CLAIMS

1. A composition comprising:

a matrix;

a plurality of cell surface receptor binding agents, wherein the plurality of cell surface receptor binding agents are bound to the matrix and comprise:

a CD4 receptor binding agent; optionally wherein the CD4 receptor binding agent binds to an amino acid sequence that is at least 80% identical to SEQ ID NO: 1;

a T cell receptor (TCR) binding agent; optionally wherein the TCR binding agent binds to an amino acid sequence that is at least 80% identical to SEQ ID NO: 2;

and

a population of single positive CD4 T cells ($CD4^+$ $CD8^-$).

2. A composition comprising:

a matrix;

a plurality of cell surface receptor binding agents, wherein the plurality of cell surface receptor binding agents are bound to the matrix and comprise:

a CD4 receptor binding agent; and

a T cell receptor (TCR) binding agent; and

a mixed population of *in vitro*-differentiated T cells, wherein the mixed population of *in vitro*-differentiated T cells comprise:

a population of single positive $CD4^+$ T cells; and

a population of double positive ($CD4^+$ $CD8^+$) T cells,

wherein the population of single positive $CD4^+$ T cells is at least about 10% of a total number of cells within the mixed population of *in vitro*-differentiated T cells.

3. The composition of claim 2, wherein the population of single positive $CD4^+$ T cells is:

(a) at least about 50% of the total number of cells within the mixed population of *in vitro*-differentiated T cells; or

(b) at least about 60% of the total number of cells within the mixed population of *in vitro*-differentiated T cells; or

(c) at least about 70% of the total number of cells within the mixed population of *in vitro*-differentiated T cells or

(d) at least about 80% of the total number of cells within the mixed population of *in vitro*-differentiated T cells; or

(e) at least about 90% of the total number of cells within the mixed population of *in vitro*-differentiated T cells.

4. A method of producing a population of single positive $CD4^+$ T cells from a population of *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cells, the method comprising:

contacting a population of *in vitro*-differentiated ($CD4^+$ $CD8^+$) T cells for a period of time with a composition comprising:

(i) a matrix;

(ii) a CD4 receptor binding agent that is bound to the matrix; and

(iii) a TCR binding agent that is bound to the matrix,

thereby producing a population of single positive $CD4^+$ T cells.

5. A method for producing a single positive $CD4^+$ T cell from a double positive ($CD4^+$ $CD8^+$) T cell, the method comprising:

contacting an *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cell with a CD4 binding agent tethered to a TCR binding agent.

6. The composition of claim 1 or 2, or the method of claim 4 or 5, wherein:

(a) the CD4 receptor binding agent comprises an antibody or a functional fragment thereof, a small molecule, a protein, a nucleic acid, or an aptamer; or

(b) the TCR binding agent comprises an antibody or a functional fragment thereof, a small molecule, a protein, a nucleic acid, or an aptamer,

optionally wherein the antibody or the functional fragment thereof comprises: a single domain antibody, a diabody, a scFv, an scFv dimer, a BsFv, a dsFv, a (dsFv)2, a dsFv-dsFv′, an Fv fragment, a Fab, a Fab′, a F(ab′)2, a ds-diabody, a nanobody, a domain antibody, or a bivalent domain antibody.

7. The composition of claim 1 or 2, wherein:

(a) the plurality of cell surface receptor binding agents bind to a single positive $CD4^+$ $CD8^-$ T cell; or

(b) the CD4 receptor binding agent binds to:

i) a CD4 receptor on an *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cell; or

ii) a CD4 receptor on an *in vitro*-differentiated $CD4^+$ $CD8^-$ T cell; or

iii) an amino acid sequence that is at least 80% identical to SEQ ID NO: 1;

or

(c) the TCR binding agent binds to:

i) a TCR alpha beta chain on an *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cell; or

ii) a TCR alpha beta chain on a single positive $CD4^+$ $CD8^-$ T cell; or

iii) an amino acid sequence that is at least 80% identical to SEQ ID NO: 2;

or

iv) an engineered T cell receptor expressed by an engineered T cell; or

v) a gamma delta TCR expressed by an engineered T cell.

8. The composition of claim 1 or 2, wherein:

(a) the population of single positive $CD4^+$ T cells or the mixed population of *in vitro*-differentiated T cells are derived from a population of stem cells, optionally wherein:

i) the stem cells are human induced pluripotent stem cells (iPSC); or

ii) the stem cells are human embryonic stem cells (ESC); or

iii) the single positive CD4+ T cells are engineered T cells derived by differentiation of engineered iPSCs; or

(b) the stem cells are human adult stem cells, optionally wherein:

(I) the human adult stem cells are extracted from or isolated from a healthy subject; or

(II) the human adult stem cells are extracted from or isolated from a subject that has, is suspected of having, or is at risk of developing a disease; or

(c) the population of single positive $CD4^+$ T cells or the mixed population of *in vitro*-differentiated T cells are derived from cord blood, placental cells, bone marrow, blood, or a tissue biopsy; or

(d) the single positive $CD4^+$ T cells are obtained by differentiation of genetically modified cells; or

(e) the mixed population of *in vitro*-differentiated T cells are derived from genetically modified cells; or

(f) the population of single positive $CD4^+$ T cells the mixed population of *in vitro*-differentiated T cells are derived from a mesodermal stem cell lineage; or

(g) the population of single positive $CD4^+$ T cells the mixed population of *in vitro*-differentiated T cells are derived from a CD34+ progenitor cell; or

(h) further comprising one or more cytokines, optionally wherein the one or more cytokines are selected from the group consisting of: interleukin 7 (IL-7), interleukin 21 (IL-21), interleukin 2 (IL-2), and any combination thereof; or

(i) the population of single positive $CD4^+$ T cells the mixed population of *in vitro*-differentiated T cells are reversibly bound to the plurality of cell surface receptor binding agents; or

(j) the population of single positive $CD4^+$ T cells express a CD4 receptor and one or more T cell markers, optionally wherein the one or more T cell markers are selected from the group consisting of: ZBTB7B (ThPOK), CD3, TBX21, IL-2, IFNG, TNFα, GATA3, IL-4, IL-13, RORC, IL-17, FOXP3, HELIOS, IL-10, BCL6, IL-21, IL-9 and IL-22.

9. The composition of claim 1 or 2, wherein:

(a) the matrix is in a form of a bead, optionally wherein the bead comprises an average diameter of at least about 1 nanometers (nm) up to 1500 micrometers (μm); or

(b) the matrix further comprises biotin; or

(c) the matrix comprises a flexible material, optionally wherein the flexible material comprises a dextran, a dextrin, an agarose, a gelatin, a polydimethylsiloxane (PDMS), a silicon, a silicone, a rubber, a collagen, a purified protein, a mixture of different purified proteins, a extrapolysaccharide, a glycosaminoglycan, a cellulose, a chitosan, a hyaluronic acid, an alginate, a polyester, a polyether, a polyanhydride, a polyalkylcyanoacrylate, a polyacrylamide, a polyorthoester, a polyphosphazene, a polyvinylacetate, a block copolymer, a polypropylene, a polytetrafluorethylene (PTFE), a polyurethane, a poly(lactic-co-glycolic acid (PLGA), a copolymer, or any combination thereof; or

(d) the matrix is in a form of a solid support, optionally wherein the solid support comprises a superparamagnetic colloidal material, a metal, an acrylic, a glass, a plastic, a polystyrene, a thermoplastic polymer, a polytetrafluoroethylene, a polyvinylchloride (PVC), a polyethersulfone, a polyphenylene oxide, a polyhexamethylene adipamide, a polycarbonate, a polycaprolactam, a polyethylene terephthalate, a polymethylcethacrylate, an acrylonitrile butadiene styrene (ABS), or any combination thereof; or

(e) the matrix is biocompatible; or

(f) the matrix is biodegradable; or

(g) the composition further comprises an extracellular matrix (ECM) composition, optionally wherein the ECM composition comprises fibronectin, vitronectin, retronectin, laminin, collagen, elastin, hyaluronic acid, methylcellulose, or any combination thereof.

10. The method of claim 4:

(a) further comprising contacting the population of *in vitro*-differentiated ($CD4^+$ $CD8^+$) T cells with one or more cytokines, optionally wherein the one or more cytokines comprise IL-7, IL-21, IL-2, or any combination thereof; or

(b) wherein the period of time is at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, up to 20 days; or

(c) comprising an additional step wherein the population of $CD4^+$ single positive T cells are isolated and purified; or

(d) wherein the composition further comprises an extracellular matrix (ECM) composition, optionally wherein the ECM composition comprises fibronectin, vitronectin, retronectin, laminin, collagen, elastin, hyaluronic acid, methylcellulose, or any combination thereof; or

(e) further comprising contacting the single positive $CD4^+$ T cells with a second composition comprising: a matrix and further comprising:

(i) a CD3 binding agent that is bound to the matrix;

(ii) a CD3 binding agent that is bound to the matrix and a CD28 binding agent that is bound to the matrix; or

(iii) a CD3 binding agent that is bound to the matrix; a CD28 binding agent that is bound to the matrix; and a CD2 binding agent that is bound to the matrix,

optionally further comprising contacting the single positive $CD4^+$ T cells with a second composition comprising: a matrix and further comprising:

(i) a CD3 binding agent that is bound to the matrix;

(ii) a CD3 binding agent that is bound to the matrix and a CD28 binding agent that is bound to the matrix; or

(iii) a CD3 binding agent that is bound to the matrix; a CD28 binding agent that is bound to the matrix; and a CD2 binding agent that is bound to the matrix; and/or

(f) wherein the method further comprises contacting the population of single positive $CD4^+$ T cells with IL-7, IL-2 and IL-21 at a final concentration of at least about 5 ng/mL up to 100 ng/mL; or

(g) wherein a surface of the matrix is coated with a plurality of antibodies; or

(h) wherein the contacting stimulates the population of *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cells to increase CD4 expression and decrease CD8 expression relative to a population of double positive ($CD4^+$ $CD8^+$) T cells that have not been contacted; or

(i) wherein the method does not comprise a step of cell sorting;

(j) wherein the population of single positive $CD4^+$ T cells comprises at least 40%, 50%, 60%, 70%, 80%, 90%, up to 100% of the population of total cells relative to the population of *in vitro*-differentiated double positive T cells; or

(k) wherein the *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cells are obtained by differentiating a population of human pluripotent stem cells, optionally wherein:

i) the population of human pluripotent stem cells comprise human induced pluripotent stem cells (iPSC); or

ii) the population of human pluripotent stem cells comprise human embryonic stem cells (ESC); or

(l) wherein the *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cells are obtained by differentiation of cells obtained from cord blood, placental cells, bone marrow, blood, or a tissue biopsy; or

(m) wherein the *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cells are obtained by differentiation of genetically modified cells.

11. The method of claim 5, wherein:

(a) the CD4 binding agent binds to an amino acid sequence that is at least 80% identical to SEQ ID NO: 1; or

(b) the TCR binding agent binds to:

i) an amino acid sequence that is at least 80% identical to SEQ ID NO: 2; or

ii) an engineered T cell receptor expressed by an engineered T cell; or

iii) a gamma delta TCR expressed by an engineered T cell.

iv) a TCR alpha chain, a TCR beta chain, or a TCR alpha and beta chain; or

(c) a TCR gamma chain, TCR delta chain, or a TCR gamma and delta chain.

12. A composition comprising a CD4 binding agent:

(a) tethered to a TCR binding agent; or

(b) fixed proximal to a TCR binding agent.

13. The composition of claim 12, wherein:

(a) the CD4 binding agent comprises an antibody or a functional fragment thereof, a small molecule, a protein, a nucleic acid, or an aptamer; or

(b) the TCR binding agent comprises an antibody or a functional fragment thereof, a small molecule, a protein, a nucleic acid, or an aptamer.

14. A fusion protein comprising:

(a) a CD4 binding agent that binds to SEQ ID NO: 1 or a functional fragment thereof; and a TCR binding agent that binds to SEQ ID NO: 2 or a functional fragment thereof; or

(b) a CD8 binding agent that binds to SEQ ID NO: 1 or a functional fragment thereof; and a TCR binding agent that binds to SEQ ID NO: 2 or a functional fragment thereof.

15. An *in vitro*-differentiated T cell, wherein the *in vitro*-differentiated T cell is bound to the fusion protein of claim 14, optionally wherein the *in vitro*-differentiated T cell is a single positive $CD4^+$ T cell, a single positive $CD8^+$ T cell, a double positive $CD4^+$ $CD8^+$ T cell, or a derivative thereof, optionally wherein:

(a) the single positive $CD4^+$ T cell expresses a CD4 receptor and one or more marker selected from the group consisting of: ZBTB7B (ThPOK), CD3, TBX21, IL-2, IFNG, TNFα, GATA3, IL-4, IL-13, RORC, IL-17, FOXP3, HELIOS, IL-10, BCL6, IL-21, IL-9 and IL-22; or

(b) the single positive $CD8^+$ T cell expresses a CD8 receptor; and a Notch receptor or a variant thereof, an intracellular Notch (ICN), or an effector molecule of the Notch receptor, optionally wherein:

i) the effector molecule of the notch receptor comprises ADAM-family metalloprotease (ADAM10), gamma (γ)-secretase, RBP-Jκ, Mastermind-like family (MAML1-3), or a histone acetyltransferase; or

ii) the Notch receptor or the variant thereof comprises a Notch 1 receptor or a functional fragment thereof, a Notch 2 receptor or a functional fragment thereof, or a Notch 3 receptor or a functional fragment thereof.

16. The composition of any one of claims 1-3, 6-9, 12 or 13 for use in a method for treatment of a disease or a condition in a subject in need thereof, the method comprising:

administering to the subject a therapeutically effective amount of the composition, optionally wherein:

(a) the disease or the condition is a cancer, an autoimmune disease, or diabetes; or

(b) the subject in need thereof has a solid tumor or a blood cancer; or

(c) the administering is:

i) local or systemic; or

ii) topical administration; or

iii) intravenous;

iv) via intratumoral injection.

17. A system for producing a single positive $CD4^+$ T cell comprising:

a cell culture vessel configured for localizing a plurality of cell surface receptor binding agents to an *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cell or a population thereof comprising;

a matrix;

a plurality of cell surface receptor binding agents, wherein the plurality of cell surface receptor binding agents are bound to the matrix and comprise:

a CD4 receptor binding agent;

a TCR binding agent; and

an *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cell or a population thereof,

wherein the plurality of cell surface receptor binding agents bind to the *in vitro*-differentiated double positive ($CD4^+$ $CD8^+$) T cell for a period of time,

and wherein the cell culture vessel is configured to facilitate contact between the plurality of cell surface receptor binding agents and the *in vitro*-differentiated double positive $CD4^+$ $CD8^+$ T cell or population thereof,, thereby producing a single positive $CD4^+$ T cell within at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, up to 20 days.