02
ENSG00000178538	CA8	3.24E+00	3.21E+00	3.94E+00	3.06E-03
ENSG00000211698	<NA>	3.24E+00	5.86E-01	2.22E+00	3.35E-02
ENSG00000170275	CRTAP	3.23E+00	2.99E+00	4.04E+00	2.69E-03
ENSG00000244405	ETV5	3.22E+00	1.93E+00	2.13E+00	3.87E-02
ENSG00000276449	<NA>	3.22E+00	1.01E+00	2.27E+00	3.12E-02
ENSG00000112290	WASF1	3.21E+00	3.56E+00	3.81E+00	3.61E-03
ENSG00000128039	SRD5A3	3.20E+00	1.66E+00	2.04E+00	4.39E-02
ENSG00000197134	ZNF257	3.19E+00	2.33E+00	2.33E+00	2.86E-02
ENSG00000280832	GSEC	3.17E+00	2.18E+00	3.06E+00	9.86E-03
ENSG00000180592	SKIDA1	3.16E+00	-4.99E-02	2.01E+00	4.61E-02
ENSG00000108861	DUSP3	3.16E+00	6.88E-01	2.06E+00	4.30E-02
ENSG00000188171	ZNF626	3.14E+00	2.06E+00	2.43E+00	2.44E-02
ENSG00000138650	PCDH10	3.14E+00	5.03E+00	5.13E+00	7.57E-04
ENSG00000159399	HK2	3.14E+00	4.08E+00	3.06E+00	9.78E-03
ENSG00000184014	DENND5A	3.13E+00	5.08E+00	3.90E+00	3.23E-03
ENSG00000254535	PABPC4L	3.13E+00	2.76E+00	3.13E+00	8.91E-03
ENSG00000237854	<NA>	3.13E+00	1.64E+00	2.77E+00	1.49E-02
ENSG00000183735	TBK1	3.11E+00	1.40E+00	2.31E+00	2.90E-02
ENSG00000160062	ZBTB8A	3.10E+00	1.43E+00	2.61E+00	1.86E-02
ENSG00000270550	<NA>	3.09E+00	-9.06E-02	1.95E+00	4.99E-02
ENSG00000106605	BLVRA	3.09E+00	7.64E-01	2.11E+00	3.93E-02
ENSG00000034053	APBA2	3.08E+00	4.11E+00	2.43E+00	2.44E-02
ENSG00000213468	FIRRE	3.08E+00	4.11E+00	2.46E+00	2.34E-02
ENSG00000153707	PTPRD	3.07E+00	5.36E+00	5.26E+00	6.60E-04
ENSG00000133460	SLC2A11	3.07E+00	1.61E+00	2.42E+00	2.46E-02
ENSG00000126947	ARMCX1	3.07E+00	1.91E+00	2.19E+00	3.46E-02
ENSG00000213949	ITGA1	3.07E+00	2.42E+00	3.06E+00	9.75E-03
ENSG00000085871	MGST2	3.05E+00	1.53E+00	2.26E+00	3.11E-02
ENSG00000107815	TWNK	3.04E+00	2.06E+00	3.00E+00	1.07E-02
ENSG00000211829	<NA>	3.03E+00	4.48E+00	3.90E+00	3.19E-03
ENSG00000134940	ACRV1	3.03E+00	1.26E+00	2.35E+00	2.72E-02
ENSG00000272512	<NA>	3.00E+00	9.39E-01	1.98E+00	4.77E-02
ENSG00000234912	SNHG20	2.99E+00	1.14E+00	2.17E+00	3.58E-02
ENSG00000198298	ZNF485	2.99E+00	8.08E-01	2.18E+00	3.49E-02
ENSG00000134504	KCTD1	2.99E+00	1.61E+00	2.45E+00	2.34E-02
ENSG00000177398	UMODL1	2.98E+00	2.78E+00	2.85E+00	1.31E-02
ENSG00000128394	APOBEC3F	2.97E+00	2.33E+00	2.91E+00	1.20E-02
ENSG00000153404	PLEKHG4B	2.97E+00	2.71E+00	3.17E+00	8.40E-03

ENSG00000139926	FRMD6	2.96E+00	3.95E+00	3.95E+00	2.98E-03
ENSG00000182557	SPNS3	2.96E+00	2.19E+00	2.80E+00	1.41E-02
ENSG00000185304	RGPD2	2.96E+00	2.62E+00	3.22E+00	7.82E-03
ENSG00000114757	PEX5L	2.94E+00	3.84E+00	3.95E+00	3.00E-03
ENSG00000103550	KNOP1	2.93E+00	1.45E+00	2.13E+00	3.79E-02
ENSG00000234345	<NA>	2.91E+00	1.12E+00	2.18E+00	3.51E-02
ENSG00000112218	GPR63	2.91E+00	1.13E+00	2.21E+00	3.33E-02
ENSG00000217527	<NA>	2.91E+00	1.21E+00	2.11E+00	3.89E-02
ENSG00000152990	ADGRA3	2.91E+00	4.10E+00	3.67E+00	4.26E-03
ENSG00000204287	HLA-DRA	2.90E+00	3.68E+00	1.96E+00	4.87E-02
ENSG00000196549	MME	2.90E+00	6.02E+00	3.29E+00	7.06E-03
ENSG00000099194	SCD	2.89E+00	5.69E+00	2.67E+00	1.71E-02
ENSG00000100350	FOXRED2	2.88E+00	3.00E+00	3.31E+00	6.90E-03
ENSG00000275209	<NA>	2.88E+00	4.79E+00	3.82E+00	3.53E-03
ENSG00000235492	LINC01221	2.88E+00	3.10E+00	2.72E+00	1.57E-02
ENSG00000278090	<NA>	2.88E+00	3.04E+00	2.28E+00	3.01E-02
ENSG00000106484	MEST	2.87E+00	3.00E+00	2.78E+00	1.44E-02
ENSG00000152207	CYSLTR2	2.87E+00	1.56E+00	2.39E+00	2.54E-02
ENSG00000105737	GRIK5	2.87E+00	1.56E+00	2.44E+00	2.37E-02
ENSG00000206190	ATP10A	2.86E+00	2.81E+00	3.08E+00	9.46E-03
ENSG00000171132	PRKCE	2.85E+00	3.72E+00	2.72E+00	1.57E-02
ENSG00000060138	YBX3	2.85E+00	5.94E+00	2.23E+00	3.23E-02
ENSG00000174749	FAM241A	2.85E+00	3.45E+00	3.31E+00	6.90E-03
ENSG00000144893	MED12L	2.84E+00	4.01E+00	4.18E+00	2.24E-03
ENSG00000266074	BAHCC1	2.84E+00	6.06E+00	4.48E+00	1.56E-03
ENSG00000101082	SLA2	2.83E+00	3.63E+00	2.87E+00	1.26E-02
ENSG00000138722	MMRN1	2.83E+00	1.10E+00	1.99E+00	4.65E-02
ENSG00000164932	CTHRC1	2.83E+00	2.24E+00	2.87E+00	1.27E-02
ENSG00000181631	P2RY13	2.82E+00	1.34E+00	2.03E+00	4.38E-02
ENSG00000254087	LYN	2.82E+00	1.45E+00	2.25E+00	3.14E-02
ENSG00000141447	OSBPL1A	2.82E+00	3.37E+00	3.20E+00	8.02E-03
ENSG00000267886	ZNF730	2.81E+00	2.43E+00	2.90E+00	1.22E-02
ENSG00000227403	LINC01806	2.80E+00	2.09E+00	2.63E+00	1.79E-02
ENSG00000179583	CIITA	2.80E+00	4.18E+00	4.43E+00	1.67E-03
ENSG00000143179	UCK2	2.78E+00	4.78E+00	3.09E+00	9.37E-03
ENSG00000181481	RNF135	2.78E+00	7.66E-01	1.96E+00	4.84E-02
ENSG00000145220	LYAR	2.78E+00	2.36E+00	2.73E+00	1.56E-02
ENSG00000111696	NT5DC3	2.77E+00	1.16E+00	2.13E+00	3.72E-02
ENSG00000168899	VAMP5	2.76E+00	1.60E+00	1.98E+00	4.68E-02
ENSG00000226950	DANCR	2.76E+00	3.74E+00	3.52E+00	5.18E-03
ENSG00000136161	RCBTB2	2.76E+00	3.94E+00	3.28E+00	7.12E-03

ENSG00000228223	HCG11	2.76E+00	2.22E+00	2.32E+00	2.83E-02
ENSG00000109576	AADAT	2.75E+00	2.87E+00	2.90E+00	1.22E-02
ENSG00000037241	RPL26L1	2.75E+00	1.76E+00	2.33E+00	2.77E-02
ENSG00000112195	TREML2	2.75E+00	3.27E+00	3.40E+00	6.06E-03
ENSG00000128602	SMO	2.74E+00	1.65E+00	2.30E+00	2.89E-02
ENSG00000279620	<NA>	2.73E+00	1.40E+00	2.07E+00	4.07E-02
ENSG00000260855	<NA>	2.73E+00	2.41E+00	2.10E+00	3.92E-02
ENSG00000163297	ANTXR2	2.73E+00	3.93E+00	2.49E+00	2.17E-02
ENSG00000247081	BAALC-AS1	2.73E+00	1.48E+00	2.11E+00	3.84E-02
ENSG00000253325	<NA>	2.73E+00	1.25E+00	1.94E+00	4.96E-02
ENSG00000095932	SMIM24	2.72E+00	3.77E+00	3.36E+00	6.42E-03
ENSG00000117016	RIMS3	2.71E+00	3.96E+00	3.74E+00	3.90E-03
ENSG00000164292	RHOBTB3	2.71E+00	3.91E+00	3.97E+00	2.92E-03
ENSG00000073060	SCARB1	2.70E+00	2.24E+00	2.44E+00	2.37E-02
ENSG00000134897	BIVM	2.69E+00	4.11E+00	3.18E+00	8.24E-03
ENSG00000007237	GAS7	2.68E+00	5.25E+00	4.80E+00	1.08E-03
ENSG00000146530	VWDE	2.66E+00	2.38E+00	1.94E+00	4.95E-02
ENSG00000106560	GIMAP2	2.66E+00	4.48E+00	3.45E+00	5.66E-03
ENSG00000044459	CNTLN	2.63E+00	4.15E+00	3.59E+00	4.74E-03
ENSG00000113658	SMAD5	2.63E+00	3.74E+00	2.64E+00	1.75E-02
ENSG00000151376	ME3	2.62E+00	2.41E+00	2.36E+00	2.64E-02
ENSG00000086730	LAT2	2.60E+00	3.01E+00	2.56E+00	1.97E-02
ENSG00000101911	PRPS2	2.59E+00	5.71E+00	4.87E+00	9.90E-04
ENSG00000130635	COL5A1	2.58E+00	1.84E+00	2.02E+00	4.34E-02
ENSG00000179958	DCTPP1	2.58E+00	2.26E+00	2.24E+00	3.13E-02
ENSG00000100522	GNPNAT1	2.57E+00	1.53E+00	1.98E+00	4.64E-02
ENSG00000166825	ANPEP	2.56E+00	2.07E+00	2.07E+00	4.06E-02
ENSG00000121858	TNFSF10	2.54E+00	1.94E+00	1.96E+00	4.79E-02
ENSG00000169126	ARMC4	2.51E+00	1.76E+00	2.03E+00	4.30E-02
ENSG00000170017	ALCAM	2.51E+00	2.62E+00	1.99E+00	4.52E-02
ENSG00000175711	B3GNTL1	2.51E+00	2.80E+00	2.66E+00	1.68E-02
ENSG00000080493	SLC4A4	2.51E+00	3.65E+00	3.41E+00	6.00E-03
ENSG00000232677	LINC00665	2.51E+00	3.82E+00	3.48E+00	5.45E-03
ENSG00000153165	RGPD3	2.50E+00	1.85E+00	1.94E+00	4.91E-02
ENSG00000060982	BCAT1	2.49E+00	5.09E+00	3.70E+00	4.07E-03
ENSG00000183077	AFMID	2.49E+00	2.07E+00	2.23E+00	3.19E-02
ENSG00000138449	SLC40A1	2.49E+00	2.92E+00	2.65E+00	1.73E-02
ENSG00000279496	<NA>	2.47E+00	2.66E+00	2.22E+00	3.20E-02
ENSG00000105270	CLIP3	2.46E+00	4.39E+00	2.59E+00	1.87E-02
ENSG00000275004	ZNF280B	2.44E+00	2.79E+00	2.53E+00	2.03E-02
ENSG00000117322	CR2	2.44E+00	5.29E+00	3.37E+00	6.28E-03

ENSG00000105810	CDK6	2.40E+00	9.77E+00	5.50E+00	5.04E-04
ENSG00000061918	GUCY1B1	2.39E+00	4.17E+00	3.33E+00	6.59E-03
ENSG00000106772	PRUNE2	2.38E+00	3.47E+00	2.16E+00	3.50E-02
ENSG00000109458	GAB1	2.38E+00	4.32E+00	3.52E+00	5.17E-03
ENSG00000168904	LRRC28	2.37E+00	5.98E+00	3.39E+00	6.09E-03
ENSG00000180385	<NA>	2.37E+00	3.92E+00	2.85E+00	1.29E-02
ENSG00000171867	PRNP	2.36E+00	2.69E+00	2.12E+00	3.71E-02
ENSG00000136997	MYC	2.35E+00	5.63E+00	2.12E+00	3.73E-02
ENSG00000124217	MOCS3	2.35E+00	2.41E+00	2.23E+00	3.15E-02
ENSG00000282851	BISPR	2.35E+00	2.24E+00	1.94E+00	4.87E-02
ENSG00000076248	UNG	2.35E+00	3.75E+00	2.55E+00	1.97E-02
ENSG00000260260	SNHG19	2.32E+00	2.94E+00	2.08E+00	3.96E-02
ENSG00000188227	ZNF793	2.32E+00	2.78E+00	2.09E+00	3.87E-02
ENSG00000021355	SERPINB1	2.31E+00	4.53E+00	2.89E+00	1.21E-02
ENSG00000205336	ADGRG1	2.30E+00	5.94E+00	3.81E+00	3.53E-03
ENSG00000189283	FHIT	2.30E+00	4.32E+00	2.06E+00	4.05E-02
ENSG00000185252	ZNF74	2.29E+00	1.95E+00	1.92E+00	4.97E-02
ENSG00000158220	ESYT3	2.28E+00	4.30E+00	2.52E+00	2.07E-02
ENSG00000133142	TCEAL4	2.27E+00	4.16E+00	3.18E+00	8.07E-03
ENSG00000149485	FADS1	2.26E+00	4.99E+00	2.59E+00	1.85E-02
ENSG00000182095	TNRC18	2.26E+00	4.82E+00	3.56E+00	4.90E-03
ENSG00000172172	MRPL13	2.25E+00	2.59E+00	1.95E+00	4.75E-02
ENSG00000143479	DYRK3	2.25E+00	3.23E+00	2.42E+00	2.38E-02
ENSG00000143162	CREG1	2.25E+00	2.28E+00	2.02E+00	4.27E-02
ENSG00000165028	NIPSNAP3B	2.24E+00	2.13E+00	1.97E+00	4.63E-02
ENSG00000109787	KLF3	2.23E+00	4.00E+00	2.06E+00	4.05E-02
ENSG00000278730	<NA>	2.23E+00	4.08E+00	2.09E+00	3.87E-02
ENSG00000132382	MYBBP1A	2.23E+00	3.43E+00	1.98E+00	4.57E-02
ENSG00000157557	ETS2	2.22E+00	5.40E+00	3.15E+00	8.40E-03
ENSG00000065923	SLC9A7	2.22E+00	4.07E+00	2.80E+00	1.39E-02
ENSG00000132849	PATJ	2.20E+00	3.33E+00	2.58E+00	1.89E-02
ENSG00000154447	SH3RF1	2.20E+00	3.00E+00	2.16E+00	3.46E-02
ENSG00000164930	FZD6	2.20E+00	4.20E+00	2.56E+00	1.94E-02
ENSG00000177425	PAWR	2.20E+00	3.93E+00	2.54E+00	1.99E-02
ENSG00000163006	CCDC138	2.19E+00	4.82E+00	2.97E+00	1.08E-02
ENSG00000197965	MPZL1	2.19E+00	5.10E+00	3.20E+00	7.94E-03
ENSG00000197128	ZNF772	2.18E+00	4.04E+00	2.38E+00	2.51E-02
ENSG00000118640	VAMP8	2.17E+00	3.57E+00	2.08E+00	3.90E-02
ENSG00000226806	LOC100507600	2.17E+00	6.35E+00	3.01E+00	1.02E-02
ENSG00000138326	RPS24	2.17E+00	6.50E+00	3.33E+00	6.61E-03
ENSG00000161551	ZNF577	2.17E+00	3.74E+00	2.62E+00	1.77E-02

ENSG00000111640	GAPDH	2.16E+00	9.25E+00	2.84E+00	1.30E-02
ENSG00000083845	RPS5	2.12E+00	7.39E+00	2.45E+00	2.26E-02
ENSG00000213160	KLHL23	2.12E+00	4.02E+00	2.64E+00	1.73E-02
ENSG00000143494	VASH2	2.11E+00	3.39E+00	1.97E+00	4.56E-02
ENSG00000123600	METTL8	2.09E+00	3.82E+00	2.11E+00	3.71E-02
ENSG00000173141	MRPL57	2.09E+00	4.21E+00	2.75E+00	1.48E-02
ENSG00000103489	XYLT1	2.09E+00	5.30E+00	2.56E+00	1.93E-02
ENSG00000166086	JAM3	2.08E+00	5.62E+00	3.47E+00	5.44E-03
ENSG00000138758	SEPT11	2.07E+00	4.97E+00	2.92E+00	1.16E-02
ENSG00000233410	<NA>	2.06E+00	3.81E+00	2.17E+00	3.39E-02
ENSG00000184117	NIPSNAP1	2.06E+00	3.63E+00	2.33E+00	2.70E-02
ENSG00000084207	GSTP1	2.06E+00	4.29E+00	2.11E+00	3.74E-02
ENSG00000205133	TRIQK	2.06E+00	3.16E+00	2.10E+00	3.77E-02
ENSG00000148400	NOTCH1	2.06E+00	6.89E+00	4.04E+00	2.62E-03
ENSG00000111885	MAN1A1	2.06E+00	5.23E+00	2.55E+00	1.97E-02
ENSG00000093217	XYLB	2.05E+00	3.59E+00	2.24E+00	3.09E-02
ENSG00000164253	WDR41	2.05E+00	4.53E+00	2.79E+00	1.39E-02
ENSG00000128791	TWSG1	2.05E+00	3.60E+00	2.39E+00	2.47E-02
ENSG00000059573	ALDH18A1	2.04E+00	4.55E+00	2.67E+00	1.65E-02
ENSG00000115268	RPS15	2.04E+00	7.19E+00	2.23E+00	3.13E-02
ENSG00000231500	RPS18	2.03E+00	6.63E+00	2.33E+00	2.69E-02
ENSG00000140563	MCTP2	2.03E+00	4.35E+00	2.46E+00	2.24E-02
ENSG00000168961	LGALS9	2.03E+00	3.70E+00	2.35E+00	2.62E-02
ENSG00000159199	ATP5MC1	2.03E+00	2.88E+00	1.93E+00	4.89E-02
ENSG00000186918	ZNF395	2.03E+00	3.12E+00	1.97E+00	4.59E-02
ENSG00000133935	ERG28	2.02E+00	3.58E+00	2.05E+00	4.05E-02
ENSG00000114942	EEF1B2	2.02E+00	7.00E+00	2.80E+00	1.37E-02
ENSG00000151914	DST	2.02E+00	5.62E+00	2.41E+00	2.39E-02
ENSG00000183527	PSMG1	2.01E+00	4.52E+00	1.97E+00	4.55E-02
ENSG00000228474	OST4	2.00E+00	4.80E+00	2.74E+00	1.50E-02
ENSG00000188636	RTL6	1.99E+00	5.23E+00	2.99E+00	1.05E-02
ENSG00000119979	FAM45A	1.98E+00	4.55E+00	2.15E+00	3.48E-02
ENSG00000071082	RPL31	1.98E+00	8.20E+00	3.26E+00	7.22E-03
ENSG00000281103	TRG-AS1	1.98E+00	4.54E+00	2.63E+00	1.74E-02
ENSG00000059588	TARBP1	1.98E+00	6.37E+00	2.64E+00	1.72E-02
ENSG00000100823	APEX1	1.97E+00	7.13E+00	3.27E+00	7.11E-03
ENSG00000100129	EIF3L	1.97E+00	6.23E+00	3.08E+00	9.31E-03
ENSG00000172687	ZNF738	1.95E+00	3.90E+00	2.32E+00	2.75E-02
ENSG00000144711	IQSEC1	1.95E+00	4.79E+00	2.64E+00	1.71E-02
ENSG00000108298	RPL19	1.92E+00	7.69E+00	3.05E+00	9.72E-03
ENSG00000169855	ROBO1	1.91E+00	4.39E+00	2.40E+00	2.43E-02

ENSG00000144354	CDCA7	1.90E+00	6.46E+00	2.89E+00	1.21E-02
ENSG00000142657	PGD	1.90E+00	5.08E+00	2.59E+00	1.86E-02
ENSG00000152556	PFKM	1.89E+00	3.46E+00	1.96E+00	4.66E-02
ENSG00000157657	ZNF618	1.89E+00	4.46E+00	2.45E+00	2.26E-02
ENSG00000174989	FBXW8	1.88E+00	3.76E+00	1.96E+00	4.62E-02
ENSG00000135272	MDFIC	1.87E+00	5.31E+00	2.32E+00	2.72E-02
ENSG00000105373	NOP53	1.87E+00	5.42E+00	2.80E+00	1.37E-02
ENSG00000149639	SOGA1	1.87E+00	5.24E+00	2.75E+00	1.46E-02
ENSG00000135446	CDK4	1.85E+00	3.66E+00	1.94E+00	4.73E-02
ENSG00000142937	RPS8	1.84E+00	7.87E+00	1.99E+00	4.44E-02
ENSG00000114738	MAPKAPK3	1.83E+00	5.09E+00	2.55E+00	1.96E-02
ENSG00000003400	CASP10	1.83E+00	5.19E+00	2.34E+00	2.64E-02
ENSG00000163597	SNHG16	1.82E+00	3.88E+00	2.04E+00	4.12E-02
ENSG00000145741	BTF3	1.82E+00	5.95E+00	2.76E+00	1.46E-02
ENSG00000184635	ZNF93	1.82E+00	3.73E+00	1.96E+00	4.64E-02
ENSG00000148840	PPRC1	1.82E+00	5.44E+00	2.08E+00	3.87E-02
ENSG00000115850	LCT	1.81E+00	4.20E+00	2.12E+00	3.65E-02
ENSG00000117174	ZNHIT6	1.81E+00	5.43E+00	2.66E+00	1.68E-02
ENSG00000118276	B4GALT6	1.80E+00	4.99E+00	2.27E+00	2.95E-02
ENSG00000186468	RPS23	1.80E+00	4.93E+00	1.93E+00	4.81E-02
ENSG00000167085	PHB	1.79E+00	4.91E+00	2.01E+00	4.32E-02
ENSG00000147403	RPL10	1.78E+00	8.07E+00	2.39E+00	2.45E-02
ENSG00000144736	SHQ1	1.78E+00	5.46E+00	2.16E+00	3.45E-02
ENSG00000198561	CTNND1	1.78E+00	5.95E+00	2.04E+00	4.12E-02
ENSG00000118495	PLAGL1	1.77E+00	4.75E+00	2.20E+00	3.24E-02
ENSG00000181163	NPM1	1.77E+00	9.24E+00	2.06E+00	3.96E-02
ENSG00000198755	RPL10A	1.75E+00	7.60E+00	2.03E+00	4.19E-02
ENSG00000136636	KCTD3	1.75E+00	5.00E+00	2.28E+00	2.90E-02
ENSG00000149554	CHEK1	1.74E+00	5.33E+00	1.95E+00	4.69E-02
ENSG00000148303	RPL7A	1.74E+00	7.94E+00	1.91E+00	4.94E-02
ENSG00000118181	RPS25	1.73E+00	7.59E+00	2.57E+00	1.89E-02
ENSG00000102221	JADE3	1.73E+00	4.55E+00	1.95E+00	4.71E-02
ENSG00000143641	GALNT2	1.73E+00	6.43E+00	2.57E+00	1.91E-02
ENSG00000141076	UTP4	1.72E+00	4.90E+00	2.10E+00	3.78E-02
ENSG00000104738	MCM4	1.72E+00	6.16E+00	2.07E+00	3.91E-02
ENSG00000174444	RPL4	1.71E+00	9.07E+00	2.33E+00	2.68E-02
ENSG00000172716	SLFN11	1.69E+00	5.87E+00	2.39E+00	2.47E-02
ENSG00000102898	NUTF2	1.69E+00	4.97E+00	1.92E+00	4.88E-02
ENSG00000134986	NREP	1.64E+00	8.64E+00	2.75E+00	1.47E-02
ENSG00000121210	TMEM131L	1.63E+00	8.51E+00	2.54E+00	2.00E-02
ENSG00000204472	AIF1	1.63E+00	5.51E+00	1.95E+00	4.71E-02

ENSG00000197961	ZNF121	1.61E+00	5.53E+00	2.01E+00	4.28E-02
ENSG00000115365	LANCL1	1.60E+00	5.70E+00	2.11E+00	3.73E-02
ENSG00000139083	ETV6	1.60E+00	7.27E+00	2.54E+00	1.98E-02
ENSG00000197635	DPP4	1.57E+00	6.22E+00	2.05E+00	4.06E-02
ENSG00000103222	ABCC1	1.54E+00	7.15E+00	1.93E+00	4.84E-02
ENSG00000153283	CD96	1.51E+00	7.74E+00	1.94E+00	4.76E-02
ENSG00000110700	RPS13	1.50E+00	7.69E+00	2.06E+00	3.99E-02
ENSG00000113648	H2AFY	1.48E+00	8.26E+00	2.18E+00	3.33E-02

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Claims:

1. A method of generating stem or progenitor cells unable to undergo T cell receptor (TCR) gene rearrangements (TCR), the method comprising:

(a) culturing a sample comprising stem cells or progenitor cells,

wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells.

2. The method of claim 1, wherein the method further comprises (b) isolating cells of the T cell lineage.

3. The method of claim 1 or 2, wherein the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

4. The method of claim 1 or 2, wherein the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

5. The method of any one of claims 1-4, wherein the stem cells are pluripotent stem cells.

6. The method of claim 5, wherein the pluripotent stem cells are embryonic stem cells or induced pluripotent stem cells (iPSCs).

7. The method of any one of claims 1-6, wherein the stem cells or progenitor cells are human cells.

8. The method of any one of claims 1-7, wherein the cells of the T cell lineage are progenitor T (proT) cells.

9. The method of any one of claims 1-7, wherein the cells of the T cell lineage are CD4+CD8+ double positive cells or CD4+CD8+CD3+ double positive cells.

10. The method of any one of claims 1-7, wherein the cells of the T cell lineage are CD8+CD3+ single positive cells or CD4+CD3+ single positive cells.

11. The method of any one of claims 1-10, further comprising engineering the stem cells or progenitor cells or the cells of the T cell lineage to comprise at least one of a nucleic acid encoding a T cell receptor (TCR), a TCRβ chain and a chimeric antigen receptor (CAR).

12. The method of claim 11, wherein the stem cells or progenitor cells or the cells of the T cell lineage express the at least one of the T cell receptor (TCR), the TCRβ chain and the chimeric antigen receptor (CAR).

13. The method of any one of claims 1-10, further comprising engineering the stem cells or progenitor cells or the cells of the T cell lineage to comprise a nucleic acid encoding a TCRβ chain.

14. The method of claim 13, wherein the stem cells or progenitor cells or the cells of the T cell lineage further comprise a nucleic acid encoding a CAR.

15. The method of claim 11 or 12, wherein the TCR or CAR confers specificity to an antigen, optionally a tumor-associated antigen, viral antigen or self antigen.

16. A cell of the T cell lineage, wherein the cell is generated by the method of any one of claims 1-15.

17. The cell of claim 16, wherein the cell of the T cell lineage is a CD4+CD8+ double positive cell or a CD4+CD8+CD3+ double positive cell.

18. The cell of claim 16, wherein the cell is a CD45+CD34+CD7+ progenitor T cell, CD8+CD3+ single positive cell or CD4+CD3+ single positive cell.

19. A stem or progenitor cell, wherein expression of at least one gene or protein required for V(D)J recombination in the stem cell or progenitor cell is reduced or eliminated compared to a wild-type stem cell or progenitor cell.

20. The stem or progenitor cell of claim 19, wherein the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

21. The stem or progenitor cell of claim 20, wherein the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4

(XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

22. The stem or progenitor cell of any one of claims 19-21, further comprising at least one of a nucleic acid encoding a T cell receptor (TCR), a TCRβ chain and a chimeric antigen receptor (CAR).

23. The stem or progenitor cell of any one of claims 19-21, further comprising a nucleic acid encoding a TCRβ chain.

24. The stem or progenitor cell of any one of claims 19-23, wherein the stem cell is a pluripotent stem cell.

25. The stem or progenitor cell of claim 24, wherein the pluripotent stem cell is an embryonic stem cell or induced pluripotent stem cell (iPSC).

26. The stem or progenitor cell of any one of claims 19-25, wherein the stem cell or progenitor cell is a human cell.

27. A use of the stem or progenitor cell of any one of claims 19-26 for generating cells of the T cell lineage.

28. A kit comprising (i) a stem or progenitor cell of any one of claims 19-27 and (ii) instructions for use of the stem or progenitor cell of any one of claims 19-27 for generating cells of the T cell lineage.

29. A method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells or progenitor cells to a subject in need thereof,

wherein the stem cells or progenitor cells are engineered to comprise at least one of a nucleic acid encoding a T cell receptor (TCR) and a chimeric antigen receptor (CAR) that confers specificity to an antigen.

30. A method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, and isolating cells of the T cell lineage wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells of the T cell lineage to a subject in need thereof,

wherein the stem cells or progenitor cells or the cells of the T cell lineage are engineered to comprise at least one of a nucleic acid encoding a T cell receptor (TCR) and a chimeric antigen receptor (CAR) that confers specificity to an antigen.

31. The method of claim 29 or 30, wherein the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

32. The method of claim 29 or 30, wherein the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

33. The method of any one of claims 29-32, wherein the disease is cancer and the antigen is a tumor-associated antigen.

34. A method of generating stem or progenitor cells unable to undergo T cell receptor (BCR) gene rearrangements, the method comprising:

(a) culturing a sample comprising stem cells or progenitor cells,

wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells.

35. The method of claim 34, wherein the method further comprises (b) isolating cells of the B cell lineage.

36. The method of claim 34 or 35, wherein the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

37. The method of claim 34 or 35, wherein the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

38. The method of any one of claims 34-37, wherein the stem cells are pluripotent stem cells.

39. The method of claim 38, wherein the pluripotent stem cells are embryonic stem cells or induced pluripotent stem cells (iPSCs).

40. The method of any one of claims 34-39, wherein the stem cells or progenitor cells are human cells.

41. The method of any one of claims 34-40, wherein the cells of the B cell lineage are CD20+ or CD19+ cells.

42. The method of any one of claims 34-40, wherein the cells of the B cell lineage are Tumor-Infiltrating B Cells (TIBs).

43. The method of any one of claims 34-42, further comprising engineering the stem cells or progenitor cells or the cells of the B cell lineage to comprise at least one of a nucleic acid encoding a B cell receptor (BCR), chimeric antigen receptor (CAR) or BCRβ chain.

44. The method of claim 43, wherein the stem cells or progenitor cells or the cells of the B cell lineage express the B cell receptor (BCR), chimeric antigen receptor (CAR) or BCRβ chain.

45. The method of claim 43 or 44, wherein the BCR or CAR confers specificity to an antigen, optionally a tumor-associated antigen, viral antigen or self antigen.

46. A cell of the B cell lineage, wherein the cell is generated by the method of any one of claims 35-45.

47. The cell of claim 46, wherein the cell of the B cell lineage is a CD20+ or CD19+ cell.

48. The cell of claim 46, wherein the cell of the B cell lineage is a Tumor-Infiltrating B Cell (TIB).

49. A use of the stem or progenitor cell of any one of claims 19-21 for generating cells of the B cell lineage.

50. A kit comprising (i) a stem or progenitor cell of any one of claims 19-21 and (ii) instructions for use of the stem or progenitor cell of any one of claims 19-21 for generating cells of the B cell lineage.

51. A method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the stem cells or progenitor cells to a subject in need thereof,

wherein the stem cells or progenitor cells are engineered to comprise least one nucleic acid encoding a B cell receptor (BCR) or chimeric antigen receptor (CAR) that confers specificity to an antigen.

52. A method of treating a disease or condition in a subject comprising:

(i) culturing a sample comprising stem cells or progenitor cells, and isolating cells of the B cell lineage wherein expression of at least one gene or protein required for V(D)J recombination in the stem cells or progenitor cells is reduced or eliminated compared to wildtype stem cells or progenitor cells, and

(ii) administering an effective amount of the cells of the B cell lineage to a subject in need thereof,

wherein the stem cells or progenitor cells or the cells of the B cell lineage are engineered to comprise least one nucleic acid encoding a B cell receptor (BCR) or chimeric antigen receptor (CAR) that confers specificity to an antigen.

53. The method of claim 51 or 52, wherein the at least one gene or protein required for V(D)J recombination is RAG1 and/or RAG2.

54. The method of claim 51 or 52, wherein the at least one gene or protein required for V(D)J recombination is selected from the group consisting of Artemis, DNA-dependent protein kinase (DNA-PK), X-ray repair cross-complementing protein 4 (XRCC4), DNA ligase IV, non-homologous end-joining factor 1 (NHEJ1), Paralog of XRCC4 and XLF (PAXX), DNA polymerase λ and DNA polymerase μ.

55. The method of any one of claims 51-54, wherein the disease is cancer and the antigen is a tumor-associated antigen.

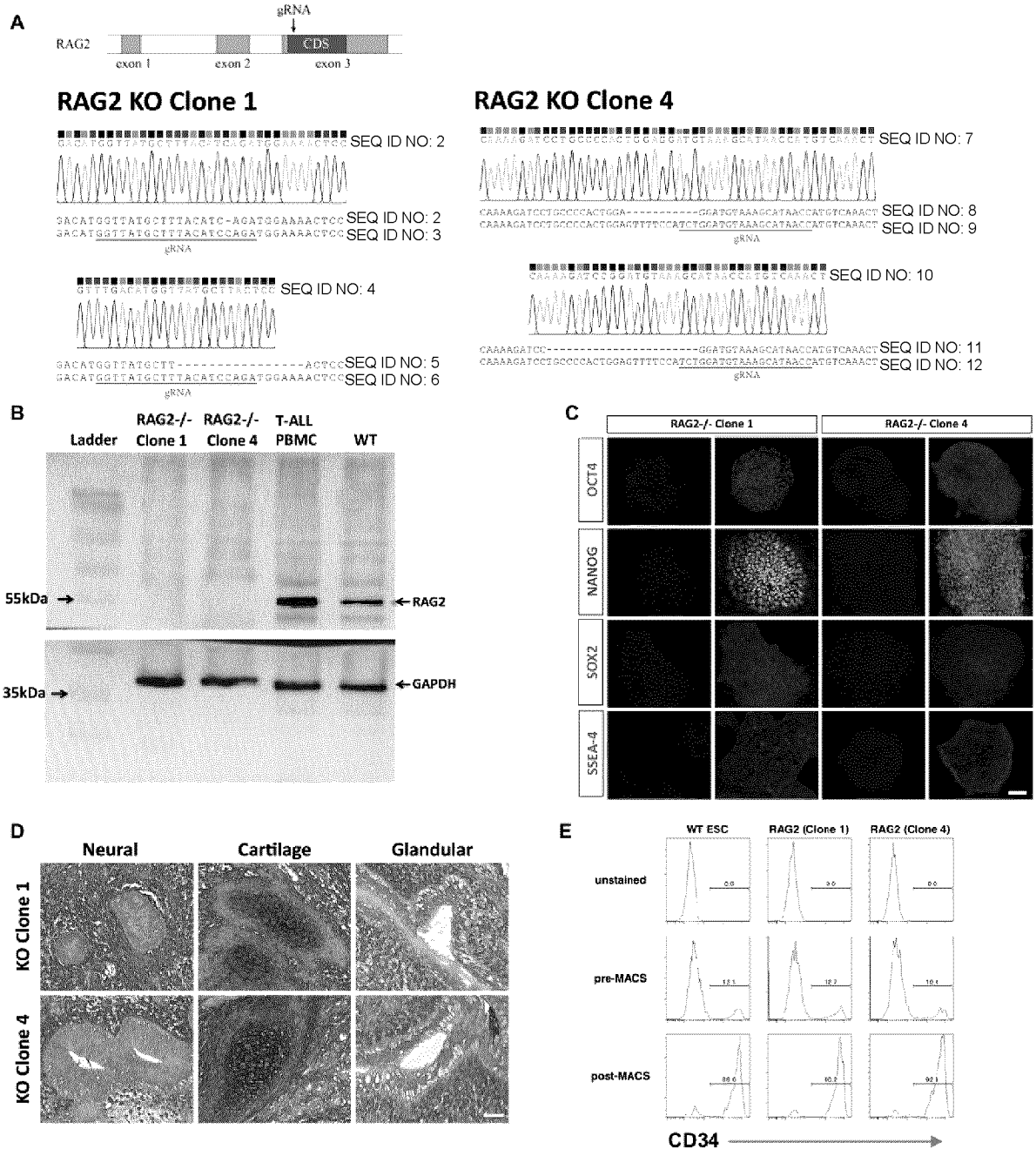

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exon 1
exon 2
exon 3
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RAG2 KO Clone 4
SEQ ID NO: 7
SEQ ID NO: 8
SEQ ID NO: 9
gRNA
SEQ ID NO: 10
SEQ ID NO: 11
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gRNA
B
Ladder
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RAG2-/- Clone 4
T-ALL PBMC
WT
55kDa
RAG2
35kDa
GAPDH
C
RAG2-/- Clone 1
RAG2-/- Clone 4
OCT4
NANOG
SOX2
SSEA-4
D
Neural
Cartilage
Glandular
KO Clone 1
KO Clone 4
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RAG2 (Clone 4)
unstained
pre-MACS
post-MACS
CD34

Fig. 1

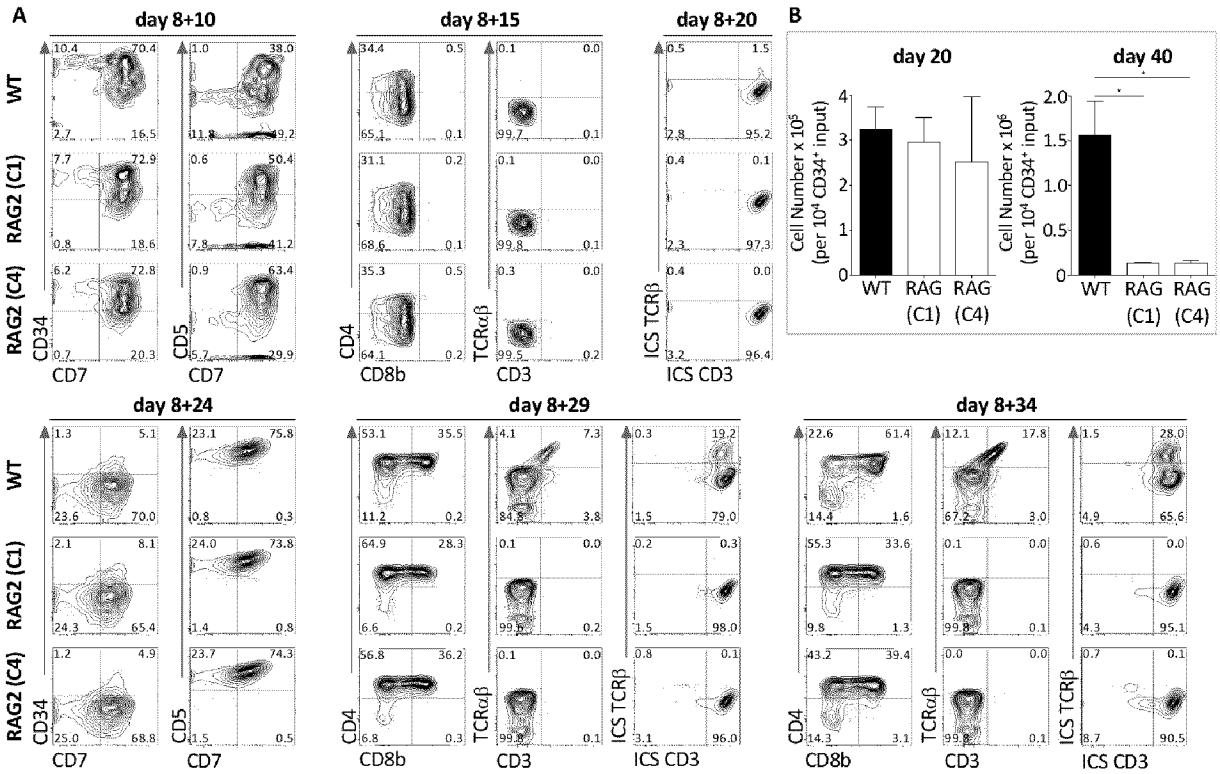

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day 8+15
day 8+20
WT
RAG2 (C1)
RAG2 (C4)
CD34
CD7
CD5
CD4
CD8b
TCRαβ
CD3
ICS TCRβ
ICS CD3
B
day 20
day 40
Cell Number x 10^5 (per 10^4 CD34+ input)
WT
RAG (C1)
RAG (C4)
day 8+24
day 8+29
day 8+34

Fig. 2

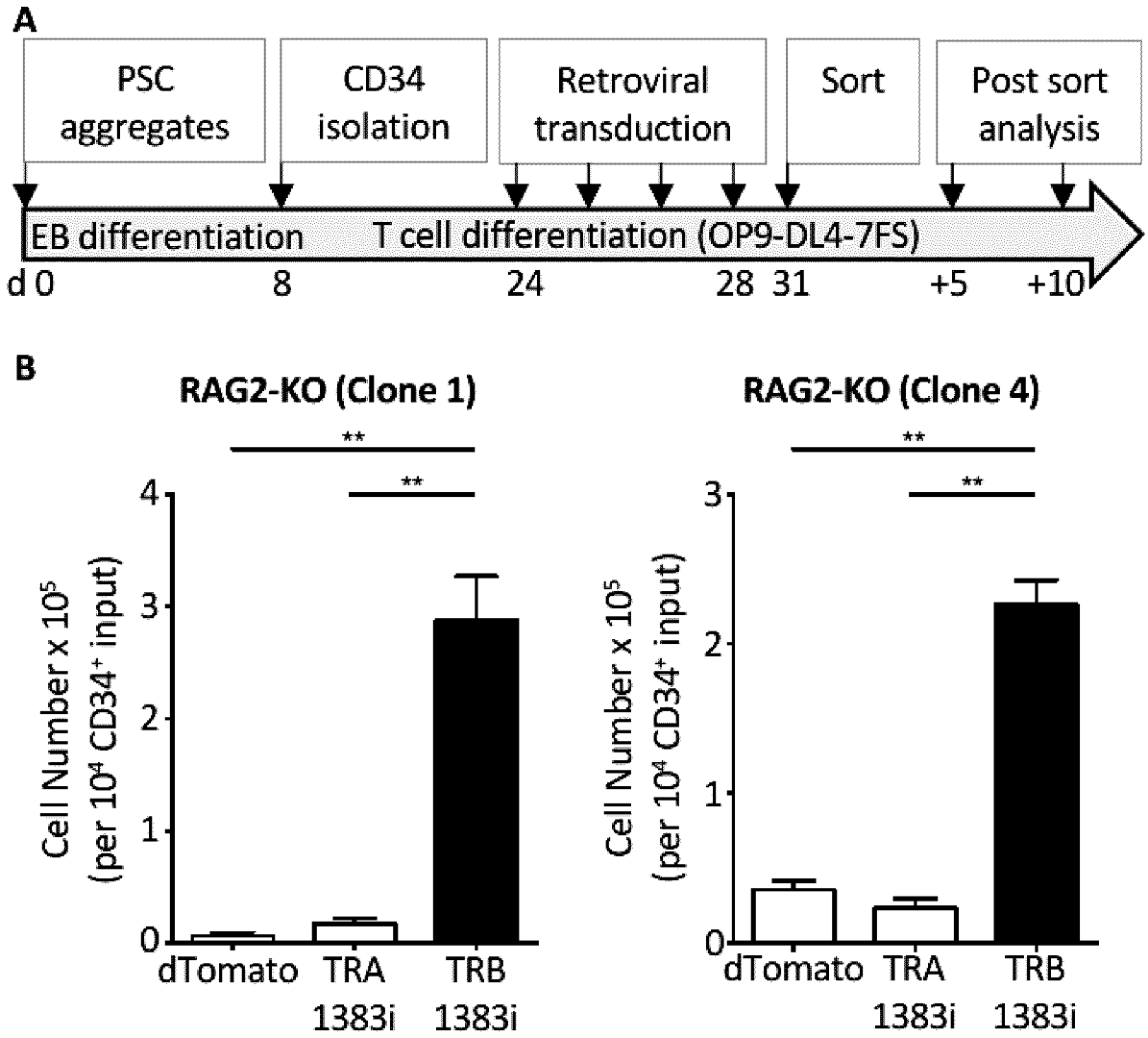

A
PSC aggregates
CD34 isolation
Retroviral transduction
Sort
Post sort analysis
EB differentiation
T cell differentiation (OP9-DL4-7FS)
d 0
8
24
28
31
+5
+10
B
RAG2-KO (Clone 1)
RAG2-KO (Clone 4)
Cell Number x 10⁵ (per 10⁴ CD34⁺ input)
**
**
dTomato
TRA 1383i
TRB 1383i
0
1
2
3
4

Fig. 3

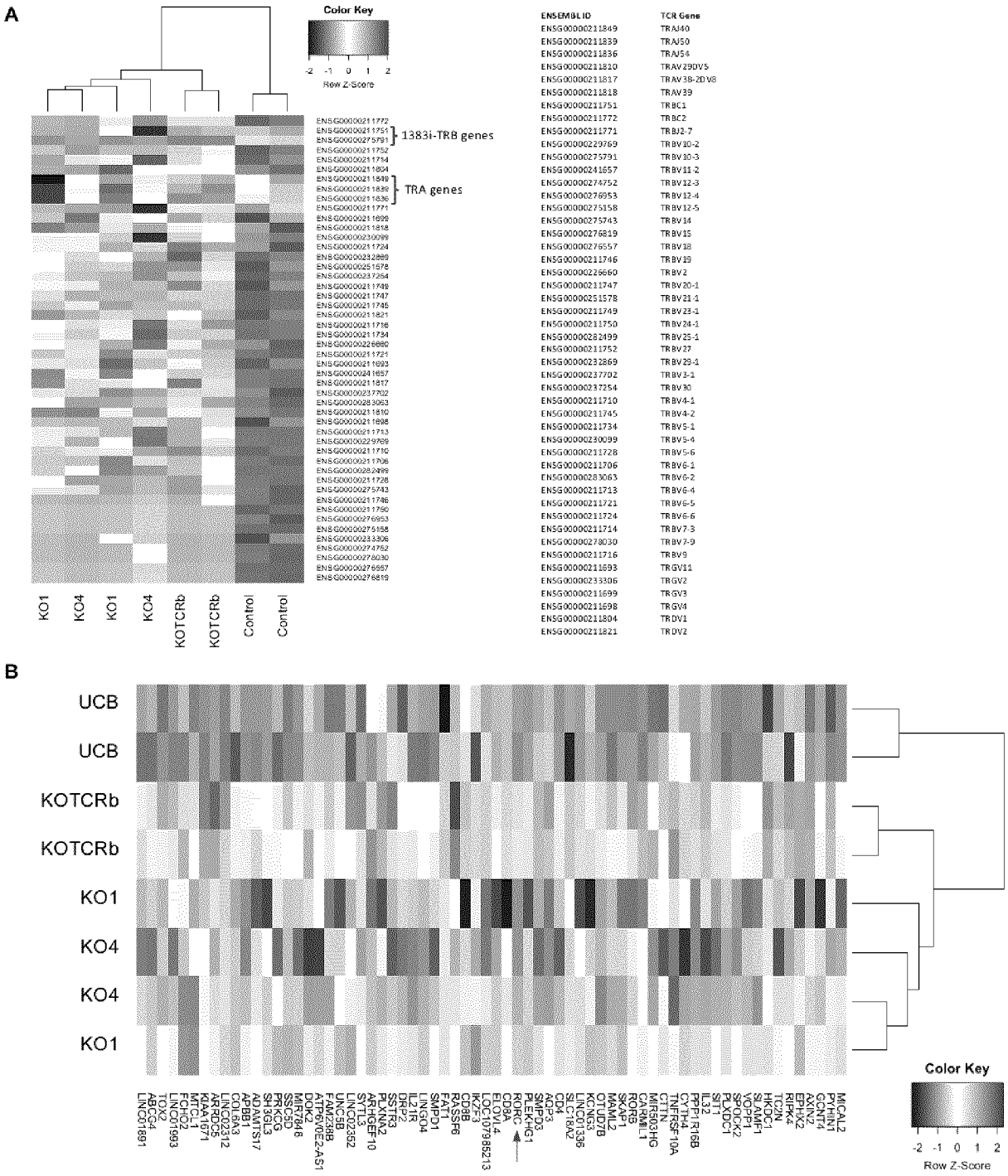

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Control
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KOTCRb
KO1
KO4
KO4
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Fig. 4

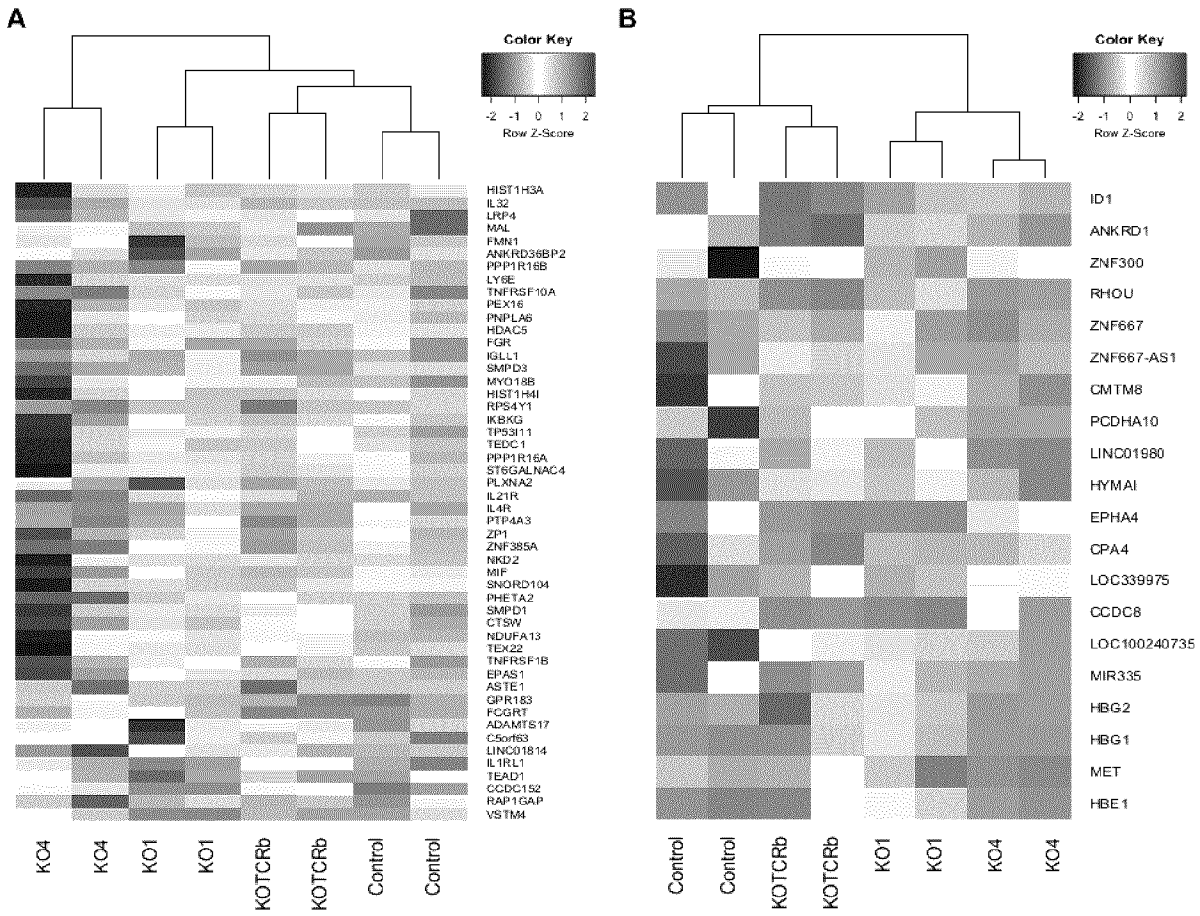

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PEX16
PNPLA6
HDAC5
FGR
IGLL1
SMPD3
MYO18B
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RPS4Y1
IKBKG
TP53I11
TEDC1
PPP1R16A
ST6GALNAC4
PLXNA2
IL21R
IL4R
PTP4A3
ZP1
ZNF385A
NKD2
MIF
SNORD104
PHETA2
SMPD1
CTSW
NDUFA13
TEX22
TNFRSF1B
EPAS1
ASTE1
GPR183
FCGRT
ADAMTS17
C5orf63
LINC01814
IL1RL1
TEAD1
CCDC152
RAP1GAP
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Row Z-Score
ID1
ANKRD1
ZNF300
RHOU
ZNF667
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CMTM8
PCDHA10
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CPA4
LOC339975
CCDC8
LOC100240735
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MET
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Control
Control
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KOTCRb
KO1
KO1
KO4
KO4

Fig. 5

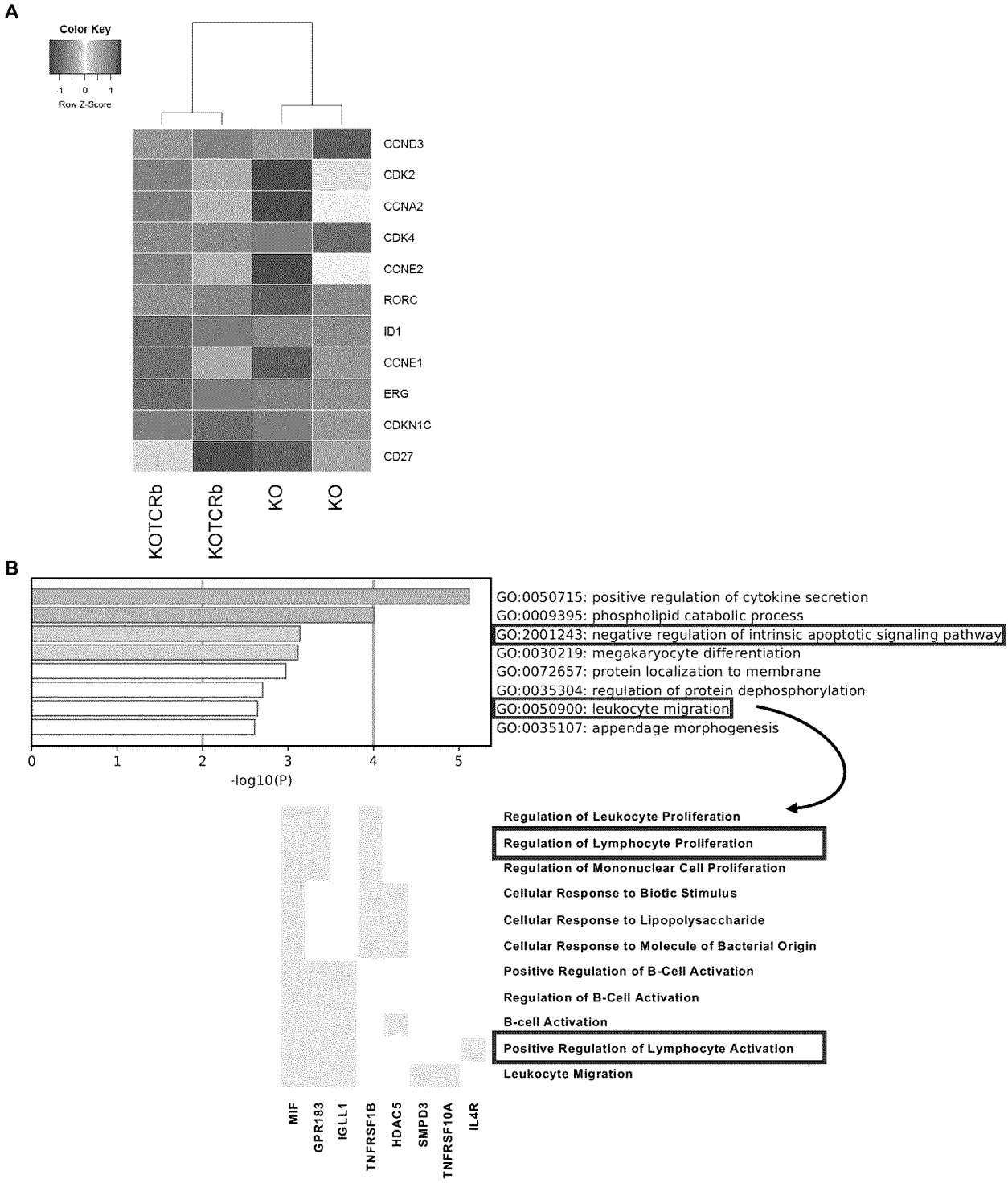

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KOTCRb
KO
KO
B
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GO:0009395: phospholipid catabolic process
GO:2001243: negative regulation of intrinsic apoptotic signaling pathway
GO:0030219: megakaryocyte differentiation
GO:0072657: protein localization to membrane
GO:0035304: regulation of protein dephosphorylation
GO:0050900: leukocyte migration
GO:0035107: appendage morphogenesis
0 1 2 3 4 5
-log10(P)
Regulation of Leukocyte Proliferation
Regulation of Lymphocyte Proliferation
Regulation of Mononuclear Cell Proliferation
Cellular Response to Biotic Stimulus
Cellular Response to Lipopolysaccharide
Cellular Response to Molecule of Bacterial Origin
Positive Regulation of B-Cell Activation
Regulation of B-Cell Activation
B-cell Activation
Positive Regulation of Lymphocyte Activation
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IL4R

Fig. 6

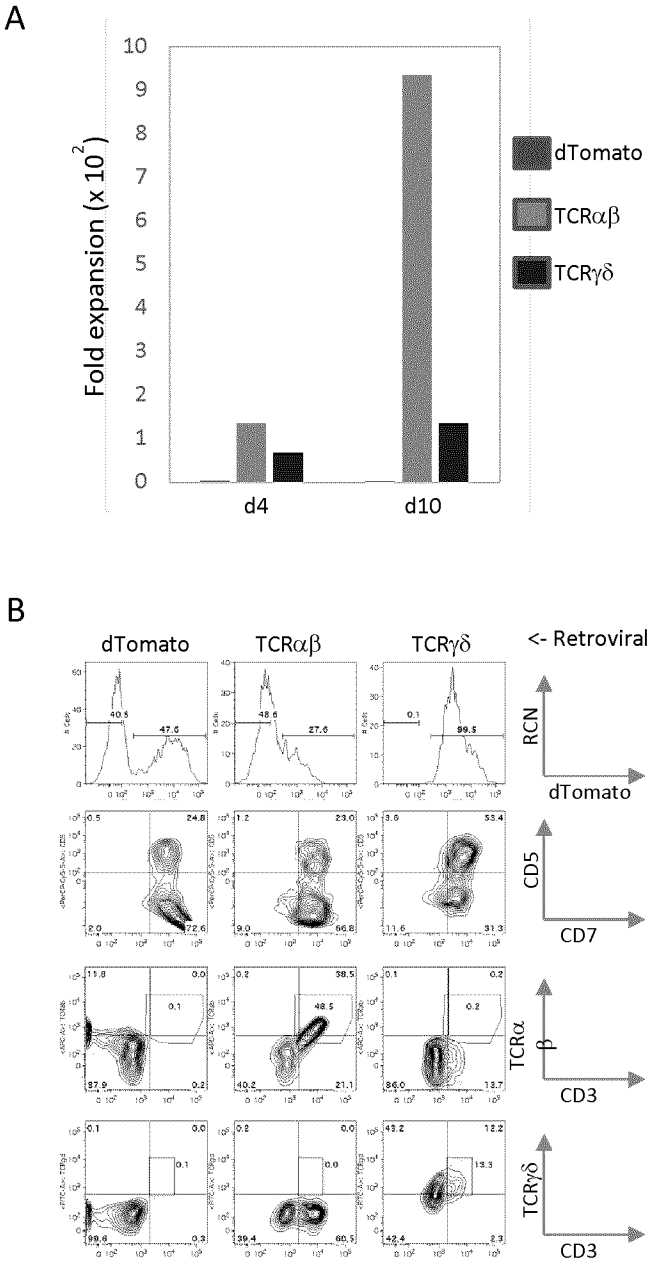

A
Fold expansion (x 10^2)
10
9
8
7
6
5
4
3
2
1
0
d4
d10
dTomato
TCRαβ
TCRγδ
B
dTomato
TCRαβ
TCRγδ
<- Retroviral
RCN
dTomato
CD5
CD7
TCRα
β
CD3
TCRγδ
CD3

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2022/050622

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12N 5/10*** (2006.01), ***A61K 35/12*** (2015.01), ***A61K 35/17*** (2015.01), ***C07K 14/725*** (2006.01), ***C07K 19/00*** (2006.01), ***C12N 5/071*** (2010.01), ***C12N 5/0783*** (2010.01), ***C12N 15/10*** (2006.01)

CPC: A61K 35/12 (2020.01), A61K 35/17 (2020.01), C07K 14/705 (2020.01), C12N 5/06 (2022.05), C12N 5/10 (2020.05) C12N 5/0602 (2020.01), C12N 5/0635 (2020.01), C12N 5/0636 (2020.01), C12N 15/10 (2022.05), C12N 2510/00 (2020.01)

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)
IPC: ALL

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
None

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Google, PubMed, STN (keywords: RAG2, mutation, development, T cell, TCR, human, edit*, progenitor, TCR beta, isolate*, stem, RAG, knockout, chimeric antigen receptor, VDJ, deficien*, deficiency, CAR, recomb*, reduc*, treatment of disease and Zuniga-Pflucker, JC)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	MINAWAGA, A. et al. "Enhancing T Cell Receptor Stability in Rejuvenated iPSC-Derived T Cells Improves Their Use in Cancer Immunotherapy". 2018. Cell Stem Cell. Vol. 23, pp 850-858 ISSN: 1875-9777 (Electronic)	1-3, 5-9, 11-13, 15-17, 19, 20, 22-31, 33 and 34 14
X	LEE, J. H. et al. "Differences between Immunodeficient mice generated by classical gene targeting and CRISPR/Cas9-mediated gene knockout". 2018. Transgenic Research. Vol. 27, pp 245-51 ISSN: 1573-9368 (Electronic)	10, 16-19 and 20
X	KIM, J-I. et al. "CRISPR/Cas9-mediated knockout of Rag-2 causes systemic lymphopenia with hypoplastic lymphoid organs in FVB mice". 2018. Laboratory Animal Research. Vol. 34, pp 166-75 ISSN: 2233-7660 (Electronic)	16, 17, 19 and 20

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

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"D"	document cited by the applicant in the international application	"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
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 13 June 2022 (13-06-2022)	Date of mailing of the international search report 29 June 2022 (29-06-2022)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 81	Authorized officer Philip Marshall (819) 639-5251

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2022/050622

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim 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. ☐ Claim Nos.:
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:

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-34 are directed to a method of generating stem or progenitor cells unable to undergo T cell receptor rearrangements wherein expression of gene encoding VDJ recombination is eliminated or reduced, cell produced by said method, a stem cell or progenitor cell that is missing a gene required for VDJ recombination, use of the stem or progenitor and a kit comprising said stem or progenitor cell, a method for treating a disease in a subject comprising administering said cells wherein said cells comprising a TCR or a CAR conferring specificity to an antigen; and

(continued on page 6)

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 claim 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 claim Nos.:

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.

Continued from box III on page 3

Group B - Claims 35-55 are directed to a method of generating a stem or progenitor cell unable to undergo B cell receptor gene rearrangement, a cell of the B lineage generated by said method, a use of stem or progenitor cell for generating cells of the B lineage, a kit comprising a stem or progenitor cell comprising said cell, a method for treating a disease or condition in a subject comprising administering said cells to a subject.

There is no single inventive concept linking claims of groups A and B *a posteriori*. Claims of group A are directed to a method of generating a stem or progenitor cell unable to undergo T cell receptor gene rearrangement whereas claims of group B are directed to a method of generating a stem or progenitor cell unable to undergo B cell receptor gene rearrangement. The common feature to both methods is a progenitor cell that is deficient for RAG-2 which is well known in the art as exemplified by Minawaga, A. et al. and by Kim, J-I. et al. Therefore, each claim group is considered a separate invention. The claims must be limited to one inventive concept as set out in PCT Rule 13.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHINKAI, Y. et al. "RAG-2 Deficient Mice lack Mature Lymphocytes Owing to Inability to Initiate V(D)J Rearrangement". 1992. Vol 68, pp 855-867 ISSN: 1097-4172 (Electronic)	19 and 20
P, X	CHEN, E. L. Y. et al. "Cutting Edge: TCR-β Selection Is Required at the CD4+ CD8+ stage of Human T Cell Development". 2021. Vol. 206, pp 2271-2276 ISSN: 1550-6606 (Electronic)	
Y	ZHAO, Q et al. "Engineered TCR-T Cell Immunotherapy in AntiCancer Precision Medicine: Pros and Cons". Published March 30th, 2021. Frontiers in Immunology. Vol. 12, Article 658753 ISSN: 1664-3224 (Electronic)	14