WHAT IS CLAIMED IS:

1. A method of preparing a population of hematopoietic stem cells (HSCs) comprising Long Term (LT)-HSCs, the method comprising:

providing a population comprising endothelial and/or hemogenic endothelial (HE) cells,

decreasing expression or activity of two or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells;

increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2 and mllt3 in the endothelial and/or HE cells, so as to stimulate formation of the HSCs including LT-HSCs.

2. The method of claim 1, comprising decreasing expression or activity of three or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells; and increasing expression or activity of three or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in the endothelial and/or HE cells, so as to stimulate formation and optionally expansion of the HSCs.

3. The method of claim 1, comprising decreasing expression or activity of vegfa, hey2, grp116, gna13, cdh5, and plxnd1 in the endothelial and/or HE cells; and increasing expression or activity of runx1, spi1, cebpa, tal1, and gata2 in the endothelial and/or HE cells, so as to stimulate formation and optionally expansion of the HSCs.

4. The method of any one of claims 1 to 3, wherein the increasing of activity or expression of the hematopoietic genes comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, and introducing a genetic modification of expression elements or introducing a gain-of-function mutation to the hematopoietic gene.

5. The method of any one of claims 1 to 4, wherein the decreasing of expression or activity of endothelial genes comprises one or more of: introducing a full or partial gene deletion, RNA silencing, antisense oligonucleotide inhibition, pharmacological

inhibition, and introducing a genetic modification of expression elements or introducing a loss-of-function mutation to the endothelial gene.

6. The method of any one of claims 1 to 3, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Dnmt3b at an effective level and duration.

7. The method of claim 6, wherein increasing the expression or activity of Dnmt3b comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, and introducing a genetic modification of expression elements or gain-of-function mutation to the Dnmt3b gene.

8. The method of any one of claims 1 to 3, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Gimap6 at an effective level and duration.

9. The method of claim 8, wherein increasing the expression or activity of Gimap6 comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or an episome, and introducing a genetic modification of expression elements or gain-of-function mutation to the Gimap6 gene.

10. The method of any one of claims 1 to 3, wherein the said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by contacting the endothelial cells or HE cells with 2D or 3D cyclic strain, an agonist of Piezo1 at an effective concentration and duration, or combinations thereof.

11. The method of claim 10, wherein the Piezo1 agonist is Yoda1, Jedi1, and/or Jedi2.

12. The method of claim 11, wherein the effective amount of the Piezo1 agonist is in the range of 0.1 to 500 uM, or in the range of 0.1 to 100 μM.

13. The method of claim 10, wherein the agonist of Piezo1 is identified in a chemical library, based on: decreasing expression or activity of endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in endothelial and/or

HE cells; and increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in endothelial and/or HE cells upon contact with a candidate compound.

14. The method of any one of claims 1 to 13, wherein the method comprises providing a population comprising embryonic bodies, endothelial cells, hemogenic endothelial (HE) cells , or combinations thereof to a bioreactor.

15. The method of claim 14, wherein the bioreactor provides a cyclic-strain biomechanical stretching, an agonist of Piezo1 at an effective concentration and duration, or combinations thereof.

16. The method of claim 15, wherein the cyclic-strain biomechanical stretching decreases expression or activity of three or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells; and increases expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in the endothelial and/or HE cells, so as to stimulate formation, and optionally expansion, of the HSCs.

17. The method of any one of claims 1 to 16, wherein the HSCs engraft in a hematopoietic niche and reconstitute to functional, multi-lineage adult blood.

18. The method of any one of claims 1 to 17, wherein HE cells are obtained from induced pluripotent stem cells (iPSCs), non-hematopoietic stem cells, somatic cells, or endothelial cells.

19. The method of any one of claims 1 to 18, wherein the hematopoietic stem cells comprise at least 1% long term hematopoietic stem cells (LT-HSCs), or at least 5% LT-HSCs.

20. The method of claim 19, wherein the hematopoietic stem cells comprise at least 0.1% long term hematopoietic stem cells (LT-HSCs).

21. The method of any one of claim 1 to 20, wherein the endothelial and/or HE cells are derived from HLA-modified or HLA-null cells, and/or transgene-free cells, gene-corrected, transgene-overexpressed, and are optionally derived by genetic or chemical induction of iPS cells or somatic cells.

22. The method of any one of claims 1 to 21, wherein source cells are obtained or derived from a subject, off-the-shelf library of cells, wherein the subject is optionally a universally compatible donor.

23. The method of claim 22, wherein the source cells are obtained or derived from a subject who has a blood, bone marrow, lysosomal storage, mitochondrial, metabolic, or immune disease.

24. The method of claim 23, wherein the subject does not have a hematological or non-hematological malignancy.

25. The method of any one of claims 1 to 24, further comprising, recovering and optionally expanding the HSCs.

26. The method of claim 25, wherein the population of HSCs are administered to a recipient, wherein the recipient is optionally the donor subject.

27. The method of claim 26, wherein at least about 10^2 HSCs are administered.

28. The method of claim 26, wherein at least about 10^3 HSCs are administered.

29. The method of claim 26, wherein at least about 10^4 HSCs are administered.

30. The method of claim 26, wherein at least about 10^5 HSCs are administered.

31. A method for transitioning a population of cells to hematopoietic endothelial (HE) cells, the method comprising:

providing a population comprising embryonic bodies or endothelial cells, and providing a genetic, pharmacological, and/or mechanical stimulus selected from one or more of:

decreasing expression or activity of one or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the cells; and increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2 and mllt3 in the cells;

applying a 2D or 3D cyclic strain, and

contacting the cells with an agonist of Piezo1 at an effective concentration and duration; so as to transition the cells to HE cells.

32. The method of claim 31, wherein the increasing of activity or expression of the hematopoietic genes comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements; and introducing gain-of-function mutation to the hematopoietic gene.

33. The method of claim 32, wherein the decreasing of expression or activity of endothelial genes comprises one or more of: introducing a full or partial gene deletion, RNA silencing; antisense oligonucleotide inhibition, pharmacological inhibition, introducing a genetic modification of expression elements, and introducing a loss-of-function mutation to the endothelial gene.

34. The method of claim 33 or 33, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Dnmt3b at an effective level and duration.

35. The method of claim 34, wherein increasing the expression or activity of Dnmt3b comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements, and incorporating a gain-of-function mutation to the Dnmt3b gene.

36. The method of any one of claims 32 to 35, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Gimap6 at an effective level and duration.

37. The method of claim 36, wherein increasing the expression or activity of Gimap6 comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or an episome, introducing a genetic modification of expression elements, and introducing gain-of-function mutation(s) to the Gimap6 gene.

38. The method of claim 31, wherein the cells are contacted with an effective amount of a Piezo1 agonist selected from Yoda1, Jedi1, and/or Jedi2.

39. The method of claim 38, wherein the effective amount of the Piezo1 agonist is in the range of 0.1 to 500 uM, or in the range of 0.1 to 100 μM.

40. The method of any one of claims 31 to 39, wherein the method comprises providing the population to a bioreactor, where the bioreactor provides a cyclic-strain biomechanical stretching.

41. The method of claim 40, wherein the HE cells are recovered, or are transitioned to HSCs, optionally by applying said genetic, pharmacological, and/or mechanical stimulus.

42. The method of claim 41, wherein the HE cells are transitioned to HSCs that engraft in a hematopoietic niche and reconstitute to functional, multi-lineage adult blood.

43. The method of any one of claims 31 to 42, wherein embryonic bodies or endothelial cells are derived from induced pluripotent stem cells (iPSCs).

44. The method of any one of claims 31 to 42, wherein the endothelial cells are derived from non-hematopoietic stem cells.

45. The method of any one of claims 31 to 44, wherein the HE cells are transitioned to hematopoietic stem cells comprising long term hematopoietic stem cells (LT-HSCs).

46. The method of any one of claims 31 to 45, wherein the population of cells are derived from HLA-modified or HLA-null cells.

47. The method of any one of claims 31 to 45, wherein the population of cells are transgene-free cells.

48. The method of any one of claims 31 to 47, further comprising expanding the HSCs in a process that comprises applying said genetic, pharmacological, and/or mechanical stimulus to the HSC cells.

49. The method of claim 48, wherein the HSCs are administered to a recipient, wherein the recipient is optionally the donor subject.

50. A method for expanding a population of hematopoietic stem cells (HSCs), the method comprising:

providing a population of HSCs, and providing a genetic, pharmacological, and/or mechanical stimulus selected from one or more of:

decreasing expression or activity of one or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the cells; and increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2 and mllt3 in the cells;

applying a 2D or 3D cyclic strain, and

contacting the cells with an agonist of Piezo1 at an effective concentration and duration; so as to expand the HSCs.

51. The method of claim 50, wherein the increasing of activity or expression of the hematopoietic genes comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements; and introducing gain-of-function mutation to the hematopoietic gene.

52. The method of claim 51, wherein the decreasing of expression or activity of endothelial genes comprises one or more of: introducing a full or partial gene deletion, RNA silencing, antisense oligonucleotide inhibition, pharmacological inhibition, introducing a genetic modification of expression elements, and introducing a loss-of-53 mutation to the endothelial gene.

53. The method of claim 51 or 52, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Dnmt3b at an effective level and duration.

54. The method of claim 53, wherein increasing the expression or activity of Dnmt3b comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements; and incorporating a gain-of-function mutation to the Dnmt3b gene.

55. The method of any one of claims 50 to 54, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Gimap6 at an effective level and duration.

56. The method of claim 55, wherein increasing the expression or activity of Gimap6 comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or an episome, introducing a genetic modification of expression elements, and introducing gain-of-function mutation(s) to the Gimap6 gene.

57. The method of claim 50, wherein the cells are contacted with an effective amount of a Piezo1 agonist selected from Yoda1, Jedi1, and/or Jedi2.

58. The method of claim 57, wherein the effective amount of the Piezo1 agonist is in the range of 0.1 to 500 uM, or in the range of 0.1 to 100 μM.

59. The method of any one of claims 50 to 58, wherein the method comprises providing the population to a bioreactor, where the bioreactor provides a cyclic-strain biomechanical stretching.

60. The method of claim 59, wherein the HSCs engraft in a hematopoietic niche and reconstitute to functional, multi-lineage adult blood.

61. The method of any one of claims 50 to 60, wherein the HSCs are transitioned from endothelial cells or HE cells.

62. The method of any one of claims 50 to 61, wherein the HSCs comprise long term hematopoietic stem cells (LT-HSCs).

63. The method of any one of claims 50 to 62, wherein the population of HSCs are derived from HLA-modified or HLA-null cells.

64. The method of claim 63, wherein the population of cells are transgene-free cells.

65. A pharmaceutical composition comprising a population of HSCs prepared by the method of any one of claims 1 to 64, and a pharmaceutically acceptable vehicle.

66. The pharmaceutical composition of claim 65, comprising at least 10^4 LT-HSC cells.

67. A method of treating a subject in need of hematopoietic stem cell therapy or transplantation, the method comprising administering to the subject a therapeutically

effective amount of hematopoietic stem cells (HSCs) prepared by the method of any one of claims 1 to 64 or administering the pharmaceutical composition of claims 65 or 66.

68. The method of claim 67, wherein the subject has malignant or non-malignant form of blood, bone marrow, lysosomal storage, mitochondrial, metabolic, or immune disease.

69. The method of claim 67 or 68, wherein the subject has a condition selected from acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia, a myeloproliferative disorder; myelodysplastic syndrome; multiple myeloma; non-Hodgkin lymphoma, Hodgkin disease, neuroblastoma, a germ cell tumor, or amyloidosis.

70. The method of claim 69, wherein the subject has a condition selected from an autoimmune disorder such as systemic lupus erythematosus (SLE) or systemic sclerosis; aplastic anemia; pure red-cell aplasia; paroxysmal nocturnal hemoglobinuria, Fanconi anemia; thalassemia major; sickle cell anemia; severe combined immunodeficiency (SCID); Wiskott-Aldrich syndrome; Hemophagocytic lymphohistiocytosis; inborn errors of metabolism; epidermolysis bullosa; severe congenital neutropenia; Shwachman-Diamond syndrome; Diamond-Blackfan anemia; Pearson Syndrome, and Leukocyte adhesion deficiency.

71. A method for making hematopoietic stem cells (HSCs), comprising:

contacting a panel of chemical compounds with embryonic bodies, endothelial cells and/or hemogenic endothelial cells, and determining a change in expression level induced by said chemical compounds of: Dnmt3b or Gimap6; at least two of vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln; and at least two of runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3;

selecting a compound that induces the following changes in gene expression:

increase in expression of Dnmt3b and/or Gimap6,

decrease in expression of two of more of vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln; and

increase in expression of two or more runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3; and

inducing the transition of endothelial cells and/or hemogenic endothelial cells to HSCs by contacting the selected compound with endothelial cells and/or hemogenic endothelial cells, thereby making self-renewing HSCs that can engraft and reconstitute multi-lineage adult blood.

72. The method of claim 71, wherein the selected compound decreases expression of five or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells; and increases expression of five or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in the endothelial and/or HE cells, so as to stimulate formation and optionally expansion of the HE cells, HSCs, or combinations thereof.

73. The method of claim 72, wherein the selected compound decreases expression of vegfa, hey2, grp116, gna13, cdh5, and plxnd1 in the endothelial and/or HE cells; and increases expression of runx1, spi1, cebpa, tal1, and gata2 in the endothelial and/or HE cells.

74. The method of any one of claims 71 to 73, wherein the selected compound increases the expression of Dnmt3b.

75. The method of any one of claims 71 to 73, wherein the selected compound is a Piezo1 agonist.

76. The method of claim 74, wherein the selected compound is a derivative of Yoda1, Jedi1, and/or Jedi2.

77. The method of any one of claims 71 to 76, wherein embryonic bodies, endothelial cells or HE cells are obtained from induced pluripotent stem cells (iPSCs), non-hematopoietic stem cells, somatic cells, or endothelial cells.

78. The method of any one of claims 71 to 76, wherein the hematopoietic stem cells obtained comprise at least 0.1% long term hematopoietic stem cells (LT-HSCs).

79. The method of claim 78, wherein the hematopoietic stem cells obtained comprise at least about 1% or at least about 10% long term hematopoietic stem cells (LT-HSCs).

80. The method of claim 78, wherein the hematopoietic stem cells obtained comprise from about 2% to about 25% LT-HSCs.

81. The method of any one of claims 71 to 80, wherein the endothelial and/or HE cells are derived from HLA-modified or HLA-null cells, transgene-overexpressed, and/or transgene-free cells, and are optionally derived by genetic or chemical induction of iPS cells or somatic cells.

82. The method of any one of claims 71 to 81, wherein source cells are obtained or derived from a subject, wherein the subject is optionally a patient, matched or unmatched donor, or a universally compatible donor.

83. The method of any one of claims 71 to 82, further comprising, recovering the HSCs.

84. A composition for cellular therapy produced according to this disclosure, and comprising at least about 10^3 or at least about 10^4 LT-HSC cells.

WHAT IS CLAIMED IS:

1. An *ex vivo* or *in vitro* method for transitioning a population of cells to hematopoietic endothelial (HE) cells, the method comprising:

providing a genetic, pharmacological, and/or mechanical stimulus to a population comprising embryonic bodies or endothelial cells, wherein the genetic, pharmacological, and/or mechanical stimulus is selected from one or more of:

decreasing expression or activity of one or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the cells; and increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2 and mllt3 in the cells;

applying a 2D or 3D cyclic strain, and

contacting the cells with an agonist of Piezo1 at an effective concentration and duration; so as to transition the cells to HE cells.

2. The method of claim 1, wherein the increasing of activity or expression of the hematopoietic genes comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements; and introducing gain-of-function mutation to the hematopoietic gene; optionally,

wherein the decreasing of expression or activity of endothelial genes comprises one or more of: introducing a full or partial gene deletion, RNA silencing; antisense oligonucleotide inhibition, pharmacological inhibition, introducing a genetic modification of expression elements, and introducing a loss-of-function mutation to the endothelial gene; and/or,

wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Dnmt3b at an effective level and duration; optionally,

wherein increasing the expression or activity of Dnmt3b comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements, and incorporating a gain-of-function mutation to the Dnmt3b gene; and/or,

wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Gimap6 at an effective level and duration; optionally,

wherein increasing the expression or activity of Gimap6 comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or an episome, introducing a genetic modification of expression elements, and introducing gain-of-function mutation(s) to the Gimap6 gene.

3. The method of claim 1, wherein the cells are contacted with an effective amount of a Piezo1 agonist selected from Yoda1, Jedi1, and/or Jedi2; optionally,

wherein the effective amount of the Piezo1 agonist is in the range of 0.1 to 500 uM, or in the range of 0.1 to 100 μM.

4. The method of any one of claims 1 to 3, wherein the method comprises providing the population to a bioreactor, where the bioreactor provides a cyclic-strain biomechanical stretching; optionally,

wherein the HE cells are recovered, or are transitioned to HSCs, optionally

by applying said genetic, pharmacological, and/or mechanical stimulus; optionally,

wherein the HE cells are transitioned to HSCs that engraft in a hematopoietic niche and reconstitute to functional, multi-lineage adult blood; and/or,

wherein embryonic bodies or endothelial cells are derived from induced pluripotent stem cells (iPSCs); or,

wherein the endothelial cells are derived from non-hematopoietic stem cells; and/or,

wherein the HE cells are transitioned to hematopoietic stem cells comprising long term hematopoietic stem cells (LT-HSCs), and/or,

wherein the population of cells are derived from HLA-modified or HLA-null cells; or, wherein the population of cells are transgene-free cells; and/or,

further comprising expanding the HSCs in a process that comprises applying said genetic, pharmacological, and/or mechanical stimulus to the HSC cells;

optionally,

wherein the HSCs are to be administered to a recipient, wherein the recipient is optionally the donor subject.

5. An *in vitro* or *ex vivo* method for expanding a population of hematopoietic stem cells (HSCs), the method comprising:

providing a genetic, pharmacological, and/or mechanical stimulus to a population of HSCs, wherein the genetic, pharmacological, and/or mechanical stimulus is selected from one or more of:

decreasing expression or activity of one or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the cells; and increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2 and mllt3 in the cells;

applying a 2D or 3D cyclic strain, and

contacting the cells with an agonist of Piezo1 at an effective concentration and duration; so as to expand the HSCs.

6. The method of claim 5, wherein the increasing of activity or expression of the hematopoietic genes comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements; and introducing gain-of-function mutation to the hematopoietic gene; optionally,

wherein the decreasing of expression or activity of endothelial genes comprises one or more of: introducing a full or partial gene deletion, RNA silencing, antisense oligonucleotide inhibition, pharmacological inhibition, introducing a genetic modification of expression elements, and introducing a loss-of-53 mutation to the endothelial gene; and/or,

wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Dnmt3b at an effective level and duration; optionally,

wherein increasing the expression or activity of Dnmt3b comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, introducing a genetic modification of expression elements; and

incorporating a gain-of-function mutation to the Dnmt3b gene; and/or,

wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Gimap6 at an effective level and duration; optionally,

wherein increasing the expression or activity of Gimap6 comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or an episome, introducing a genetic modification of expression elements, and introducing gain-of-function mutation(s) to the Gimap6 gene.

7. The method of claim 5, wherein the cells are contacted with an effective amount of a Piezo1 agonist selected from Yoda1, Jedi1, and/or Jedi2; optionally,

wherein the effective amount of the Piezo1 agonist is in the range of 0.1 to 500 uM, or in the range of 0.1 to 100 μM.

8. The method of any one of claims 5 to 7, wherein the method comprises providing the population to a bioreactor, where the bioreactor provides a cyclic-strain biomechanical stretching; optionally,

wherein the HSCs engraft in a hematopoietic niche and reconstitute to functional, multi-lineage adult blood; and/or

wherein the HSCs are transitioned from endothelial cells or HE cells; and/or,

wherein the HSCs comprise long term hematopoietic stem cells (LT-HSCs); and/or,

wherein the population of HSCs are derived from HLA-modified or HLA-null cells; optionally,

wherein the population of cells are transgene-free cells.

9. An *ex vivo* or *in vitro* method of preparing a population of hematopoietic stem cells (HSCs) comprising Long Term (LT)-HSCs, the method comprising:

decreasing expression or activity of two or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in a population of cells comprising endothelial and/or hemogenic endothelial (HE) cells;

increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2 and mllt3 in the endothelial and/or HE cells, so as to stimulate formation of the HSCs including LT-HSCs.

10. The method of claim 9, comprising decreasing expression or activity of three or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells; and increasing expression or activity of three or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in the endothelial and/or HE cells, so as to stimulate formation and optionally expansion of the HSCs or

comprising decreasing expression or activity of vegfa, hey2, grp116, gna13, cdh5, and plxnd1 in the endothelial and/or HE cells; and increasing expression or activity of runx1, spi1, cebpa, tal1, and gata2 in the endothelial and/or HE cells, so as to stimulate formation and optionally expansion of the HSCs.

11. The method of claim 9 or 10, wherein the increasing of activity or expression of the hematopoietic genes comprises one or more of: introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, and introducing a genetic modification of expression elements or introducing a gain-of-function mutation to the hematopoietic gene; and/or,

wherein the decreasing of expression or activity of endothelial genes comprises one or more of: introducing a full or partial gene deletion, RNA silencing, antisense oligonucleotide inhibition, pharmacological inhibition, and introducing a genetic modification of expression elements or introducing a loss-of-function mutation to the endothelial gene.

12. The method of any one of claims 9 to 11, wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Dnmt3b at an effective level and duration; optionally,

wherein increasing the expression or activity of Dnmt3b comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or episome, and introducing a genetic modification of expression elements or gain-of-function mutation to the Dnmt3b gene; or,

wherein said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by increasing the expression or activity of Gimap6 at an effective level and duration; optionally,

wherein increasing the expression or activity of Gimap6 comprises one or more of introducing an encoding mRNA or an mRNA derivative, introducing an encoding transgene or an episome, and introducing a genetic modification of expression elements or gain-of-function mutation to the Gimap6 gene; and/or,

wherein the said decreasing expression or activity of endothelial genes and said increasing expression or activity of hematopoietic genes is conducted by contacting the endothelial cells or HE cells with 2D or 3D cyclic strain, an agonist of Piezo1 at an effective concentration and duration, or combinations thereof; optionally,

wherein the Piezo1 agonist is Yoda1, Jedi1, and/or Jedi2; optionally,

wherein the effective amount of the Piezo1 agonist is in the range of 0.1 to 500 uM, or in the range of 0.1 to 100 μM; or,

wherein the agonist of Piezo1 is identified in a chemical library, based on: decreasing expression or activity of endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in endothelial and/or HE cells; and increasing expression or activity of two or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in endothelial and/or HE cells upon contact with a candidate compound; and/or,

wherein the method comprises providing a population comprising embryonic bodies, endothelial cells, hemogenic endothelial (HE) cells, or combinations thereof to a bioreactor; optionally

wherein the bioreactor provides a cyclic-strain biomechanical stretching, an agonist of Piezo1 at an effective concentration and duration, or combinations thereof; optionally,

wherein the cyclic-strain biomechanical stretching decreases expression or activity of three or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells; and increases expression or activity of two or more hematopoietic genes selected from runx1, spi1,

cebpa, tal1, gfi1, gata2, and mllt3 in the endothelial and/or HE cells, so as to stimulate formation, and optionally expansion, of the HSCs; and/or,

wherein the HSCs engraft in a hematopoietic niche and reconstitute to functional, multi-lineage adult blood; and/or,

wherein HE cells are obtained from induced pluripotent stem cells (iPSCs), non- hematopoietic stem cells, somatic cells, or endothelial cells and/or,

wherein the hematopoietic stem cells comprise at least 1% long term hematopoietic stem cells (LT-HSCs), or at least 5% LT-HSCs; optionally,

wherein the hematopoietic stem cells comprise at least 0.1% long term hematopoietic stem cells (LT-HSCs); and/or,

wherein the endothelial and/or HE cells are derived from HLA-modified or HLA-null cells, and/or transgene-free cells, gene-corrected, transgene-overexpressed, and are optionally derived by genetic or chemical induction of iPS cells or somatic cells; and/or,

wherein source cells are obtained or derived from a subject, off-the-shelf library of cells, wherein the subject is optionally a universally compatible donor; optionally,

wherein the source cells are obtained or derived from a subject who has a blood, bone marrow, lysosomal storage, mitochondrial, metabolic, or immune disease; optionally,

wherein the subject does not have a hematological or non-hematological malignancy; and/or, further comprising, recovering and optionally expanding the HSCs; optionally,

wherein the population of HSCs are to be administered to a recipient, wherein the recipient is optionally the donor subject; optionally,

wherein at least about 10^2 HSCs are to be administered, or,

wherein at least about 10^3 HSCs are to be administered, or,

wherein at least about 10^4 HSCs are to be administered, or,

wherein at least about 10^5 HSCs are to be administered.

13. A pharmaceutical composition comprising a population of HSCs prepared by the method of any one of claims 1 to 12, and a pharmaceutically acceptable vehicle; optionally, comprising at least 10^4 LT-HSC cells.

14. Use of the hematopoietic stem cells (HSCs) prepared by the method of any one of claims 1 to 12 or the pharmaceutical composition of claim 13 in the manufacture of a medicament for the treatment of hematopoietic stem cell therapy or transplantation in a subject in need thereof, wherein a therapeutically effective amount of said HSCs or said pharmaceutical composition is to be administered to the subject.

15. The use of claim 14, wherein the subject has malignant or non-malignant form of blood, bone marrow, lysosomal storage, mitochondrial, metabolic, or immune disease and/or,

wherein the subject has a condition selected from acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia, a myeloproliferative disorder; myelodysplastic syndrome; multiple myeloma; non-Hodgkin lymphoma, Hodgkin disease, neuroblastoma, a germ cell tumor, or amyloidosis; optionally,

wherein the subject has a condition selected from an autoimmune disorder such as systemic lupus erythematosus (SLE) or systemic sclerosis; aplastic anemia; pure red-cell aplasia; paroxysmal nocturnal hemoglobinuria, Fanconi anemia; thalassemia major; sickle cell anemia; severe combined immunodeficiency (SCID); Wiskott-Aldrich syndrome; Hemophagocytic lymphohistiocytosis; inborn errors of metabolism; epidermolysis bullosa; severe congenital neutropenia; Shwachman-Diamond syndrome; Diamond-Blackfan anemia; Pearson Syndrome, and Leukocyte adhesion deficiency.

16. An *ex vivo* or *in vitro* method for making hematopoietic stem cells (HSCs), comprising:

contacting a panel of chemical compounds with embryonic bodies, endothelial cells and/or hemogenic endothelial cells, and determining a change in expression level

induced by said chemical compounds of: Dnmt3b or Gimap6; at least two of vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln; and at least two of runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3;

selecting a compound that induces the following changes in gene expression: increase in expression of Dnmt3b and/or Gimap6,

decrease in expression of two of more of vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln; and

increase in expression of two or more runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3; and

inducing the transition of endothelial cells and/or hemogenic endothelial cells to HSCs by contacting the selected compound with endothelial cells and/or hemogenic endothelial cells, thereby making self-renewing HSCs that can engraft and reconstitute multi-lineage adult blood.

17. The method of claim 16, wherein the selected compound decreases expression of five or more endothelial genes selected from vegfa, hey2, grp116, gna13, sox17, cdh5, plxnd1, bcl6, and apln in the endothelial and/or HE cells; and increases expression of five or more hematopoietic genes selected from runx1, spi1, cebpa, tal1, gfi1, gata2, and mllt3 in the endothelial and/or HE cells, so as to stimulate formation and optionally expansion of the HE cells, HSCs, or combinations thereof; optionally,

wherein the selected compound decreases expression of vegfa, hey2, grp116, gna13, cdh5, and plxnd1 in the endothelial and/or HE cells; and increases expression of runx1, spi1, cebpa, tal1, and gata2 in the endothelial and/or HE cells; and/or,

wherein the selected compound increases the expression of Dnmt3b; optionally,

wherein the selected compound is a derivative of Yoda1, Jedi1, and/or Jedi2; or, wherein the selected compound is a Piezo1 agonist; and/or,

wherein embryonic bodies, endothelial cells or HE cells are obtained from induced pluripotent stem cells (iPSCs), non-hematopoietic stem cells, somatic cells, or endothelial cells; or, wherein the hematopoietic stem cells obtained comprise at least 0.1% long term hematopoietic stem cells (LT-HSCs); optionally,

wherein the hematopoietic stem cells obtained comprise at least about 1% or at

least about 10% long term hematopoietic stem cells (LT-HSCs); or,

wherein the hematopoietic stem cells obtained comprise from about 2% to about 25% LT-HSCs; and/or,

wherein the endothelial and/or HE cells are derived from HLA-modified or HLA-null cells, transgene-overexpressed, and/or transgene-free cells, and are optionally derived by genetic or chemical induction of iPS cells or somatic cells; and/or,

wherein source cells are obtained or derived from a subject, wherein the subject is optionally a patient, matched or unmatched donor, or a universally compatible donor; and/or,

further comprising, recovering the HSCs.