SUMMARY

[0007] The present invention relates generally to cell culture conditions, media, culture platforms, and methods for culturing and differentiating stem cells to a hematopoietic cell fate.

[0008] Specifically, the present invention provides methods and compositions for the generation of hematopoietic cell lineages through definitive hemogenic endothelium (HE) and definitive hematopoietic stem cells (HSC) derived from pluripotent stem cells, including hiPSCs under serum/feeder-free conditions and in a scalable and monolayer culturing platform without the need of EB formation. Cells that may be differentiated according to the methods of the invention range from pluripotent stem cells, to progenitor cells that are committed to a particular terminally differentiated cell and transdifferentiated cells, cells of various lineages directly transitioned to hematopoietic fate withoug going through a pluripotent intermediate. Similarly, the cells produced by differentiation of stem cells range from multipotent stem or progenitor cells to terminally differentiated stem cells, and all intervening hematopoietic cell lineages.

[0009] The present invention provides methods and compositions for differentiating and expanding cells of the hematopoietic lineage from pluripotent stem cells in monolayer culturing, which comprises contacting the pluripotent sterm cells with a BMP pathway activator, and optionally, bFGF. As such, pluripotent stem cell-derived mesodermal cells are obtained and expanded without forming embryoid bodies from pluripotent stem cells. The mesodermal cells are then subjected to contact with a BMP pathway activator, bFGF, and a WNT pathway activator to obtain expanded mesodermal cells having definitive hemogenic endothelium (HE) potential without forming embryoid bodies from the pluripotent stem cells. By subsequent contact with bFGF, and optionally, a ROCK inhibitor, and/or a WNT pathway activator, the mesodermal cells having definitive HE potential are differentiated to definitive HE cells, which are also expanded during differentiation.

[00010] The methods provided herein for obtaining cells of the hematopoietic lineage are superior to EB-mediated pluripotent stem cell differentiation, because EB formation leads to modest to minimal cell expansion, does not allow monolayer culturing which is important for many applications requiring homogeneous expansion, and homogeneous differentiation of the cells in a population, and is laborious and low efficiency.

[00010a] In one aspect, the present invention refers to an *ex vivo* method for directing differentiation of pluripotent stem cells to hematopoietic lineage cells, comprising: (i) contacting the pluripotent stem cells with a composition comprising a BMP pathway activator, and optionally bFGF, to obtain mesodermal cells; and (ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential, wherein the mesodermal cells having definitive hemogenic endothelium (HE) potential are capable of providing hematopoietic lineage cells comprising hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, or B cells; and wherein mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without forming embryoid bodies.

[00010b] In another aspect, the present invention refers to an *ex vivo* method for directing differentiation of pluripotent stem cells to cells of hematopoietic lineage, comprising: (i) contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, and a BMP activator, to obtain mesodermal cells; (ii) contacting the mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to obtain hemogenic endothelium; and (iii) contacting the hemogenic endothelium with a composition comprising one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; and a BMP activator, to obtain definitive HSCs, wherein the composition is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors.

[00010c] In another aspect, the present invention refers to an *ex vivo* method for generating pluripotent stem cell-derived T lineage cells comprising: (I) contacting pluripotent stem cell-derived T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators, to obtain T cells; wherein the composition is free of BMP activators; wherein the pluripotent stem cell-derived T cell progenitors are obtained by contacting pluripotent stem cell-derived definitive HSCs with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; and one or more Notch pathway activators to allow differentiation

and expansion; wherein the pluripotent stem cell-derived definitive HSCs are obtained by contacting pluripotent stem cell-derived hemogenic endothelium with a composition comprising a BMP activator; and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, to allow differentiation and expansion, wherein the composition is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors; wherein the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor to allow differentiation and expansion; and wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to allow differentiation and expansion; or (II) contacting pluripotent stem cell-derived pre-T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, to obtain pluripotent stem cell-derived T cell progenitors or T cells, wherein the composition is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; wherein the pluripotent stem cell-derived pre-T cell progenitors are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, to allow cell differentiation and expansion; wherein the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11, to allow cell differentiation and expansion; wherein the composition is free of TGFβ receptor/ALK inhibitor; wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor, and wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

[00010d] In yet another aspect, the present invention refers to an *ex vivo* method for generating pluripotent stem cell-derived NK lineage cells comprising: (I) contacting pluripotent stem cell-derived NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, to obtain pluripotent stem cell-derived NK cells, wherein the composition is free of BMP activators; wherein the pluripotent stem cell-derived NK cell progenitors are obtained by contacting pluripotent stem cell-derived definitive HSCs with a composition comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15, to allow cell differentiation and expansion; wherein the pluripotent stem cell-derived definitive HSCs are obtained by contacting pluripotent stem cell-derived hemogenic endothelium with a composition comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF and TPO, to allow cell differentiation and expansion, wherein the composition is free of Wnt pathway activators and TGFβ receptor/ALK inhibitors; wherein the pluripotent stem cell-derived hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to initiate differentiation of pluripotent stem cell-derived mesodermal cells to allow cell differentiation and expansion; and wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to allow cell differentiation and expansion; or (II) contacting pluripotent stem cell-derived pre-NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, to obtain pluripotent stem cell-derived NK cell progenitors or NK cells, wherein the composition is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; wherein the pluripotent stem cell-derived pre-NK cell progenitors are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7 and IL15, to allow cell differentiation and expansion; wherein pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK

inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11, to allow cell differentiation and expansion, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor; wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor; and wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

[00010e] In yet another aspect, the present invention refers to *ex vivo* method for generating pluripotent stem cell-derived definitive hemogenic endothelium, comprising: contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11, to obtain pluripotent stem cell-derived definitive hemogenic endothelium, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor; wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor; and wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

[00010f] In still another aspect, the present invention refers to an *ex vivo* method for generating pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage, comprising: contacting pluripotent stem cell-derived pre-HSC with a composition comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to obtain pluripotent stem cell-derived multipotent progenitors, wherein the composition is free of ROCK inhibitor; wherein the pluripotent stem cell-derived pre-HSC are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK

inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to allow cell differentiation and expansion; wherein the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor; wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor; wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor; and wherein contacting pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

[00010g] In still another aspect, the present invention refers to a culture platform for obtaining pluripotent stem cell-derived hematopoietic lineage cells, comprising: I: (i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding pluripotent stem cell-derived definitive HSCs from pluripotent stem cell-derived definitive hemogenic endothelium; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; or, II: a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF,

IL6, and IL11, wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, and wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential; a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, and wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in pluripotent stem cell-derived mesodermal cells; and a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells.

[00010h] In a further aspect, the present invention refers to a composition for obtaining and expanding pluripotent stem cell-derived hematopoietic cells, comprising one or more of: I: (i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for obtaining pluripotent stem cell-derived definitive HSCs from pluripotent stem cell-derived definitive hemogenic endothelium; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells, wherein the culture medium is suitable for obtaining pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium comprising a GSK3 inhibitor, a BMP activator; and iPSC, wherein the culture medium is suitable for obtaining pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; or, II: (i) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; and the culture medium is optionally free of TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells with definitive hemogenic endothelium potential, wherein the medium is suitable for obtaining pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells with definitive hemogenic endothelium potential; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the culture medium is free of TGFβ receptor/ALK inhibitor; and pluripotent

stem cell-derived mesodermal cells, wherein the medium is suitable for obtaining pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential from pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium that comprises a BMP activator, and optionally bFGF; and pluripotent stem cells, wherein the medium is suitable for obtaining pluripotent stem cell-derived mesodermal cells from pluripotent stem cells.

[00010i] In yet another aspect, the present invention refers to a culture platform for generating pluripotent stem cell-derived T lineage cells comprising: I: (i) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells drom pluripotent stem cells; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells; (iii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding pluripotent stem cell-derived definitive HSC from pluripotent stem cell-derived definitive hemogenic endothelium; and (iv) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3 and IL6, and one or more Notch pathway activators, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived T cell progenitors from pluripotent stem cell-derived definitive HSC; and optionally, (iii) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; wherein the culture medium is free of BMP activator, and wherein the culture medium is suitable for differentiating pluripotent stem cell-derived T cells from pluripotent stem cell-derived T cell progenitors or, II: a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive HE potential in the pluripotent stem cell-derived mesodermal cells; a culture medium

comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, wherein the medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells with hemogenic endothelium potential; (iv) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pluripotent stem cell-derived pre-T cell progenitor; and (v) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, wherein the culture medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-T cell progenitors to pluripotent stem cell-derived T cell progenitors or T cells.

[00010j] In still another aspect, the present invention refers to a culture platform for generating pluripotent stem cell-derived NK cells comprising: I: (i) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating pluripotent stem cells to pluripotent stem cell-derived mesodermal cells; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived mesodermal cells to pluripotent stem cell-derived definitive hemogenic endothelium; (iii) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pluripotent stem cell-derived definitive HSCs; and (iv) a culture medium comprises a BMP activator, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSCs to pluripotent stem cell-derived NK cell progenitors; and optionally, a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15,

wherein the culture medium is free of BMP activators, and wherein the culture medium is suitable for differentiating pluripotent stem cell-derived NK cell progenitors to pluripotent stem cell-derived NK cells; or, II: a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from ipluripotent stem cells; a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in the pluripotent stem cell-derived mesodermal cells; a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential; (iv) a medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pre-NK cell progenitor; and (v) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors and is suitable for differentiating pluripotent stem cell-derived pre-NK cell progenitors to pluripotent stem cell-derived NK cell progenitors or NK cells.

[00010k] In another aspect, the present invention refers to a culture platform for generating pluripotent stem cell-derived definitive hemogenic endothelium, comprising: (i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in the pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive

hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential.

[00010l] In still another aspect, the present invention refers to a culture platform for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors, comprising: (i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in the pluripotent stem cell-derived mesodermal cells; (iii) a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential; (iv) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pluripotent stem cell-derived pre-HSC; and (v) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, wherein the culture medium is free of ROCK inhibitor, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-HSC to pluripotent stem cell-derived multipotent progenitors.

[00010m] In still another aspect, the present invention refers to a composition comprising pluripotent stem cell-derived hematopoietic cells and one or more culture medium selected from the group consisting of: (i) CD34+ HE cells (iCD34), and one or more culture medium selected from iCD34-C, iMPP-A, iTC-A1, iTC-A2, iTC-B1, iTC-B2, iNK-A1, iNK-A2, iNK-B1 and iNK-B2; (ii) definitive hemogenic endothelium (iHE), and one or more culture medium selected

from iCD34-C, iMPP-A, iTC-A1, iTC-A2, iTC-B1, iTC-B2, iNK-A1, iNK-A2, iNK-B1 and iNK-B2; (iii) definitive HSCs, and one or more culture medium selected from iMPP-A, iTC-A1, iTC-A2, iTC-B1, iTC-B2, iNK-A1, iNK-A2, iNK-B1 and iNK B2; (iv) multipotent progenitor cells (iMPP), and iMPP-A; (v) T cell progenitors (iproT), and one or more culture medium selected from iTC-A1, iTC-A2, iTC-B1, and iTC-B2; (vi) T cells (iTC), and iTC-B1 or iTC-B2; (vii) NK cell progenitors (iproNK), and one or more culture medium selected from iNK-A1, iNK-A2, iNK-B1, and iNK-B2; and (viii) NK cells (iNK), and iNK-B1 or iNK-B2 (ix) HSC (iHSC), and iHSC-A, iHSC-B, and iHSC-C; wherein iHSC-A comprises a Wnt pathway activator, and a BMP activator; wherein iHSC-B comprises a Wnt pathway activator, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor; wherein iHSC-C comprises a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; wherein iTC-A1 comprises a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6, and one or more Notch pathway activators selected from the group consisting of Jag1, Jag2, DLL-1, DLL-3 and DLL-4; wherein iTC-B1 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6, and one or more Notch pathway activators selected from the group consisting of Jag1, Jag2, DLL-1, DLL-3 and DLL-4; (iHSC platform); wherein iNK-A1 comprises a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; wherein iNK-B1 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15; wherein iCD34-C comprises a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein iMPP-A comprises a MP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11; wherein iTC-A2 comprises a BMP activator, a ROCK inhibitor, VEGF, and bFGF; and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7; wherein iTC-B2 comprises one or more growth factors and cytokines selectedfrom the group consisting of SCF, Flt3L, and IL7; wherein iNK-A2 comprises a BMP activator, a ROCK inhibitor, VEGF, and bFGF, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL15; and wherein iNK-B2 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7 and IL15.

[00011] Provided herein is a monolayer differentiation platform that facilitates differentiation towards definitive hemogenic endothelium resulting in the derivation of hematopoietic stem cells and differentiated progeny such as T, B, NKT and NK cells. The demonstrated monolayer differentiation strategy combines enhanced differentiation efficiency with large-scale expansion enables the delivery of therapeutically relevant number of pluripotent stem cell-derived hematopoietic cells for various therapeutic applications. Further, the present disclosure disclosed that monolayer culturing using the methods provided herein leads to functional hematopoietic lineage cells that enable full range of *in vitro* differentiation, *ex vivo* modulation, and *in vivo* long term hematopoietic self-renewal, reconstitution and engraftment.

[00012] One example of the present disclosure provides a culture platform for obtaining pluripotent stem cell-derived hematopoietic lineage cells, which comprises: group I: (i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding definitive HSCs from definitive hemogenic endothelium; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating and expanding definitive hemogenic endothelium from mesodermal cells; and (iii) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating and expanding mesodermal cells from pluripotent stem cells.

[00013] Alternatively, the culture platform for obtaining pluripotent stem cell-derived hematopoietic lineage cells comprises group II: (i) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, and is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding definitive hemogenic endothelium from mesodermal cells having definitive hemogenic endothelium potential; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining mesodermal cell having definitive hemogenic endothelium potential; and (iii) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and

expanding mesodermal cells from pluripotent stem cells. In some embodiment, the pluripotent stem cells of the above culture platform are iPSCs. In some examples, the iPSCs are naïve iPSCs. In some embodiment, the Group (II) of the above culture platform further comprises: (iv) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells.

[00014] In some examples of the above culture platform, each of the Group (I) and (II) further comprises additional culture media.

[00015] Group (I) may further comprise: (i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; wherein the culture medium is free of BMP activator, and is suitable for differentiating pluripotent stem cell-derived T cell progenitors to T cells, or (ii) a culture medium comprising a BMP activator; one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; and one or more Notch pathway activators, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSC to T cell progenitors. These additional culture media are suitable for generating pluripotent stem cell-derived T lineage cells.

[00016] Group (II) may further comprise: (i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, but free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-T cell progenitors to T cell progenitor or T cells; or (ii) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pre-T cell progenitor; and these additional culture media are suitable for generating pluripotent stem cell-derived T lineage cells.

[00017] In some examples of the above culture platform, each of the Group (I) and (II) again further comprises additional culture media:

[00018] Group (I) may further comprise: (i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, wherein the culture medium is free of BMP activators, and is suitable for differentiating pluripotent stem cell-derived NK cell progenitors to NK cells; or (ii) a culture medium comprises a BMP activator; one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSC to NK cell progenitors; and these additional media are suitable for generating pluripotent stem cell-derived NK lineage cells.

[00019] As to Group (II), in addition to above mentioned media, it may further comprise: (i) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors and is suitable for differentiating pluripotent stem cell-derived pre-NK cell progenitors to NK cell progenitors or NK cells; or (ii) a medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pre-NK cell progenitors; and these additional media are suitable for generating pluripotent stem cell-derived NK lineage cells.

[00020] In yet another embodiment, the group (II) of the provided culture platform further comprises: (i) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, but free of ROCK inhibitor, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-HSCs to hematopoietic multipotent progenitors; (ii) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, a wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pre-HSCs; and these culture media are provided for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors.

[00021] Another example of the present disclosure provides a composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells, which comprises one or more of the following Group (I) or (II).

[00022] Group (I): (i) a culture medium comprising a BMP activator; one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding definitive HSCs from pluripotent stem cell-derived definitive hemogenic endothelium; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells, wherein the culture medium is suitable for differentiating and expanding definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium comprising a GSK3 inhibitor, a BMP activator; and iPSC, wherein the culture medium is suitable for differentiating and expanding mesodermal cells from pluripotent stem cells.

[00023] Group (II): (i) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; and pluripotent stem cell-derived mesodermal cells with definitive hemogenic endothelium potential, wherein the medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells with hemogenic endothelium potential; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, but free of TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells, wherein the medium is suitable for differentiating and expanding mesodermal cells having definitive hemogenic endothelium potential from pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium that comprises a BMP activator, and optionally bFGF; and iPSCs, wherein the medium is suitable for differentiating and expanding mesodermal cells from pluripotent stem cells.

[00024] In some examples of the composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells, the pluripotent stem cells are iPSCs. In some examples, the iPSCs are naïve iPSCs.

[00025] In some examples of the composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells, the Group (II) comprises an additional composition, such as (vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors; and pluripotent stem cells; wherein the medium is suitable for seeding and expanding the pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some examples, the iPSCs are naïve iPSCs.

[00026] In some examples of the above composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells, the Group (I) additionally comprises: (i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; and pluripotent stem cell-derived T cell progenitors, wherein the culture medium is free of BMP activator, and is suitable for differentiating pluripotent stem cell-derived T cell progenitors to T cells, or (ii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; one or more Notch pathway activators; and pluripotent stem cell-derived definitive HSC, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSCs to T cell progenitors; and these additional media are suitable for generating pluripotent stem cell-derived T lineage cells.

[00027] As to Group (II) of the above composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells, in some examples, it further comprises: (i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, but free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; and pluripotent stem cell-derived pre-T cell progenitors, wherein the culture medium is suitable for differentiating the pluripotent stem cell-derived pre-T cell progenitors to T cell progenitors or T cells; or (ii) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, and pluripotent stem cell-derived definitive hemogenic

endothelium, wherein the culture medium is suitable for differentiating the definitive hemogenic endothelium into pre-T cell progenitor. These additional media are suitable for generating pluripotent stem cell-derived T lineage cells.

[00028] In yet some other embodiment of the above composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells, Group (I) further comprises (i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15; and pluripotent stem cell-derived NK cell progenitors, wherein the culture medium is free of BMP activators, and is suitable for differentiating the NK cell progenitors to NK cells; or (ii) a culture medium comprises a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; and pluripotent stem cell-derived definitive HSC, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSC to NK cell progenitors. These additional media are suitable for generating pluripotent stem cell-derived NK lineage cells. Alternatively, Group (II) of the above composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells further comprises: (i) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; and pluripotent stem cell-derived pre-NK cell progenitors, wherein the medium is suitable for differentiating the pre-NK cell progenitors to NK cell progenitors or NK cells; or (ii) a medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the medium is suitable for differentiating the definitive hemogenic endothelium into pre-NK cell progenitors. These culture media are suitable for generating pluripotent stem cell-derived NK lineage cells.

[00029] In still some other embodiment, the group (II) of the above composition for differentiating and expanding pluripotent stem cell-derived hematopoietic cells further comprises one or more medium for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors, wherein the medium comprises: (i) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, but free of ROCK inhibitor, and

pluripotent stem cell-derived pre-HSC, wherein the culture medium is suitable for differentiating the pre-HSC to hematopoietic multipotent progenitors; and/or (ii) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is suitable for differentiating the definitive hemogenic endothelium to pre-HSC.

[00030] One example of the present disclosure provides a culture platform for generating pluripotent stem cell-derived T lineage cells, which comprises: group I-(i) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells drom pluripotent stem cells; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating and expanding definitive hemogenic endothelium from mesodermal cells; (iii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding definitive HSC from definitive hemogenic endothelium; and (iv) a culture medium comprising a BMP activator; one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; and one or more Notch pathway activators, wherein the culture medium is suitable for differentiating T cell progenitors from definitive HSC; and optionally, (v) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; wherein the culture medium is free of BMP activator, and is suitable for differentiating T cells from T cell progenitors.

[00031] Alternatively, the culture platform for generating pluripotent stem cell-derived T lineage cells comprises: group II-(i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining mesodermal cells having definitive HE potential from the mesodermal cells; (iii) a culture medium comprising a ROCK

inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding definitive hemogenic endothelium from the mesodermal cells with definitive hemogenic endothelium potential; (iv) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is suitable for differentiating the definitive hemogenic endothelium into pre-T cell progenitor; and (v) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, but free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; and pluripotent stem cell-derived pre-T cell progenitors, wherein the culture medium is suitable for differentiating the pre-T cell progenitors to T cell progenitors or T cells.

[00032] In some examples of the above culture platform for generating pluripotent stem cell-derived T lineage cells, the culture platform of group II further comprises: (vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is optionally free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some examples, the iPSCs are naïve iPSCs.

[00033] Another example of the present disclosure provides a culture platform for generating pluripotent stem cell-derived NK cells, which comprises: group I- (i) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating pluripotent stem cells to mesodermal cells; (ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating mesodermal cells to definitive hemogenic endothelium; (iii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating definitive hemogenic endothelium to definitive HSCs; (iv) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; wherein the culture medium is suitable for differentiating definitive HSCs to NK cell

progenitors; and optionally, (v) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, wherein the culture medium is free of BMP activators, and is suitable for differentiating NK cell progenitors to NK cells.

[00034] Alternatively, the culture platform for generating pluripotent stem cell-derived NK cells comprises: group II- (i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding mesodermal cells from pluripotent stem cells; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining mesodermal cells having definitive hemogenic endothelium potential from mesodermal cells; (iii) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating definitive hemogenic endothelium from mesodermal cells having definitive hemogenic endothelium potential; (iv) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is suitable for differentiating definitive hemogenic endothelium into pre-NK cell progenitor; and (v) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors and is suitable for differentiating pre-NK cell progenitors to NK cell progenitors or NK cells.

[00035] In some embodiment of the above culture platform for generating pluripotent stem cell-derived NK cells, , the culture platform of group II further comprises: (vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some examples, the iPSCs are naïve iPSCs.

[00036] Yet another example of the present disclosure provides a culture platform for generating pluripotent stem cell-derived definitive hemogenic endothelium (iHE), which

comprises: (i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding mesodermal cells from pluripotent stem cells; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining mesodermal cells having definitive hemogenic endothelium potential from the pluripotent stem cell-derived mesodermal cells; and (iii) a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, IL11, wherein the medium is optionally free of TGFβ receptor/ALK inhibitor, and wherein the medium is suitable for differentiating and expanding definitive hemogenic endothelium from the mesodermal cells having definitive hemogenic endothelium potential.

[00037] In some examples of the culture platform for generating pluripotent stem cell-derived definitive hemogenic endothelium (iHE), the culture platform further comprises (iv) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells.

[00038] Still another example of the disclosure provides a culture platform for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors, which comprises: (i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; (ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining mesodermal cells having definitive hemogenic endothelium potential from the pluripotent stem cell-derived mesodermal cells; (iii) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding definitive hemogenic endothelium from mesodermal cells having definitive hemogenic endothelium potential; (iv) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11; wherein the culture medium is suitable for differentiating definitive hemogenic endothelium to pre-HSC; and (v) a culture medium comprising a BMP

activator, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, wherein the medium is free of ROCK inhibitor, wherein the culture medium is suitable for differentiating pre-HSC to hematopoietic multipotent progenitors. In some examples, the culture platform further comprises (vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some examples, the iPSCs are naïve iPSCs.

[00039] Another example of the disclosure provides a method for directing differentiation of pluripotent stem cells into cells of a definitive hematopoietic lineage, which comprises: group I- (i) contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to initiate differentiation and expansion of pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; (ii) contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of pluripotent stem cell-derived definitive hemogenic endothelium cell from the pluripotent stem cell-derived mesodermal cellss; and (iii) contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the composition is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, to initiate differentiation and expansion of the pluripotent stem cell-derived definitive HSCs from hemogenic endothelium cell.

[00040] Alternatively, the method for directing differentiation of pluripotent stem cells into cells of a definitive hematopoietic lineage comprises: group II- (i) contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to initiate differentiation and expansion of mesodermal cells from the pluripotent stem cells; (ii) contacting the mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of mesodermal cells having definitive HE potential from the mesodermal cells; (iii) contacting the mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, one or more growth factors and cytokines selected

from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the composition is optionally free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential; and optionally, subjecting pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, mesodermal cells having hemogenic endothelium, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%.

[00041] In some examplesof the method for directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage, the method of group (II) further comprises contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, wherein the composition is free of TGFβ receptor/ALK inhibitors, to seed and expand the pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some examples, the iPSCs are naïve iPSCs.

[00042] In some examples of the method for directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage, the differentiation of the pluripotent stem cells into cells of hematopoietic lineage is void of generation of embryoid bodies, and is in a monolayer culturing form.

[00043] In some examples of the above method, the obtained pluripotent stem cell-derived definitive hemogenic endothelium cells are CD34+. In some examples, the obtained definitive hemogenic endothelium cells are CD34+CD43-. In some examples, the definitive hemogenic endothelium cells are CD34+CD43-CXCR4-CD73-.

[00044] In some other examples of the above method, group I further comprises (i) contacting pluripotent stem cell-derived definitive HSCs with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6, and one or more Notch pathway activators, to initiate the differentiation of the pluripotent stem cell-derived definitive HSCs to T cell progenitors, and optionally, contacting the T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators, to initiate the differentiation of the T cell progenitors to T cells. In some examples, group II of the method further comprises (i) contacting pluripotent

stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, to initiate the differentiation of the definitive hemogenic endothelium to pre-T cell progenitors; and optionally, (ii) contacting the pre-T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, but free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, to initiate the differentiation of the pre-T cell progenitors to T cell progenitors or T cells. In some examples of the method, the pluripotent stem cell-derived T cell progenitors are CD34+CD7+.

[00045] In yet some other examples of the above method for directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage, group I of the method further comprises: (i) contacting pluripotent stem cell-derived definitive HSCs with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15, to initiate differentiation of the definitive HSC to NK cell progenitors; and optionally, (ii) contacting the NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, but free of BMP activators, to initiate differentiation of the NK cell progenitors to NK cells; or group II of the method further comprises: (i) contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, a BMP activator, to initiate differentiation of the definitive hemogenic endothelium to pre-NK cell progenitor; and optionally, (ii) contacting pluripotent stem cells-derived pre-NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, to initiate differentiation of the pre-NK cell progenitors to NK cell progenitors or NK cells. In some examples, group II of the method further comprises: (i) contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to initiate

differentiation of definitive hemogenic endothelium to pre-HSCs; (ii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, but free of ROCK inhibitor, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-HSC to hematopoietic multipotent progenitors. In some examples, the pluripotent stem cell-derived definitive HSCs obtained using the above method are CD34+CD45+, and are suitable for long-term engraftment.

[00046] Another example of the disclosure provides a method for generating pluripotent stem cell-derived T lineage cells, which comprises: group I- (i) contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to initiate differentiation and expansion of mesodermal cells from pluripotent stem cells; (ii) contacting mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of definitive hemogenic endothelium from the mesodermal cells; (iii) contacting definitive hemogenic endothelium with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; wherein the composition is free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, to initiate differentiation and expansion of definitive HSCs from definitive hemogenic endothelium; and (iv) contacting definitive HSCs with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6, and one or more Notch pathway activators, to initiate differentiation of the definitive HSC to T cell progenitors; and optionally, (v) contacting the T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; one or more Notch pathway activators; but free of BMP activators, to initiating differentiation of T cell progenitors to T cells.

[00047] Alternatively, the method for generating pluripotent stem cell-derived T lineage cells comprises: group II-(i) contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to initiate differentiation and expansion of mesodermal cells from pluripotent stem cells; (ii) contacting the mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, but free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of the mesodermal cells having definitive HE potential

from the mesodermal cells; (iii) contacting mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the composition is free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of definitive hemogenic endothelium from mesodermal cells having definitive HE potential; (iv) contacting definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, to initiate differentiation of the definitive hemogenic endothelium to pre-T cell progenitors; and (v) contacting the pre-T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, wherein the composition is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; to initiate differentiation of the pre-T cell progenitors to T cell progenitors or T cells; and optionally, the seeded pluripotent stem cells, mesodermal cells, mesodermal cells having definitive HE potential, and/or definitive hemogenic endothelium may be subject to low oxygen tension between about 2% to about 10%. In some examples, group II of the above method further comprises: contacting iPSCs with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, but free of TGFβ receptor/ALK inhibitors, to seed and expand pluripotent stem cells; and/or wherein the pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some embidiments, the iPSCs are naïve iPSC. In some examples of the method, the differentiation of the pluripotent stem cells into T cell lineages is void of generation of embryoid bodies, and is in a monolayer culturing format.

[00048] Yet another example of the disclosure provides a method for generating pluripotent stem cell-derived NK lineage cells, which comprises: group I- (i) contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to initiate differentiation of the pluripotent stem cells to mesodermal cells; (ii) contacting the mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to initiate differentiation of mesodermal cells to definitive hemogenic endothelium; (iii) contacting the definitive hemogenic endothelium with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the composition is

free of Wnt pathway activators and TGFβ receptor/ALK inhibitors; to initiate differentiation of pluripotent stem cells-derived definitive hemogenic endothelium to definitive HSCs; and (iv) contacting definitive HSCs with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15 to initiate differentiation of definitive HSCs to NK cell progenitors; and optionally, (v) contacting pluripotent stem cell-derived NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, but free of BMP activators, to initiate differentiation NK cell progenitors to NK cells. Alternatively, the method for generating pluripotent stem cell-derived NK lineage cells comprises: group II- (i) contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to initiate differentiation and expansion of mesodermal cells from the pluripotent stem cells; (ii) contacting mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of mesodermal cells having definitive HE potential from mesodermal cells; (iii) contacting mesodermal cells having definitive HE potential with a composition comprising one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; and a ROCK inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells having definitive HE potential; (iv) contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, a BMP activator, and a ROCK inhibitor, to initiate differentiation of the pluripotent stem cell-derived definitive hemogenic endothelium to pre-NK cell progenitors; and (v) contacting pluripotent stem cell-derived pre-NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, but free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, to initiate differentiation of the pluripotent stem cell-derived pre-NK cell progenitors to pluripotent stem cell-derived NK cell progenitors or NK cells; and optionally, subjecting seeded pluripotent stem cells, pluripotent stem cell-derived-mesodermal cells, and/or definitive hemogenic endothelium under low oxygen tension

between about 2% to about 10%. In some examples, the method for generating pluripotent stem cell-derived NK lineage cells of group II further comprises contacting iPSCs with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, but free of TGFβ receptor/ALK inhibitors, to seed and expand the iPSCs. In some examples, the iPSCs are naïve iPSCs. In some examples, the method for generating pluripotent stem cell-derived NK lineage cells is void of generation of embryoid bodies, and is in a monolayer culturing format.

[00049] Another example of the disclosure provides a method for generating pluripotent stem cell-derived definitive hemogenic endothelium, comprising: (i) contacting iPSCs with a composition comprising a BMP activator, and optionally bFGF, to initiate differentiation and expansion of pluripotent stem cell-derived mesodermal cells from pluripotent stem cells; (ii) contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of pluripotent stem cell-derived mesodermal cells having definitive HE potential from pluripotent stem cell-derived mesodermal cells; (iii) contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; and a ROCK inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells having definitive HE potential; and optionally, subjecting seeded pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%. In some examples, the above method for generating pluripotent stem cell-derived definitive hemogenic endothelium, further comprises: contacting iPSCs with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, but free of TGFβ receptor/ALK inhibitors, to seed and expand the iPSCs; and/or wherein the iPSCs are naïve iPSCs. In some examples, the above method of differentiating iPSCs into cells of a definitive hemogenic endothelium is void of generation of embryoid bodies, and is in monolayer culturing format.

[00050] Another example of the disclosure provides a method for generating pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage, comprising: (i) contacting iPSCs with a composition comprising a BMP activator, and optionally bFGF, to initiate differentiation

and expansion of pluripotent stem cell-derived mesodermal cells from iPSCs; (ii) contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, but free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of the mesodermal cells having definitive HE potential from the mesodermal cells; (iii) contacting mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, wherein the composition is free of TGFβ receptor/ALK inhibitor, to initiate differentiation and expansion of definitive hemogenic endothelium from mesodermal cells having definitive HE potential; (iv) contacting definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to initiate differentiation of definitive hemogenic endothelium to pre-HSC; and (v) contacting pre-HSC with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, but free of ROCK inhibitor, to initiate differentiation of the pre-HSC to hematopoietic multipotent progenitors; and optionally, subjecting seeded pluripotent stem cells, mesodermal cells, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%. In some examples, the above method for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors further comprises contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, but free of TGFβ receptor/ALK inhibitors, to seed and expand the pluripotent stem cells. In some examples, the pluripotent stem cells are iPSCs. In some embodiments, the iPSCs are naïve iPSCs. In some examples, the differentiation of the pluripotent stem cells into hematopoiesis multipotent progenitors using the above method is void of generation of embryoid bodies, and is in monolayer culturing format.

[00051] A further example of the disclosure provides a composition comprising: one or more cell populations generated from the culture platform disclosed herein: pluripotent stem cells-derived (i) CD34+ definitive hemogenic endothelium (iCD34), wherein the iCD34 cells have capacity to differentiate into multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells and NK cells, and wherein the iCD34 cells are CD34+CD43-; (ii) definitive

hemogenic endothelium (iHE), wherein the iHE cells are CD34+; (iii) pluripotent stem cell-derived definitive HSCs, wherein the iHSC is CD34+CD45+; (iv) hematopoietic multipotent progenitor cells, wherein the iMPP cells are CD34+CD45+; (v) T cell progenitors, wherein the T cell progenitors are CD34+CD7+; (vi) T cells, wherein the T cells are CD4+ or CD8+; (vii) NK cell progenitors, wherein the NK cell progenitors are CD56+CD7+CD161+; and (viii) NK cells, wherein the NK cells are CD56+CD57+CD16+CD94-.

[00052] Still a further example of the disclosure provides one or more cell lines, or clonal cells generated using the methods disclosed herein: pluripotent stem cell-derived (i) CD34+ definitive hemogenic endothelium (iCD34), wherein the iCD34 cells have capacity to differentiate into multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells and NK cells, and wherein the iCD34 cells are CD34+CD43-; (ii) definitive hemogenic endothelium (iHE), wherein the iHE cell line or clonal cells are CD34+; (iii) definitive HSCs, wherein the iHSCs is CD34+CD45+; (iv) hematopoietic multipotent progenitor cells (iMPP), wherein the iMPP cells are CD34+CD45+; (v) T cell progenitors, wherein the T cell progenitors are CD34+CD7+; (vi) T cells, wherein the T cells are CD4+ or CD8+; (vii) NK cell progenitors, wherein the NK cell progenitors are CD56+CD7+CD161+; and (viii) NK cells, wherein the NK cells are CD56+CD57+CD16+CD94-.

[00053] Another example of the present disclosure provides a method of promoting hematopoietic self-renewal, reconstitution or engraftment using one or more of cell populations, cell lines or clonal cells generated using methods as disclosed: pluripotent stem cell-derived (i) CD34+ definitive hemogenic endothelium (iCD34), wherein the iCD34 cells have capacity to differentiate into multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells and NK cells, and wherein the iCD34 cells are CD34+CD43-; (ii) definitive hemogenic endothelium (iHE), wherein the iHE cell line or clonal cells are CD34+; (iii) definitive HSCs, wherein the iHSCs are CD34+CD45+; (iv) hematopoietic multipotent progenitor cells, wherein the iMPP cells are CD34+CD45+; (v) T cell progenitors, wherein the T cell progenitors are CD34+CD7+; (vi) T cells, wherein the T cells are CD4+ or CD8+; (vii) NK cell progenitors,

wherein the NK cell progenitors are CD56+CD7+CD161+; and (viii) NK cells, wherein the NK cells are CD56+CD57+CD16+CD94-.

[00054] In summary, the present disclosure provides methods and compositions enabling a direct differentiation of pluripotent stem cells in monolayer without generating embryoid bodies from pluripotent stem cells, thereby achieving differentiation and expansion of mesodermal cells, definitive HE, and definitive HSCs, from which other hematopoietic lineage cells can be obtained in a scalable, reliable manner with a very high level of efficiency.

BRIEF DESCRIPTION OF THE DRAWINGS

[00055] Fig. 1 shows an exemplary schematic diagram for a multi-staged process for the hematopoietic differentiation of human induced pluripotent stem cells (hiPSCs) to fully differentiated T cells.

[00056] Fig. 2 shows an exemplary schematic diagram for a multi-staged process for the hematopoietic differentiation of hiPSCs to fully differentiated NK cells.

[00057] Figs. 3A-3E show morphological changes over a 9 day course demonstrating the transition from hiPSCs towards hematopoietic cells.

[00058] Figs. 4A-4D show the expression profile of stem cells as they completely transition away from pluripotency towards a hematopoietic fate.

[00059] Figs. 5A-5C show CD34/ CD45 expression profile of hematopoietic cells differentiated from hiPSCs generated from various methods and starting input.

[00060] Fig. 6A-6C show improvement in CD34 differentiation efficiency when mediated as a monolayer in described differentiation culture media.

[00061] Figs. 7 shows survival and proliferation of hematopoietic differentiated cells under ROCK inhibition after single cell disassociation.

[00062] Fig. 8 shows maintenance of CD34 expression of hematopoietic cells differentiated from hiPSC after 22 days in culture.

[00063] Figs. 9A-9B show *in vivo* reconstitution of engrafted CD34 positive cells as seen with the presence of cells exclusively expressing human CD45 marker and reconstitution of CD34+ cells with the presence of both T and B cells. The CD34 positive cells are derived from naïve

CLAIMS

What is claimed is:

1. An *ex vivo* method for directing differentiation of pluripotent stem cells to hematopoietic lineage cells, comprising: (i) contacting the pluripotent stem cells with a composition comprising a BMP pathway activator, and optionally bFGF, to obtain mesodermal cells; and (ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential, wherein the mesodermal cells having definitive hemogenic endothelium (HE) potential are capable of providing hematopoietic lineage cells comprising hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, or B cells; and wherein mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without forming embryoid bodies.

2. The method of claim 1, further comprising: contacting the mesodermal cells having definitive HE potential with a composition comprising bFGF and a ROCK inhibitor, to obtain definitive HE cells.

3. The method of claim 2, further comprising: contacting the definitive HE cells with a composition comprising a BMP activator, and optionally a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11 to obtain hematopoietic multipotent progenitor cells (MPP).

4. The method of claim 2, further comprising: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7; and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-T cell progenitors, T cell progenitors, and/or T cells.

5. The method of claim 2, further comprising: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7 and IL15, and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, and/or NK cells.

6. The method of claim 1, further comprising: contacting the pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells.

7. The method of claim 1, further comprising: subjecting the pluripotent stem cells, the mesodermal cells, and/or the mesodermal cells having definitive hemogenic endothelium potential under low oxygen tension between about 2% to about 10%.

8. The method of claim 1, wherein the pluripotent stem cell differentiation to hematopoietic lineage cells is free of, or essentially free of, TGFβ receptor/ALK inhibitors.

9. The method of claim 1, wherein the pluripotent stem cell differentiation to hematopoietic lineage cells is under feeder-free condition.

10. The method of claim 1, wherein the pluripotent stem cell differentiation to hematopoietic lineage cells is under stromal-free condition.

11. The method of claim 1, wherein the pluripotent stem cell comprises induced pluripotent stem cell (iPSC).

12. The method of claim 11, wherein the iPSC is naïve iPSC.

13. The method of claim 1, wherein the WNT pathway activator is a GSK3 inhibitor.

14. The method of claim 13, wherein the GSK3 inhibitor is CHIR99021.

15. The method of claim 1, wherein the BMP pathway activator is BMP4.

16. The method of claim 2, wherein the ROCK inhibitor is Thiazovivin or Y-27632.

17. The method of claim 2, wherein the ROCK inhibitor is Y-27632.

18. An *ex vivo* method for directing differentiation of pluripotent stem cells to cells of hematopoietic lineage, comprising:

(i) contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, and a BMP activator, to obtain mesodermal cells;

(ii) contacting the mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to obtain hemogenic endothelium; and

(iii) contacting the hemogenic endothelium with a composition comprising one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; and a BMP activator, to obtain definitive HSCs, wherein the composition is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors.

19. The method of claim 18, further comprising:

(iv) contacting definitive HSCs with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; a BMP activator, and one or more Notch pathway activators, to obtain T cell progenitors, and optionally,

(v) contacting T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators, to obtain T cells;

or

(vi) contacting definitive HSCs with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; and a BMP activator, to obtain NK cell progenitors; and optionally,

(vii) contacting NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, to obtain NK cells, wherein the composition is free of BMP activators.

20. The method of claim 18, wherein the definitive HSCs are CD34+CD45+.

21. The method of claim 20, wherein the definitive HSCs are suitable for long-term engraftment.

22. The method of claim 19, wherein the T cell progenitors are CD34+CD7+.

23. The method of claim 19, wherein the NK cell progenitors are CD56+CD7+CD161+.

24. The method of claim 18, wherein directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage is void of generation of embryoid bodies.

25. The method of claim 18, wherein directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage is under monolayer culturing.

26. The method of claim 18, wherein directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage is under feeder-free condition.

27. The method of claim 18, wherein directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage is under stromal-free condition.

28. The method of claim 18, wherein the pluripotent stem cells are iPSCs.

29. The method of claim 28, where in the iPSCs are naïve iPSCs.

30. An *ex vivo* method for generating pluripotent stem cell-derived T lineage cells comprising:

(I) contacting pluripotent stem cell-derived T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators, to obtain T cells; wherein the composition is free of BMP activators;

wherein the pluripotent stem cell-derived T cell progenitors are obtained by contacting pluripotent stem cell-derived definitive HSCs with a composition comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; and one or more Notch pathway activators to allow differentiation and expansion;

wherein the pluripotent stem cell-derived definitive HSCs are obtained by contacting pluripotent stem cell-derived hemogenic endothelium with a composition comprising a

BMP activator; and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, to allow differentiation and expansion, wherein the composition is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors;

wherein the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor to allow differentiation and expansion; and

wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to allow differentiation and expansion;

Or,

(II) contacting pluripotent stem cell-derived pre-T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, to obtain pluripotent stem cell-derived T cell progenitors or T cells, wherein the composition is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors;

wherein the pluripotent stem cell-derived pre-T cell progenitors are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, to allow cell differentiation and expansion;

wherein the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11, to allow cell differentiation and expansion; wherein the composition is free of TGFβ receptor/ALK inhibitor;

wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor, and

wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

31. The method of claim 30, further comprising:

contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor to seed and expand the pluripotent stem cells, wherein the composition is free of TGFβ receptor/ALK inhibitors.

32. The method of claim 30, wherein generating pluripotent stem cell-derived T lineage cells is void of generation of embryoid bodies.

33. The method of claim 30, wherein generating pluripotent stem cell-derived T lineage cells is under monolayer culturing.

34. The method of claim 30, wherein generating pluripotent stem cell-derived T lineage cells is under feeder-free condition.

35. The method of claim 30, wherein generating pluripotent stem cell-derived T lineage cells is under stromal-free condition.

36. The method of claim 30, wherein the pluripotent stem cells are iPSCs.

37. The method of claim 36, where in the iPSCs are naïve iPSCs.

38. An *ex vivo* method for generating pluripotent stem cell-derived NK lineage cells comprising:

(I) contacting pluripotent stem cell-derived NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, to obtain pluripotent stem cell-derived NK cells, wherein the composition is free of BMP activators;

wherein the pluripotent stem cell-derived NK cell progenitors are obtained by contacting pluripotent stem cell-derived definitive HSCs with a composition comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15, to allow cell differentiation and expansion;

wherein the pluripotent stem cell-derived definitive HSCs are obtained by contacting pluripotent stem cell-derived hemogenic endothelium with a composition comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF and TPO, to allow cell differentiation and expansion, wherein the composition is free of Wnt pathway activators and TGFβ receptor/ALK inhibitors;

wherein the pluripotent stem cell-derived hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, to initiate differentiation of pluripotent stem cell-derived mesodermal cells to allow cell differentiation and expansion; and

wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a GSK3 inhibitor, a BMP activator, to allow cell differentiation and expansion;

or

(II) contacting pluripotent stem cell-derived pre-NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, to obtain pluripotent stem cell-derived NK cell progenitors or NK cells, wherein the composition is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors;

wherein the pluripotent stem cell-derived pre-NK cell progenitors are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7 and IL15, to allow cell differentiation and expansion;

wherein pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11, to allow cell differentiation and expansion, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor;

wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor; and

wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

39. The method of claim 38, further comprising subjecting seeded pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, mesodermal cells with hemogenic endothelium potential, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%.

40. The method of claim 38, further comprising contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells, wherein the composition is free of TGFβ receptor/ALK inhibitors.

41. The method of claim 38, wherein generating pluripotent stem cell-derived NK lineage cells is void of generation of embryoid bodies.

42. The method of claim 38, wherein generating pluripotent stem cell-derived NK lineage cells is under monolayer culturing.

43. The method of claim 38, wherein generating pluripotent stem cell-derived NK lineage cells is under feeder-free condition.

44. The method of claim 38, wherein generating pluripotent stem cell-derived NK lineage cells is under stromal-free condition.

45. The method of claim 38, wherein the pluripotent stem cells are iPSCs.

46. The method of claim 45, where in the iPSCs are naïve iPSCs.

47. An *ex vivo* method for generating pluripotent stem cell-derived definitive hemogenic endothelium, comprising:

contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11, to obtain pluripotent stem cell-derived definitive hemogenic endothelium, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor;

wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is optionally free of TGFβ receptor/ALK inhibitor; and

wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

48. The method of claim 47, further comprising:

contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the pluripotent stem cells, wherein the composition is free of TGFβ receptor/ALK inhibitors.

~~49. The method of claim 47 and 48, further comprising subjecting seeded pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, mesodermal cells having definitive hemogenic endothelium potential, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%.~~

49. The method of claim 47, wherein generating pluripotent stem cell-derived definitive hemogenic endothelium is void of generation of embryoid bodies.

50. The method of claim 47, wherein generating pluripotent stem cell-derived definitive hemogenic endothelium is under monolayer culturing.

51. The method of claim 47, wherein generating pluripotent stem cell-derived definitive hemogenic endothelium is under feeder-free condition.

52. The method of claim 47, wherein generating pluripotent stem cell-derived definitive hemogenic endothelium is under stromal-free condition.

53. The method of claim 47, wherein the pluripotent stem cells are iPSCs.

54. The method of claim 53, where in the iPSCs are naïve iPSCs.

55. An *ex vivo* method for generating pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage, comprising:

contacting pluripotent stem cell-derived pre-HSC with a composition comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to obtain pluripotent stem cell-derived multipotent progenitors, wherein the composition is free of ROCK inhibitor;

wherein the pluripotent stem cell-derived pre-HSC are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to allow cell differentiation and expansion;

wherein the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor;

wherein the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell

differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor;

wherein the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, wherein the composition is free of TGFβ receptor/ALK inhibitor; and

wherein contacting pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

56. The method of claim 55, further comprising:

contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the pluripotent stem cells, wherein the composition is free of TGFβ receptor/ALK inhibitors.

~~57. The method of claim 56 and 57, further comprising subjecting seeded pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, mesodermal cells having definitive hemogenic endothelium potential, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%.~~

~~58. The method of claim 58, wherein pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage is void of generation of embryoid bodies.~~

~~59. The method of claim 58, wherein pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage is under monolayer culturing.~~

~~60. The method of claim 58, wherein pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage is under feeder-free condition.~~

~~61. The method of claim 58, wherein pluripotent stem cell-derived multipotent progenitors of hematopoietic lineage is under stromal-free condition.~~

~~62. The method of claim 58, wherein the pluripotent stem cells are iPSCs.~~

~~63. The method of claim 63, wherein the iPSCs are naïve iPSCs.~~

~~64. A method of promoting hematopoietic self-renewal, reconstitution or engraftment using a composition comprising one or more of cell populations, cell lines or clonal cells generated using methods of claims 1-64, wherein the cell populations, cell lines or clonal cells are selected from:~~

~~(i) pluripotent stem cell-derived CD34+ definitive hemogenic endothelium (iCD34 HE), wherein the iCD34 cells have capacity to differentiate into multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells and NK cells, and wherein the iCD34 cells are CD34+CD43-;~~

~~(ii) pluripotent stem cell-derived definitive hemogenic endothelium (iHE), wherein the iHE cell line or clonal cells are CD34+;~~

~~(iii) pluripotent stem cell-derived definitive HSCs, wherein the iHSCs are CD34+CD45+;~~

~~(iv) pluripotent stem cell-derived multipotent progenitor cells, wherein the iMPP cells are CD34+CD45+;~~

~~(v) pluripotent stem cell-derived T cell progenitors, wherein the T cell progenitors are CD34+CD7+;~~

~~(vi) pluripotent stem cell-derived T cells, wherein the T cells are CD4+ or CD8+;~~

~~(vii) pluripotent stem cellderived NK cell progenitors, wherein the NK cell progenitors are CD56+CD7+CD161+; and~~

~~(viii) pluripotent stem cell-derived NK cells, wherein the NK cells are CD56+CD57+CD16+CD94-.~~

57. A culture platform for obtaining pluripotent stem cell-derived hematopoietic lineage cells, comprising:

I:

(i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the culture medium is optionally free of Wnt pathway activators and

TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding pluripotent stem cell-derived definitive HSCs from pluripotent stem cell-derived definitive hemogenic endothelium;

(ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells; and

(iii) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells;

or, II:

(i) a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, and wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential;

(ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, and wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in pluripotent stem cell-derived mesodermal cells; and

(iii) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells.

58. The culture platform of claim 57, wherein Group II further comprising:

(iv) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, wherein the culture medium is free of TGFβ receptor/ALK inhibitors, and wherein the medium is suitable for seeding and expanding pluripotent stem cells.

59. The culture platform of claim 57, further comprising:

I:

(i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; wherein the culture medium is free of BMP activator, and is suitable for differentiating pluripotent stem cell-derived T cell progenitors to pluripotent stem cell-derived T cells, or

(ii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; and one or more Notch pathway activators, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSC to pluripotent stem cell-derived T cell progenitors; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived T lineage cells;

Or II:

(i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, wherein the culture medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, and wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-T cell progenitors to pluripotent stem cell-derived T cell progenitor or T cells; or

(ii) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L and IL7, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pluripotent stem cell-derived pre-T cell progenitor; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived T lineage cells.

60. The culture platform of claim 57, further comprising:

I:

(i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, wherein the culture medium is free of BMP activators, and is suitable for differentiating pluripotent stem cell-derived NK cell progenitors to pluripotent stem cell-derived NK cells; or

(ii) a culture medium comprises a BMP activator, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSC to pluripotent stem cell-derived NK cell progenitors; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived NK lineage cells;

Or II:

(i) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors and is suitable for differentiating pluripotent stem cell-derived pre-NK cell progenitors to pluripotent stem cell-derived NK cell progenitors or NK cells; or

(ii) a medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7 and IL15, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pre-NK cell progenitors; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived NK lineage cells.

61. The culture platform of claim 57, wherein group (II) further comprises:

(i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, wherein the culture medium is free of ROCK inhibitor, and wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-HSCs to pluripotent stem cell-derived multipotent progenitors; or

(ii) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pluripotent stem cell-derived pre-HSCs; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors.

62. The culture platform of claim 57, wherein the pluripotent stem cells are iPSCs.

63. The culture platform of claim 62, wherein the iPSCs are naïve iPSCs.

64. A composition for obtaining and expanding pluripotent stem cell-derived hematopoietic cells, comprising one or more of:

I:

(i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for obtaining pluripotent stem cell-derived definitive HSCs from pluripotent stem cell-derived definitive hemogenic endothelium;

(ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells, wherein the culture medium is suitable for obtaining pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells; and

(iii) a culture medium comprising a GSK3 inhibitor, a BMP activator; and iPSC, wherein the culture medium is suitable for obtaining pluripotent stem cell-derived mesodermal cells from pluripotent stem cells;

or, II:

(i) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; and the culture medium is optionally free of TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells with definitive hemogenic endothelium potential, wherein the medium is suitable for obtaining pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells with definitive hemogenic endothelium potential;

(ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the culture medium is free of TGFβ receptor/ALK inhibitor; and pluripotent stem cell-derived mesodermal cells, wherein the medium is suitable for obtaining pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential from pluripotent stem cell-derived mesodermal cells; and

(iii) a culture medium that comprises a BMP activator, and optionally bFGF; and pluripotent stem cells, wherein the medium is suitable for obtaining pluripotent stem cell-derived mesodermal cells from pluripotent stem cells.

65. The composition of claim 64, wherein Group (II) further comprises:

(vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, wherein the culture medium is free of TGFβ receptor/ALK inhibitors; and pluripotent stem cells, wherein the medium is suitable for seeding and expanding pluripotent stem cells.

66. The composition of claim 64, further comprising:

I:

(i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; and pluripotent stem cell-derived T cell progenitors, wherein the culture medium is free of BMP activator, and wherein the culture medium is suitable for differentiating pluripotent stem cell-derived T cell progenitors to pluripotent stem cell-derived T cells, or

(ii) a culture medium comprising a BMP activator; one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6; one or more Notch pathway activators; and pluripotent stem cell-derived definitive HSC, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSCs to pluripotent stem cell-derived T cell progenitors; and wherein the culture medium is suitable for generating pluripotent stem cell-derived T lineage cells;

Or II:

(i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; and pluripotent stem cell-derived pre-T cell progenitors, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-T cell progenitors to pluripotent stem cell-derived T cell progenitors or T cells; or

(ii) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L and IL7; and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pluripotent stem cell-derived pre-T cell progenitor; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived T lineage cells.

67. The composition of claim 64, further comprising:

I:

(i) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15; and pluripotent stem cell-derived NK cell progenitors, wherein the culture medium is free of BMP activators, and is suitable for differentiating pluripotent stem cell-derived NK cell progenitors to pluripotent stem cell-derived NK cells; or

(ii) a culture medium comprises a BMP activator; one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and

IL15; and pluripotent stem cell-derived definitive HSC, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSC to pluripotent stem cell-derived NK cell progenitors; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived NK lineage cells;

Or II:

(i) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors; and pluripotent stem cell-derived pre-NK cell progenitors, wherein the medium is suitable for differentiating pluripotent stem cell-derived pre-NK cell progenitors to pluripotent stem cell-derived NK cell progenitors or NK cells; or

(ii) a medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pre-NK cell progenitors; and

wherein the culture medium is suitable for generating pluripotent stem cell-derived NK lineage cells.

68. The composition of claim 64, wherein group (II) further comprises one or more medium for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors, wherein the medium comprises:

(i) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, wherein the culture medium is free of ROCK inhibitor; and pluripotent stem cell-derived pre-HSC, wherein the culture medium is suitable for obtaining pluripotent stem cell-derived pre-HSC to pluripotent stem cell-derived multipotent progenitors; and/or

(ii) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF,

VEGF, SCF, IL6 and IL11; and pluripotent stem cell-derived definitive hemogenic endothelium, wherein the culture medium is suitable for obtaining pluripotent stem cell-derived definitive hemogenic endothelium to pluripotent stem cell-derived pre-HSC.

69. The composition of claim 64, wherein the pluripotent stem cells are iPSCs.

70. The composition of claim 69, wherein the iPSCs are naïve iPSCs.

71. A culture platform for generating pluripotent stem cell-derived T lineage cells comprising:

I:

(i) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells drom pluripotent stem cells;

(ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells;

(iii) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO, wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating and expanding pluripotent stem cell-derived definitive HSC from pluripotent stem cell-derived definitive hemogenic endothelium; and

(iv) a culture medium comprising a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3 and IL6, and one or more Notch pathway activators, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived T cell progenitors from pluripotent stem cell-derived definitive HSC; and optionally,

(iii) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6; and one or more Notch pathway activators; wherein the culture medium is free of BMP activator, and wherein the

culture medium is suitable for differentiating pluripotent stem cell-derived T cells from pluripotent stem cell-derived T cell progenitors;

Or, II:

(i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells;

(ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive HE potential in the pluripotent stem cell-derived mesodermal cells;

(iii) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, wherein the medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells with hemogenic endothelium potential;

(iv) a culture medium comprising a BMP activator, a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, and IL7, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pluripotent stem cell-derived pre-T cell progenitor; and

(v) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, wherein the culture medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-T cell progenitors to pluripotent stem cell-derived T cell progenitors or T cells.

72. The culture platform of claim 71, wherein Group (II) further comprising:

(vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is optionally free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells.

73. The culture platform of claim 71, wherein the pluripotent stem cells are iPSCs.

74. The culture platform of claim 73, wherein the iPSCs are naïve iPSCs.

75. A culture platform for generating pluripotent stem cell-derived NK cells comprising:

I:

(i) a culture medium comprising a GSK3 inhibitor, a BMP activator, wherein the culture medium is suitable for differentiating pluripotent stem cells to pluripotent stem cell-derived mesodermal cells;

(ii) a culture medium comprising a GSK3 inhibitor, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived mesodermal cells to pluripotent stem cell-derived definitive hemogenic endothelium;

(iii) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO; wherein the culture medium is optionally free of Wnt pathway activators and TGFβ receptor/ALK inhibitors, and is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pluripotent stem cell-derived definitive HSCs; and

(iv) a culture medium comprises a BMP activator, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15; wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive HSCs to pluripotent stem cell-derived NK cell progenitors; and optionally,

(iii) a culture medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15, wherein the culture medium is free of BMP activators, and wherein the culture medium is suitable for differentiating pluripotent stem cell-derived NK cell progenitors to pluripotent stem cell-derived NK cells;

Or, II:

(i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from ipluripotent stem cells;

(ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in the pluripotent stem cell-derived mesodermal cells;

(iii) a culture medium comprising a ROCK inhibitor, one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential;

(iv) a medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium into pre-NK cell progenitor; and

(v) a medium comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, BMP activators and ROCK inhibitors and is suitable for differentiating pluripotent stem cell-derived pre-NK cell progenitors to pluripotent stem cell-derived NK cell progenitors or NK cells.

76. The culture platform of claim 75, wherein Group (II) further comprising:

(vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells.

77. The culture platform of claim 75, wherein the pluripotent stem cells are iPSCs.

78. The culture platform of claim 77, wherein the iPSCs are naïve iPSCs.

79. A culture platform for generating pluripotent stem cell-derived definitive hemogenic endothelium, comprising:

(i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells;

(ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, and optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in the pluripotent stem cell-derived mesodermal cells; and

(iii) a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from the pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential.

80. The culture platform of claim 79, further comprising:

(iv) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and is free of TGFβ receptor/ALK inhibitors, wherein the medium is suitable for seeding and expanding pluripotent stem cells.

81. The culture platform of claim 79, wherein the pluripotent stem cells are iPSCs.

82. The culture platform of claim 81, wherein the iPSCs are naïve iPSCs.

83. A culture platform for generating pluripotent stem cell-derived hematopoiesis multipotent progenitors, comprising:

(i) a culture medium that comprises a BMP activator, and optionally bFGF, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived mesodermal cells from pluripotent stem cells;

(ii) a culture medium comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for obtaining definitive hemogenic endothelium potential in the pluripotent stem cell-derived mesodermal cells;

(iii) a culture medium comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11; wherein the culture medium is optionally free of TGFβ receptor/ALK inhibitor, wherein the medium is suitable for differentiating and expanding pluripotent stem cell-derived definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential;

(iv) a culture medium comprising a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived definitive hemogenic endothelium to pluripotent stem cell-derived pre-HSC; and

(v) a culture medium comprising a BMP activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, and IL11, wherein the culture medium is free of ROCK inhibitor, wherein the culture medium is suitable for differentiating pluripotent stem cell-derived pre-HSC to pluripotent stem cell-derived multipotent progenitors.

84. The culture platform of claim 83, further comprising:

(vi) a culture medium that comprises a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, wherein the culture medium is free of TGFβ receptor/ALK inhibitors, and wherein the medium is suitable for seeding and expanding pluripotent stem cells.

85. The culture platform of claim 83, wherein the pluripotent stem cells are iPSCs.

86. The culture platform of claim 85, wherein the iPSCs are naïve iPSCs.

87. A composition comprising pluripotent stem cell-derived hematopoietic cells and one or more culture medium selected from the group consisting of:

(i) CD34+ HE cells (iCD34), and one or more culture medium selected from iCD34-C, iMPP-A, iTC-A1, iTC-A2, iTC-B1, iTC-B2, iNK-A1, iNK-A2, iNK-B1 and iNK-B2;

(ii) definitive hemogenic endothelium (iHE), and one or more culture medium selected from iCD34-C, iMPP-A, iTC-A1, iTC-A2, iTC-B1, iTC-B2, iNK-A1, iNK-A2, iNK-B1 and iNK-B2;

(iii) definitive HSCs, and one or more culture medium selected from iMPP-A, iTC-A1, iTC-A2, iTC-B1, iTC-B2, iNK-A1, iNK-A2, iNK-B1 and iNK-B2;

(iv) multipotent progenitor cells (iMPP), and iMPP-A;

(v) T cell progenitors (iproT), and one or more culture medium selected from iTC-A1, iTC-A2, iTC-B1, and iTC-B2;

(vi) T cells (iTC), and iTC-B1 or iTC-B2;

(vii) NK cell progenitors (iproNK), and one or more culture medium selected from iNK-A1, iNK-A2, iNK-B1, and iNK-B2; and

(viii) NK cells (iNK), and iNK-B1 or iNK-B2

(ix) HSC (iHSC), and iHSC-A, iHSC-B, and iHSC-C;

wherein iHSC-A comprises a Wnt pathway activator, and a BMP activator;

wherein iHSC-B comprises a Wnt pathway activator, a BMP activator, and optionally, a TGFβ receptor/ALK inhibitor;

wherein iHSC-C comprises a BMP activator, and one or more growth factors and cytokines selected from the group consisting of VEGF, SCF, Flt3L, IL15, IL3, IL6, IGF, and TPO;

wherein iTC-A1 comprises a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL2, IL3, and IL6, and one or more Notch pathway activators selected from the group consisting of Jag1, Jag2, DLL-1, DLL-3 and DLL-4;

wherein iTC-B1 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IGF, IL2, IL3, and IL6, and one or more Notch pathway activators selected from the group consisting of Jag1, Jag2, DLL-1, DLL-3 and DLL-4; (iHSC platform);

wherein iNK-A1 comprises a BMP activator, one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, VEGF, IL2, IL3, IL6, and IL15;

wherein iNK-B1 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IGF, IL7, IL2, IL3, IL6, and IL15;

wherein iCD34-C comprises a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6, and IL11;

wherein iMPP-A comprises a BMP activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11;

wherein iTC-A2 comprises a BMP activator, a ROCK inhibitor, VEGF, and bFGF; and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7;

wherein iTC-B2 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7;

wherein iNK-A2 comprises a BMP activator, a ROCK inhibitor, VEGF, and bFGF, and one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7, IL15; and

wherein iNK-B2 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL7 and IL15.

88. The composition of claim 87, wherein iHSC-C is free of Wnt pathway activators and TGFβ receptor/ALK inhibitors; wherein iTC-A1 is free of VEGF and/or IL15; wherein iCD34-C is free of TGFβ receptor/ALK inhibitor; wherein iTC-B1 is free of BMP activator; and/or wherein iTC-B2 is free of on eor more of VEGF, bFGF, BMP activators, and ROCK inhibitors.