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(54) Title: COMPOSITIONS AND METHODS FOR ASSESSING, TREATING, OR REDUCING AGING-RELATED FUNCTIONAL DECLINE

(57) Abstract: Described herein are compositions and methods for reducing, improving, abrogating, or alleviating age- or aging-related functional decline in an older or aging subject by reducing the ratio or number of hematopoietic stem cells (HSCs) expressing high levels of the glycoprotein CD150 (CD150^{high} HSCs), which may be dysfunctional or defective, in the subject. The methods involve reducing or removing dysfunctional or defective high CD150^{high} HSCs from a sample obtained from a subject. Methods for administering to an old, aged, or aging subject having CD150^{high} HSCs a composition comprising hematopoietic stem cells expressing low levels of the glycoprotein CD150 (CD150^{low} HSCs) or reducing or removing CD150^{high} HSCs with an antibody-drug conjugate provide therapeutics that rejuvenate the differentiated hematopoietic cell types in the blood as well as improve the physical health of the aging subject to counter functional decline that occurs in aging HSCs.



COMPOSITIONS AND METHODS FOR ASSESSING, TREATING, OR REDUCING AGING-RELATED FUNCTIONAL DECLINE

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to and benefit of U.S. Provisional Application No. 63/472,759, filed on June 13, 2023, the entire contents of which are incorporated by reference herein.

BACKGROUND

10 Aging is a process accompanied by functional decline in tissues and organs with great social and medical consequences. Previous studies have demonstrated that aged hematopoietic stem cells (HSCs) are functionally compromised, which at least partly contributes to aging-related decline of the overall health of the body. However, the underlying mechanism of aging is largely unknown. Needed in the art are compositions and
15 methods for assessing, reducing, attenuating, mitigating, and/or abrogating the aging process. Such methods are important for the physical health and well-being of mammalian subjects, including humans, as described herein.

SUMMARY

20 Developing effective anti-aging strategies is of great significance. As described and demonstrated herein, transplantation of young hematopoietic stem cells (HSCs) into old mice can mitigate aging phenotypes, underscoring the crucial role played by HSCs in the aging process. Through comprehensive molecular and functional analyses, a subset of HSCs in aged mice was identified that exhibit “youthful” molecular profiles and functions, marked by
25 low expression levels of CD150 on HSCs (“CD150^{low} HSCs”). Mechanistically, CD150^{low} HSCs from old mice can effectively differentiate into downstream lineage cells, in contrast to their CD150^{high} counterparts. Notably, transplantation of old CD150^{low} HSCs attenuates aging phenotypes and prolongs lifespan of elderly mice compared to those transplanted with unselected or CD150^{high} HSCs. Reducing the dysfunctional CD150^{high} HSCs was able to
30 alleviate aging phenotypes in old recipient mice. Thus, as described herein, the presence of “youthful” HSCs is demonstrated in old mice, and rejuvenation of physical health and improvements in the aging process can be achieved by removal of the dysfunctional HSCs.

Featured and described herein are compositions comprising selected hematopoietic stem cells (HSCs) having a reduction in levels of CD150 relative to a reference, and methods

of using the same for administration, transplantation, or engraftment in a subject in need thereof. In an embodiment, the subject is an aging or aged subject. Compositions and methods for reducing and/or removing functionally defective HSCs are provided to reduce, abrogate, mitigate, and/or alleviate aging-related phenotypes in old or aged subjects, e.g.,
5 mammalian subjects, including humans.

Also featured and described herein are compositions and methods for ameliorating an old, aging, or aged phenotype, and/or rejuvenating old or aging mammalian subjects in need thereof, including human subjects, by targeted reduction, removal, depletion, or ablation of dysfunctional HSCs from a sample of the subject, such as a peripheral blood sample, bone
10 marrow, or a tissue preparation, *in vitro* or *ex vivo*, or by removal, depletion, or ablation of dysfunctional HSCs from the subject *in vivo*.

In an aspect, a method for characterizing hematopoietic stem cell (HSC) functionality is provided, in which the method involves detecting the level of CD150 polypeptide or polynucleotide in an HSC, wherein an alteration in the level of CD150 relative to a reference
15 is indicative of HSC functionality. In an embodiment of the method, a reduction in the level of the CD150 polypeptide or polynucleotide relative to the reference indicates that the HSC has increased self-renewal capacity and is capable of balanced differentiation.

In another aspect, a method for selecting a hematopoietic stem cell (HSC) for engraftment in a subject in need thereof is provided, in which the method involves detecting
20 the level of CD150 polypeptide or polynucleotide expressed in an HSC, wherein detection of a reduced level of CD150 expression relative to a reference selects the HSC for engraftment in a subject. In an embodiment of the method, a reduction in the level of CD150 polypeptide or polynucleotide relative to the reference indicates that the HSC should be selected for engraftment. In an embodiment of the method, an increase in the level of CD150 polypeptide
25 or polynucleotide relative to the reference indicates that the HSC should not be selected for engraftment.

In an embodiment of the methods of the above-delineated aspects and/or
embodiments thereof, the HSC is present in a population marked by heterogeneity in HSC
functionality. In an embodiment of the methods of the above-delineated aspects and/or
30 embodiments thereof, the method further comprises detecting the expression of one or more markers selected from the group consisting of *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2*, wherein expression of the one or more markers positively correlates with CD150 expression. In an embodiment of the methods of the above-

delineated aspects and/or embodiments thereof, the method further comprises detecting the expression of one or more markers selected from the group consisting of *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*, wherein expression of said markers negatively correlates with CD150 expression. In an embodiment of the methods of the

5 above-delineated aspects and/or embodiments thereof, the method further comprises characterizing the level of one or more of 332 HSC aging marker genes (Tables 1A, 1B), wherein detecting an increase in one or more of said genes is indicative of HSC functional decline. In an embodiment of the methods of the above-delineated aspects and/or

10 embodiments thereof, the method levels of CD150 polypeptide are detected using FACS analysis, and wherein levels of CD150 polynucleotide are detected using RNA-seq analysis.

In another aspect, a method of rejuvenating blood components of an aging or aged subject or alleviating aging-related hematopoietic cell functional decline is provided, in which the method involves administering to the subject an effective amount of hematopoietic stem cells (HSCs) expressing low levels of CD150 polypeptide (CD150^{low} HSCs) or a

15 composition thereof comprising a physiologically acceptable carrier, excipient or diluent; wherein the CD150^{low} HSCs differentiate into hematopoietic cell types which rejuvenate blood components in the aged subject. In an embodiment of the method, CD150^{low} HSCs have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell based on flow cytometry or fluorescence activated cell sorting (FACS)

20 analysis. In an embodiment of the method, the CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment of the method, CD150^{low} HSCs express marker genes *Rnase6* and *Arhgap30*.

25 In an embodiment of the above-delineated method of rejuvenating blood components of an aging or aged subject or alleviating aging-related hematopoietic cell functional decline, the administration is intravenous or is via transplant or engraftment. In an embodiment of the method, the cell types which differentiate from the CD150^{low} HSCs to rejuvenate the blood components comprise B lymphocytes, T lymphocytes, and myeloid cells; and/or wherein the

30 rejuvenated blood components are in an activated state. In an embodiment of the method, the aging or aged subject is selected by detecting hematopoietic stem cells expressing high levels of CD150 polypeptide (CD150^{high} HSCs) in a sample obtained from the subject. In an embodiment, the CD150^{high} HSCs have a mean fluorescence intensity of greater than about 4

x 10³ cell surface expressed CD150 proteins per cell based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment, the CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment, the CD150^{high} HSCs express a CD150 mRNA level of between about 0.3 to about 2 RNA Units per cell. In an embodiment of the methods, the subject is greater than 50 years of age or is greater than 70 years of age. In embodiments of the method, the sample is a blood, plasma, serum, bone marrow, or tissue sample. In an embodiment of the method, following administration of the CD150^{low} HSCs, the subject exhibits increased numbers of naïve T cells and a decrease in central memory T cell (T_{cm}) and effector memory T cell (T_{em}) ratio in the CD8⁺ and CD4⁺ subgroups of T cells.

In another aspect, a method of improving physical health and aging-related hematopoietic cell functional decline of a selected subject is provided, in which the method involves selecting the subject by detecting hematopoietic stem cells (HSC) expressing high levels of CD150 polypeptide (CD150^{high} HSCs) in a sample obtained from the subject; and administering to the subject an effective amount of hematopoietic stem cells (HSCs) expressing low levels of CD150 polypeptide (CD150^{low} HSCs) or a composition thereof comprising a physiologically acceptable carrier, excipient or diluent; wherein the CD150^{low} HSCs differentiate into hematopoietic cell types which improve physical health and aging-related hematopoietic cell functional decline in the subject. In an embodiment of the method, the CD150^{high} HSCs have a mean fluorescence intensity of greater than about 4 x 10³ cell surface expressed CD150 proteins per cell based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment of the method, the CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature in the sample based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment of the method, the CD150^{high} HSCs express a CD150 mRNA level of between about 0.3 to about 2 RNA Units per cell. In an embodiment of the method and/or embodiments thereof, the CD150^{low} HSCs administered to the subject have a mean fluorescence intensity of less than about 4 x 10³ cell surface expressed CD150 proteins per cell based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. of the method and/or embodiments thereof, the CD150^{low} HSCs administered to the subject

comprise the lowest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment of the method and/or embodiments thereof, the CD150^{low} HSCs express marker genes *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*. In an embodiment of the method and/or embodiments thereof, the CD150^{low} HSCs express marker genes *Rnase6* and *Arhgap30*. In an embodiment of the method and/or embodiments thereof, the administration to the subject is intravenous or is via transplant or explant. In an embodiment of the method and/or embodiments thereof, improving physical health and improving aging-related hematopoietic cell functional decline of the subject comprises administering CD150^{low} HSCs which differentiate into activated or activatable B lymphocytes, T lymphocytes, and myeloid cells. In an embodiment of the method and/or embodiments thereof, the subject is greater than 50 years of age or is greater than 70 years of age. In embodiment of the method and/or embodiments thereof, the sample is a blood, plasma, serum, or bone marrow sample. In an embodiment of the method and/or embodiments thereof, following administration of the CD150^{low} HSCs, the subject exhibits increased numbers of naïve T cells and a decrease in central memory T cell (T_{cm}) and effector memory T cell (T_{em}) ratio in the CD8⁺ and CD4⁺ subgroups of T cells. In an embodiment of the method and/or embodiments thereof, the subject having CD150^{high} HSCs is selected by further detecting in cells of the sample one or more age-related genes selected from *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2*.

In an embodiment of any one of the above-delineated methods and/or embodiments thereof, the CD150^{low} HSCs highly express one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*. In an embodiment of any one of the above-delineated methods and/or embodiments thereof, the CD150^{low} HSCs administered to the subject dilute or reduce the numbers of CD150^{high} HSCs in the subject, thereby rejuvenating the blood with CD150^{low} HSCs capable of differentiation into activated or activatable hematopoietic cells and cell types. In an embodiment, the CD150^{low} HSCs administered to the subject increase the numbers of activated versus quiescent CD150-expressing HSCs in the subject.

In another aspect, a composition comprising an effective amount of hematopoietic stem cells expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs) is provided, wherein said CD150^{low} HSCs have a mean fluorescence intensity of less than about

4 x 10³ cell surface expressed CD150 proteins per cell or wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment, the composition further includes a physiologically acceptable carrier, excipient or diluent. In an embodiment of the composition, the CD150^{low} HSCs express marker genes *Rnase6* and *Arhgap30*. In an embodiment of the composition and/or embodiments thereof, CD150^{low} HSCs highly express one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*. In an embodiment, the composition comprises a saline solution or a buffer solution.

In another aspect, the use of a composition comprising an effective amount of hematopoietic stem cells expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs) is provided, wherein the CD150^{low} HSCs have a mean fluorescence intensity of less than about 4 x 10³ cell surface expressed CD150 proteins per cell wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis and/or express marker genes *Rnase6* and *Arhgap30*, in the manufacture of a medicament for treating an aging or aged subject having hematopoietic stem cells expressing a high abundance of the CD150 glycoprotein (CD150^{high} HSCs); wherein the CD150^{high} HSCs have a mean fluorescence intensity of greater than about 4 x 10³ cell surface expressed CD150 proteins per cell or wherein CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis; and/or wherein the CD150^{high} HSCs express a CD150 mRNA level of between about 0.3 to about 2 RNA Units per cell. In an embodiment of the use, the CD150^{low} HSCs express high or increased levels one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30* relative to a control with low or no expression of these markers.

In another aspect, a method is provided in which a sample, such as a blood sample (e.g., a peripheral blood sample), a lymph sample, a bone marrow sample, or a tissue preparation is contacted with an anti-CD150 antibody conjugated to saporin (CD150-saporin) to eliminate CD150^{high} HSCs (e.g., potentially dysfunctional CD150^{high} HSCs having increased cell-surface expression of CD150) obtained from an old or aged subject, e.g., a

mammalian subject, including a human subject, in the absence of transplantation. A titrated dosage of the conjugated CD150-SAP may be used to specifically deplete CD150^{high} HSCs both *in vitro* and *in vivo*. The elimination of CD150^{high} HSCs in an old or aged subject, as exemplified in mice, results in an increased B cell ratio and a decreased myeloid cell ratio in peripheral blood, which supports the targeted removal of dysfunctional or defective HSCs in old or aged subjects as an advantageous and beneficial method or procedure for mitigating aging-related functional decline.

In an aspect, a method of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) in a subject is provided in which the method involves contacting a sample containing CD150-expressing hematopoietic stem cells (HSCs) obtained from a subject in need thereof with an anti-CD150 antibody conjugated to a cellular toxin in an amount effective to bind to high CD150-expressing HSCs (CD150^{high} HSCs); wherein the CD150^{high} HSCs express one or more age-related marker genes selected from the group consisting of *Sbspon*, *Ehd3*, *Chu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2* which correlate with high expression of CD150 on the cell surface; culturing the sample for a time sufficient for the anti-CD150 antibody-toxin conjugate to cause cytolysis of the CD150^{high} HSCs; and obtaining a sample in which dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) are reduced or eliminated. In an embodiment, the subject is an old, aged, or aging subject. In embodiments, the anti-CD150 antibody-toxin conjugate is used in an amount selected from 0.01 to 1 nM, 0.01 to 0.05 nM, or 0.01 nM. In an embodiment, the method further involves measuring death of cells in the culture after three days or longer. In an embodiment, the method further involves measuring a decrease or reduction in the number of CD150-expressing HSCs compared to a control and/or a decreased ratio of CD150^{high} HSCs to CD150^{low} HSCs in the sample. In an embodiment, the control is a saline-, a buffer-, or a phosphate buffered saline-treated sample. In an embodiment, the sample is selected from blood, peripheral blood, cord blood, serum, plasma, or bone marrow. In an embodiment, the method further involves administering to the subject the sample in which dysfunctional or defective high CD150-expressing hematopoietic stem cells (HSCs) are reduced or eliminated. In an embodiment, the sample is isolated and/or purified by methods known and practiced in the art. In an embodiment, the method is performed *in vitro* or *ex vivo*. In an embodiment of the method, the sample in which dysfunctional or defective high CD150-expressing hematopoietic stem cells (HSCs) are reduced or eliminated is administered to the subject.

In another aspect a method of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) in an old, aged or aging subject is provided, in which the method involves administering to a subject in need thereof an effective amount of an anti-CD150 antibody conjugated to a cellular toxin in an amount effective to bind to high CD150-expressing HSCs (CD150^{high} HSCs) in the subject; wherein the CD150^{high} HSCs express one or more age-related marker genes selected from the group consisting of *Sbspon*, *Ehd3*, *Chu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2* which correlate with high expression of CD150 on the cell surface; thereby reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) in the subject. In an embodiment, the anti-CD150 antibody-toxin conjugate is administered in an amount of 1 mg/kg to 5 mg/kg or in an amount of 1 mg/kg. In an embodiment, the anti-CD150 antibody-toxin conjugate is administered in an amount of 1 mg/kg. In an embodiment, the method further involves measuring the percentage of high CD150-expressing HSCs (CD150^{high} HSCs) remaining after administration of the anti-CD150 antibody-toxin conjugate. In an embodiment, the method further involves measuring a decreased ratio of CD150^{high} HSCs to CD150^{low} HSCs in the subject after administration of the anti-CD150 antibody-toxin conjugate. In an embodiment, the method further involves measuring a reduction of about 60% CD150^{high} HSCs in the subject compared to a control after administration of the anti-CD150 antibody-toxin conjugate. In an embodiment of the method, the control is a saline-treated subject, a buffer-treated subject, or a phosphate buffered saline-treated subject. In an embodiment of the above-delineated method and/or embodiments thereof, reducing or eliminating the functionally defective or dysfunctional CD150^{high} HSCs attenuates an aging phenotype and improves physical functioning in the old, aged, or aging subject. In an embodiment of the above-delineated method and/or embodiments thereof, the percentage of B cells is increased and the percentage of myeloid cells are decreased in the subject by about 45-90 days following administration of the anti-CD150 antibody-toxin conjugate. In an embodiment, the measuring step is performed at least three weeks following administration of the anti-CD150 antibody-toxin conjugate.

In an embodiment of any of the above-delineated methods of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) and/or embodiments thereof, the CD150-expressing HSCs are characterized as Lin-Sca-1+C-Kit+CD48-CD34-CD150+. In another embodiment of the methods and/or embodiments thereof, the anti-CD150 antibody is TC15-12F12.2. In an embodiment, the anti-CD150

antibody is conjugated to saporin. In an embodiment, the saporin is conjugated to streptavidin. In an embodiment of any of the above-delineated methods of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) and/or embodiments thereof, the subject is 50 years old or older. In an embodiment of these methods and/or embodiments thereof, the old, aged, or aging subject has a defective or dysfunctional CD150^{high}-expressing population of HSCs associated with expression of one or more HSC age-related markers selected from *Sbspon*, *Ehd3*, *Chu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2*. In an embodiment of these methods and/or embodiments thereof, amounts of HSCs comprising lower CD150 levels (CD150^{low} HSCs) are increased, wherein said CD150^{low} HSCs express one or more genes selected from the group consisting of *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*. In an embodiment of any of the above-delineated methods and/or embodiments thereof, administration to a subject is selected from parenteral, intravenous, or subcutaneous administration.

In embodiments of any of the above-delineated methods of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) and/or embodiments thereof, CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature and CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.

Provided in another aspect is a kit or article of manufacture comprising a composition comprising an effective amount of hematopoietic stem cells (HSCs) expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs), wherein said CD150^{low} HSCs have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell based on flow cytometry or fluorescence activated cell sorting (FACS) analysis; and/or wherein CD150^{low} HSCs express one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*, which correlate with CD150^{low} expressing HSCs, and instructions for use. In an embodiment, the CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature

based on flow cytometry or fluorescence activated cell sorting (FACS) analysis; In an embodiment, the composition is a physiological or pharmaceutically acceptable composition.

In embodiments related to any of the above-delineated methods, compositions, and/or embodiments thereof, based on FACS analysis of LT-HSCs to determine CD150 surface expression level, the lowest 25% subpopulation (lower level of CD150) is defined as CD150^{low} HSCs, which show youthful signatures and exhibit good function. The highest 25% subpopulation (higher level of CD150) is defined as CD150^{high} HSCs, which show aging signatures and phenotypes when administered, e.g., via transplantation, into recipient mice. In an embodiment, the LT-HSCs, which comprise a source of CD150^{low} and CD150^{high} HSCs, express the following biomarker signature on their cell surface: Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺. In an embodiment, HSCs are isolated and/or purified from bone marrow.

Compositions and articles as described in the aspects and embodiments herein were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the disclosure and embodiments will be apparent from the detailed description, and from the claims.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art related to the disclosure and embodiments described herein. The following references provide one of skill with a general definition of many of the terms used herein: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By “agent” is meant a cell, small molecule, polypeptide, polynucleotide, or a functional fragment thereof. In some embodiments, the cell is a hematopoietic stem cell (HSC) selected as having a reduced level of CD150 or a high level of CD150 relative to a reference. In some cases, the reference is a non-CD150-expressing cell. In an embodiment, the cell is a Long-Term HSC (LT-HSC), which resides in bone marrow.

By "ameliorate" is meant decrease, reduce, suppress, attenuate, diminish, arrest, abrogate, improve, or stabilize the development or progression of a disease, a dysfunction, or a disorder.

5 By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, a 20% change, a 25% change, a 40% change, or a 50% or greater change in expression levels.

10 By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

15 As used herein, "blood components" refer to hematopoietic cells or cell types, or cells or cell types derived, differentiated, or generated from hematopoietic stem cells (HSCs), in the blood, serum, plasma, and/or bone marrow of a mammalian subject or individual. Such cells include, without limitation, activated or activatable hematopoietic cells and cell types, e.g., white blood cells, such as B cells, T cells, eosinophils, neutrophils, and myeloid cells, as well as red blood cells. In an embodiment, CD150^{low} HSCs, which have improved activities and functional properties as described herein, generate significant numbers of activated or activatable hematopoietic cells and cell types, e.g., white blood cells, such as B cells, T cells, eosinophils, neutrophils, myeloid cells, as well as increase red blood cell numbers and hemoglobin levels in old, aged, or aging subjects who receive such HSCs, to boost, renew, or rejuvenate activity and numbers of the functional blood components in the subjects.

25 In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

“CD150 polypeptide” (also called Signaling Lymphocyte Activation Molecule (SLAM)), e.g., human CD150, refers to a glycoprotein having at least about 85% amino acid sequence identity to GenBank Reference No. AAI32793.1, and having CD150 antibody binding activity. CD150 is expressed on the surface of hematopoietic cells, such as immature thymocytes, T cells, B cells, natural killer, and dendritic cells. The extracellular domain of CD150 is the receptor for measles virus. CD150 acts as a co-activator on T and B cells and/or as a marker of activated B cells. Both the mouse and human CD150 genes comprise seven exons spanning approximately 32 kb. (N. Wang et al., 2001, *Immunogenetics*, 53(5):382-394. CD150 embraces an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to the human canonical SLAM (aka SLAMF1) amino acid sequence (GenBank Reference No. AAI32793.1 and UniProt Accession No. Q13291-1). The sequence of an exemplary CD150 polypeptide follows:

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1   MDPKGLLSLT FVLFLSLAFG ASYGTGGRMM NCPKILRQLG SKVLLPLTYE RINKSMNKSI
61  HIVVTMAKSL ENSVENKIVS LDPSEAGPPR YLGDRYKFYL ENLTGIGRES RKEDEGWYLM
121 TLEKNVSVQR FCLQLRLYEQ VSTPEIKVLN KTQENGCTCL ILGCTVEKGD HVAYSWSEKA
181 GTHPLNPANS SHLLSLTLGP QHADNIYICT VSNPISNNSQ TFSPPWPGCRT DPSEKTPWAV
241 YAGLLGGVIM ILIMVVILQL RRRGKTNHQY TTVEKKSLTI YAQVQKPGPL QKKLDSFPAQ
301 DPCTTIYVAA TEPVPESVQE TNSITVYASV TLPES

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By “CD150 polynucleotide” is meant a polynucleotide encoding a CD150 polypeptide. An exemplary polynucleotide sequence encoding a human CD150 Signaling Lymphocyte Activation Molecule (SLAM)) is provided below. CD150 embraces a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to the human SLAM (aka SLAMF1) nucleic acid sequence (GenBank Reference No. BC132792) as follows:

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1   ggccaggatc ccttccttct cctcattggc tgatggatcc caaggggctc ctctccttga
61  ccttcgtgct gtttctctcc ctggcctttg gggcaagcta cggaacaggt gggcgcatga
121 tgaactgccc aaagattctc cggcagttgg gaagcaaagt gctgctgccc ctgacatatg
181 aaaggataaa taagagcatg aacaaaagca tccacattgt cgtcacaatg gcaaaatcac
241 tggagaacag tgtcgagaac aaaatagtgt ctcttgatcc atccgaagca ggccctccac
301 gttatctagg agatcgctac aagtttttatc tggagaatct caccctgggg atacgggaaa
361 gcaggaagga ggatgagggg tggtacctta tgaccctgga gaaaaatgtt tcagttcagc
421 gcttttgctt gcagttgagg ctttatgagc aggtctccac tccagaaatt aaagttttaa
481 acaagaccca ggagaacggg acctgcacct tgatactggg ctgcacagtg gagaaggggg
541 acctgtggc ttacagctgg agtgaaaagg cgggcaccca cccactgaac ccagccaaca
601 gctccacact cctgtccctc accctcggcc ccagcatgc tgacaatatc tacatctgca
661 ccgtgagcaa cctatcagc aacaattccc agaccttcag cccgtggccc ggatgcagga
721 cagacccctc agaaacaaaa ccatgggcag tgtatgctgg gctgttaggg ggtgtcatca
781 tgattctcat catggtggta atactacagt tgagaagaag aggtaaaacg aaccattacc
841 agacaacagt ggaaaaaaa agccttacga tctatgcccc agtccagaaa ccaggctctc
901 ttcagaagaa acttgactcc tcccagctc aggacccttg caccaccata tatgttgetg
961 ccacagagcc tgtcccagag tctgtccagg aaacaaattc catcacagtc tatgctagtg
1021 tgacacttcc agagagctga caccagagac caacaaaggg actttctgaa ggaaaatgga
1081 aaaacaaaaa tgaacactga acttggccac agggcccaag tttcctctgg cagacatgct
1141 gcacgtctgt acccttctca gatcaactcc ctggtgatgt ttcttcaca tacatctgtg

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1201 aaatgaacaa ggaagtgagg cttcccaaga atttagcttg ctgtgcagtg gctgcaggcg
 1261 cagaacagag cgttacttga taacagcgtt ccatctttgt gttgtagcag atgaaatgga
 1321 cagtaatgtg agttcagact ttgggcatct tgctcttggc tgga

5 The amino acid sequence of CD150 from *Mus musculus* (mouse), GenBank

Accession No. AAI17100.1) is set forth below.

1 MDPKGSLSWR ILLFLSLAFE LSYGTGGGVM DCPVILQKLG QDTWLPLTNE HQINKSVNKS
 61 VRILVTMATS PGSKSNKKIV SFDLSKGSYP DHLEDGYHFQ SKNLSLKILG NRRESEGWYL
 121 VSVEENVSVQ QFCKQLKLYE QVSPPEIKVL NKTQENENGTS CSLLLACTVK KGDHVTYSWS
 10 181 DEAGTHLLSR ANRSHLLHIT LSNQHQDSIY NCTASNPVSS ISRTFNLSSQ ACKQESSSES
 241 SPWMQYTLVP LGVVIIFILV FTAIIMMKRQ GKSNNHCQPPV EEKSLTIYAQ VQKSGPQEKK
 301 LHDALTDQDP CTTIYVAATE PAPESVQEPN PTTVYASVTL PES

15 A polynucleotide sequence encoding mouse CD150 Signaling Lymphocyte Activation
 Molecule (SLAM)) is provided below. CD150 embraces a nucleic acid sequence having at
 least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to the
 mouse *SLAM* (aka *SLAMF1*) nucleic acid sequence (GenBank Reference No. BC117099.1)
 as follows:

1 ctcacagctg gggaccctgt ctgcgattgc tggctaattg atcccaaagg atccctttcc
 20 61 tggagaatac ttctgtttct ctccctggct tttgagttga gctacggaac aggtggagggt
 121 gtgatggatt gccagtgat tctccagaag ctgggacagg acacgtggct gccctgacg
 181 aatgaacatc agataaataa gagcgtgaac aaaagtgtcc gcacccctcg caccatggcg
 241 acgtccccag gaagcaaata caacaagaaa atttgtgtctt ttgatctctc taaagggagc
 301 tatccagatc acctggagga tggctaccac tttcaatcga aaaacctgag cctgaagatc
 25 361 ctcggaaca ggcgggagag tgaaggatgg tacttggtga gcgtggagga gaacgtttct
 421 gtccagcaat tctgcaagca gctgaagctt tatgaacagg tctccctctc agagattaaa
 481 gtgctaaaca aaaccagga gaacgagaat gggacctgca gcttgctgtt ggctgcaca
 541 gtgaagaaag gggaccatgt gacttacagc tggagtgtg aggcaggcac ccacctgctg
 601 agccgagcca accgtcccca cctcctgcac atcactctta gcaaccagca tcaagacagc
 30 661 atctacaact gcaccgcaag caaccctgtc agcagtatct ctaggacctt caacctatca
 721 tcgcaagcat gcaagcagga atcctcctca gaatcgagtc catggatgca atatactctt
 781 gtaccactgg gggctgttat aatcttcac ctagttttca cggcaataat aatgatgaaa
 841 agacaaggta aatcaaatca ctgccagcca ccagtggag aaaaaagcct tactatztat
 901 gccaagatc agaaatcagg gctcaagag aagaaacttc atgatgcctt aacagatcag
 35 961 gacccctgca caaccattta tgtggctgcc acagagcctg cccagagtc tgtccaggaa
 1021 ccaaacccca ccacagttta tgccagtgtg aactgccag agagctgacc catataccca
 1081 gtgaaaggac tttttgaagg aggatagaag aacaaaaatc cactactgaac tggaccccg
 1141 gtcccaagtt ctctgtgaca gaaactgcac atctgtaacc ttctccaatc agttccctgg
 1201 tgacggatct gcacaggcat gcttatgaag tagatgagaa gtgaggcttc ctgggcatgc
 40 1261 aacctgctct gctgctgaca ca

CD150 is a type I transmembrane protein belonging to the immunoglobulin superfamily. Its
 molecular weight is between 70 kDa and 95 kDa. The extracellular region of the receptor is
 composed of one Ig variable-like domain and one Ig constant 2-like domain. The intracellular
 45 region of the receptor contains two intracellular tyrosine-based switch motifs (ITSMs) that
 interact with SH2 domain-containing proteins. The *Slamf1* gene that encodes CD150 is
 located on the human chromosome 1 and consists of eight exons and

seven introns. Alternative splicing of *SLAMF1* transcripts results in several isoforms of the protein, including the conventional transmembrane isoform (mCD150), secreted isoform (sCD150) cytoplasmic isoform (cCD150), and a novel transmembrane isoform (nCD150).

Hematopoietic stem cells (HSCs), sometimes called blood stem cells, refer to multipotent immature or primitive cells that can develop into all types of blood cells, including white blood cells (lymphocytes), red blood cells, myeloid lineage cells. HSCs reside in several organs of the body, including peripheral blood (PB), or “blood” herein, bone marrow (BM), and umbilical cord blood (UCB). In an embodiment, HSCs are characterized by marker gene and/or encoded polypeptide expression. In an embodiment, HSCs are characterized by expression of lineage-Sca-1+c-Kit+ (“LSK”) CD48-CD34-CD150+ markers. In an embodiment, lineage-Sca-1+c-Kit+ (“LSK”) CD48-CD34-CD150+ HSCs are referred to as Long Term (LT)-HSCs.

“CD150^{low} HSCs” refer to hematopoietic stem cells (HSCs), or a population of HSCs as described herein which express low levels of CD150 glycoprotein on their surface relative to HSCs from older, aged, or aging animals that express higher levels of CD150 (CD150^{high} HSCs). (**FIGs. 3B, 3C**). In some embodiments, the level of CD150 protein is detected by fluorescent intensity, e.g., using FACS or flow cytometry analysis, and a reduction in CD150 is a reduction in intensity by at least about 10%, 20%, 30%, 40%, 50%, 60%, 75%, 85%, 95% or more. In some embodiments, CD150^{low} HSCs also express a lesser abundance of mRNA encoding CD150 protein compared with CD150^{high} HSCs. (**FIGs. 3B, 3C**). In an embodiment, the level of CD150 mRNA is quantified by RNA-seq. In another embodiment, the level of CD150 mRNA is reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 75%, 85%, 95% or more. As described herein, higher numbers and amounts of CD150^{low} HSCs are found in young animals (e.g., young animals include those that are from about or equal to 2-4 months of age) and were found to be in a more active state compared with CD150^{high} HSCs from older, aging animals (e.g., aged or aging animals include those that are greater than about or equal to 12 months of age, such as about or equal to 13 months or 22-24 months of age), which are in a more quiescent state. (e.g., a1, a2, **FIGs. 2F, and 2H**). In particular, CD150^{low} HSCs may be defined as having a mean fluorescence intensity of less than about 4×10^3 (<about 4×10^3) cell surface expressed CD150 proteins based on FACS analysis (**FIG. 3B**). In an embodiment, CD150^{low} HSCs typically express the marker genes *Rnase6* and *Arhgap30*, based on scRNAseq analysis (**FIG. 10C**). In an embodiment, young subjects (individuals, mammals, patients) are characterized as having a greater number or

abundance of CD150^{low} HSCs in their blood (peripheral blood (PB)) compared with older, aged, or aging subjects. In some embodiments, older, aged, or aging subjects have both CD150^{low} HSCs and CD150^{high} HSCs in peripheral blood (or a blood sample); however, older or aging subjects typically have a greater abundance or amount of CD150^{high} HSCs compared with CD150^{low} HSCs in peripheral blood (or a blood sample). In an embodiment, representative genes that are highly expressed in young HSCs (CD150^{low} HSCs) and correlate with young or youthful HSCs include, for example, one or more of *Serpina1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6* and *Arhgap30* (**FIGs. 10A, 11D**).

“CD150^{high} HSCs” refer to hematopoietic stem cells (HSCs), or a population of HSCs as described herein which express high levels of the CD150 glycoprotein on their surface relative to HSCs from young animals that express lower levels of CD150 (CD150^{low} HSCs). (**FIG. 3B**). In some embodiments, the level of CD150 protein is detected by fluorescent intensity, and an increase in CD150 is an increase in intensity of at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 75%, 85%, 95% or more. CD150^{high} HSCs also express a greater abundance of mRNA encoding CD150 protein compared with CD150^{low} HSCs. (**FIG. 3C**). In an embodiment, the level of CD150 mRNA is quantified by RNA-seq. In another embodiment, the level of CD150 mRNA is increased by at least about 10%, 20%, 30%, 40%, 50%, 60%, 75%, 85%, 95% or more. As described herein, higher numbers and amounts of CD150^{high} HSCs are found in old, aging, or aged animals (e.g., of the ages as described above and in Example 9) and were found to be in a more quiescent state compared with CD150^{low} HSCs from younger animals (e.g., of the ages as described above and in Example 9), (e.g., q1-q4, **FIGs. 2F, 2H, 11D, and 11F**). In particular, CD150^{high} HSCs may be defined as having a mean fluorescence intensity of greater than about 4×10^3 ($> \text{about } 4 \times 10^3$) cell surface expressed CD150 proteins based on flow cytometry or fluorescence activated cell sorting (FACS) analysis (**FIG. 3B**). In addition, CD150^{high} HSCs express a level of CD150 mRNA between about 0.3 to about 2 RNA Units per cell (**FIGs. 2H, 11F**). In an embodiment, old, aged, or aging subjects (individuals, mammals, patients) are characterized as having a greater number or abundance of CD150^{high} HSCs in their blood (peripheral blood (PB)) compared with young subjects. In some embodiments, older, aged, or aging subjects have both CD150^{low} HSCs and CD150^{high} HSCs in peripheral blood (or a blood sample); however, older or aging subjects typically have a greater abundance or amount of CD150^{high} HSCs compared with CD150^{low} HSCs in peripheral blood (or a blood sample). In an embodiment, representative genes that are highly expressed in old, aged,

aging HSCs (CD150^{high} HSCs) and correlate with old or aging HSCs include, for example, one or more of *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *Mt1*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2* (**FIG. 11D**). In an embodiment, representative genes that are highly expressed in old, aged, aging HSCs (CD150^{high} HSCs) and correlate with old or aging HSCs include, for example, one or more of *Clu*, *Selp*, *Mt1*, *Ramp2* and *Gpr183*.

Based on FACS analysis of LT-HSCs to determine CD150 surface expression level, the lowest 25% subpopulation (lower level of CD150) is defined as CD150^{low} HSCs, which show youthful signatures and exhibit good function. The highest 25% subpopulation (higher level of CD150) is defined as CD150^{high} HSCs, which show aging signatures and phenotypes when administered, e.g., via transplantation, into recipient mice. In an embodiment, the LT-HSCs, which comprise a source of CD150^{low} and CD150^{high} HSCs, express the following biomarker signature on their cell surface: Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺. In an embodiment, HSCs are isolated and/or purified from bone marrow.

“Detect” refers to identifying the presence, absence or amount of the analyte to be detected. CD150 polypeptides and polynucleotides are exemplary analytes.

By “detectable label” is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, or haptens.

By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include diseases or disorders involving hematopoietic stem cells, or cells of the immune lineage, e.g., lymphocytes, T lymphocytes, B lymphocytes, myeloid cells,

By “effective amount” is meant the amount of a required to ameliorate the symptoms of a disease, disorder, state, or condition, relative to an untreated patient. The effective amount of active compound(s) used to practice methods as described herein for therapeutic treatment varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "inhibitory nucleic acid" is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA nucleic acid, or a functional portion thereof, an oligonucleotide, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (e.g., by 10%, 25%, 50%, 75%, or even 90-100%) in the expression of a target gene. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein. In an embodiment, the level of CD150 polypeptide or polynucleotide is reduced using an inhibitory polynucleotide that targets a CD150 polynucleotide, thereby reducing its expression or the expression of the encoded CD150 polypeptide.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide as described herein is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example,

phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule as described herein is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide as described herein that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide as described herein. An isolated polypeptide as described herein may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "marker" is meant any protein, polynucleotide, analyte, or clinical indicator having an alteration, e.g., an alteration in expression level or activity, that is associated with a disease or disorder, or a state or condition. A marker can be a protein, polypeptide, peptide, or polynucleotide that may be detected and/or quantified in a sample obtained from a subject, e.g., a subject having a disease, disorder, state, or condition that is associated with the presence of the marker. In an embodiment, the marker can be CD150 expression in hematopoietic stem cells (HSCs). In embodiments, the HSCs can express low (decreased relative to a control) or high (increased relative to a control) levels of CD150, e.g., CD150^{low} HSCs or CD150^{high} HSCs. In embodiments, the marker positively correlates with CD150 expression; such markers include, but are not limited to, *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2* (aging-related gene expression markers). In

other embodiments, the marker negatively correlates with CD150 expression; such markers include, but are not limited to, *Serpina1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30* (young or youthful-related gene expression markers).

As used herein, “obtaining” as in “obtaining an agent” includes synthesizing,
 5 isolating, extracting, purifying, purchasing, or otherwise acquiring the agent.

“Primer set” means a set of oligonucleotides that may be used, for example, for PCR. A primer set would consist of at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500, 600, or more primers.

By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or
 10 100%.

By “reference” is meant a standard or control condition. In one embodiment, the level of CD150 polypeptide or polynucleotide in an HSC derived from an aging subject is compared to the level of CD150 polypeptide or polynucleotide in an HSC derived from a young, healthy control subject. In other embodiments, the reference is a reference value. For
 15 example, the level of CD150 polypeptide or polynucleotide in an HSC is compared to a reference value based on results obtained in a population of HSCs derived from young, healthy control subjects, where the reference value defines a range of test values (e.g., normal range).

A “reference sequence” is a defined sequence used as a basis for sequence
 20 comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids,
 25 about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

“Rejuvenation” in one embodiment refers to the provision (e.g., administration,
 30 engraftment) of CD150^{low} HSCs to a subject, e.g., having CD150^{high} HSCs, typically characteristic of an old, aged, or aging subject as described herein, by administering an effective amount of the CD150^{low} HSCs to the subject. The administration of CD150^{low}

HSCs to the subject serves to dilute or reduce the numbers of CD150^{high} HSCs in the subject, such that the CD150^{low} HSCs, having improved activities and functional properties, generate significant numbers of activated or activatable hematopoietic cells and cell types, e.g., white blood cells, such as B cells, T cells, eosinophils, neutrophils, myeloid cells, as well as

5 increase red blood cell numbers and hemoglobin levels in the old, aged, or aging subjects, to boost, renew, or rejuvenate activity and numbers of functional blood components in such subjects. In an embodiment, rejuvenation refers to reducing, depleting, or eliminating a population of dysfunctional or diseased HSCs, i.e., CD150^{high} HSCs, *in vitro* or *ex vivo* from a sample (e.g., bone marrow or blood) obtained from a subject, or *in vivo* by administering an

10 antibody that reduces, depletes, ablates, or eliminates a population of dysfunctional or diseased HSCs, i.e., CD150^{high} HSCs, resulting in an increase in a population of more youthful CD150^{low} HSCs in the sample or the subject, an improvement in the aging status of the cells in the sample or of the subject (e.g., a decrease in the number of myeloid cells and a corresponding decrease in the number of lymphoid cells), and/or improved physical functions

15 as described herein.

“Saporin” refers to ribosome-inactivating protein of type 1, which inhibits protein synthesis in animal cells. The protein is useful as an immunotoxin for pharmacological applications. In an embodiment, saporin can be conjugated to an antibody, e.g., an anti-CD150 antibody, to produce an antibody-drug conjugate. An exemplary saporin polypeptide

20 sequence is provided below. Saporin embraces an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to the ribosome-inactivating protein saporin-6 sequence having Uniprot designation P20656, as follows:

25 MKIYVVATIAWILLQFSAWTTTDAVTSITLIDLVNPTAGQYSSFVDKIRNNVKDPNLKYGGTD
IAVIGPPSKEKFLRINFQSSRGTVSLGLKRDNLVYVAYLAMDNNTNVNRAYYFRSEITS AEST
ALFPEAT TANQKALEYTEDYQSIEKNAQITQGDQSRKELGLGIDLLSTSMEAVNKKARVVKD
EARFLLIAIQMTAEAAARFRYIQNLVIKNFPNKFENSENKVIQFEVNWKKISTAIYGD AKNGVF
NKDYDFGFGKVRQVKDLQMGLLLMYLGKPKSSNEANSTVRHYGPKPTLLIT

By "siRNA" is meant a double stranded RNA. Optimally, an siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. These dsRNAs can

30 be introduced to an individual cell or to a whole animal; for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity.

By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide as described herein, but which does not substantially recognize and bind other

molecules in a sample, for example, a biological sample, which naturally includes a polypeptide as described. A biological sample may include blood, peripheral blood, serum, plasma, lymph, bone marrow, tissue or tissue preparation, saliva, tears, urine, stool, sputum, lavage, and the like. In an embodiment, an anti-CD150 antibody specifically binds to a
5 CD150 molecule on an HSC, e.g., a CD150^{low} HSC, a CD150^{high} HSC, or a LT-HSC.

Nucleic acid molecules useful in the methods as described herein include any nucleic acid molecule that encodes a polypeptide as described herein or a functional fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having
10 “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods as described herein include any nucleic acid molecule that encodes a polypeptide as described herein or a functional fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit
15 substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By “hybridize” is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987)
20 Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

60%, 80% or 85%, 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250
25 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least
30 about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization

will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.

Additional variations on these conditions will be readily apparent to those skilled in the art.

Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In embodiments, such a sequence is at least

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches

identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and
5 phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a human patient or a non-human primate, or a bovine, equine,
10 canine, ovine, or feline mammal. Other subject mammals may include, without limitation, rodents, rabbits, mice, goats, pigs, alpacas, or llamas.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
15 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "treat," "treating," "treatment," and the like refer to reducing, abrogating, alleviating, or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not
20 require that the disorder, condition or symptoms associated therewith be completely eliminated.

The terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition in a subject who does not have, but is at risk of, or susceptible to, developing a disorder or condition, e.g., one
25 associated with hematopoietic stem cells (HSCs), particularly, due to age.

In an aspect, methods of treating disease and/or disorders or symptoms thereof which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a cell or biological product or preparation, etc. to a subject (e.g., a mammal such as a human). Thus, one embodiment is a method of treating a subject who is aging or aged
30 (old) to increase the number of CD150^{low} HSCs (or dilute out the number of CD150^{high} HSCs) in blood so as to provide the subject with a source of all blood cell types that differentiate from the HSCs and to improve the overall and physical health of the subject. The method includes the step of administering to the mammal a therapeutic amount of an

amount of a therapeutic agent (e.g., CD150^{low} HSCs) herein sufficient to treat the disease, disorder, condition, physical state or symptom thereof, under conditions such that effective treatment occurs.

The methods herein include administering to the subject (including a subject identified as in need of such treatment) an effective amount of a composition or treatment product or the like as described herein to produce such effect. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or objective (e.g. measurable by an assay, test, or diagnostic method to identify or select the subject as needing the treatment), genetic test, enzyme or protein marker, or Marker,. The therapeutic methods (which include prophylactic treatment) in general comprise administration of a therapeutically effective amount of the cells, preparations, compositions containing the same, and the like) as described herein, to a subject (e.g., animal, human) in need thereof, including a mammal, particularly a human. Such treatment will be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk for a disease, disorder, condition, or symptom thereof. Determination of those subjects "at risk" can be made by any objective or subjective determination by a diagnostic test or opinion of a subject or health care provider family history, and the like).

A method of monitoring treatment progress is encompassed herein. The method includes the step of determining a level of a diagnostic marker (Marker) (e.g., any target delineated herein or modulated by a compound herein, a protein or indicator thereof, etc.) or diagnostic measurement (e.g., screen, assay) in a subject suffering from or susceptible to a disorder or symptoms thereof associated with HSCs, particularly, CD150^{high} HSCs, in which the subject has been administered a therapeutic amount of an agent, cells, composition, preparation, etc., herein sufficient to treat the disease, condition, or symptoms thereof. The level of Marker determined in the method can be compared to known levels of Marker in either healthy normal controls or in other afflicted patients to establish the disease or condition status of the subject. In preferred embodiments, a second level of Marker in the subject is determined at a time point later than the determination of the first level, and the two levels are compared to monitor the course of the disease or condition, or the efficacy of the therapy. In certain preferred embodiments, a pre-treatment level of Marker in the subject is determined prior to beginning treatment; this pre-treatment level of Marker can then be

compared to the level of Marker in the subject after the treatment commences or continues, to determine the efficacy of the treatment.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof as described herein. Any compositions or methods provided and described herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1H provide schematic diagrams, line and bar graphs, and FACS analysis plots illustrating that transplantation of young HSCs rejuvenates old mice. **FIG. 1A:**

Diagram illustration of the competitive young (2-3 months) and old (22-24 months) HSC transplantation experiment. For the first transplantation, the ratio of young to old HSCs was 1:2 (500 young and 1,000 old) while the ratio was 1:1 (1,000 young and 1,000 old) for the second transplantation. **FIG. 1B:** Peripheral blood chimerism of donor HSCs at different

times after the 1st and 2nd transplantation. n=6 for the first and n=3 for the second transplantation. **FIG. 1C:** Diagram showing the experimental design of individual

transplantation of 1,000 young (3 months) or 1,000 old (22-24 months) HSCs into middle-aged recipients (13-month-old). Five months after the transplantation, a series of hematopoietic and physical tests were performed. **FIG. 1D:** Bar graph showing the

percentage of B cells, T cells, and myeloid cells in the peripheral blood (PB) of recipient mice, n=8. **FIG. 1E:** Bar graph showing the absolute number of blood cells in the peripheral

blood (PB) of recipient mice, n=8. **FIG. 1F:** FACS plot and bar plot showing the percentage of naïve T cells in CD8⁺ T cells from recipient mice, Naïve T cells (CD44^{low}, CD62L^{high}),

Tcm (CD44^{high}, CD62L^{high}) and Tem (CD44^{high}, CD62L^{low}), n=8. **FIG. 1G:** Bar plot showing the epigenetic age of blood from recipient mice, n=4. **FIG. 1H:** Physical tests of recipient mice that received young and old HSCs. Muscle strength, motor coordination, endurance and brain function were assessed, n=8. Mean $\pm$ SD, student t test, *p<0.05, **p<0.01, ****p<0.0001.

FIGs. 2A-2H provide diagrams, plots, heat maps, graphs and violin plots demonstrating that scRNA-seq reveals increased heterogeneity of old HSCs. **FIG. 2A:** Workflow of 10X scRNA-seq of young and old HSCs. The HSCs were sorted from young (2-3 months) and old (23 months) mice. **FIG. 2B:** UMAP plot showing the distribution of young and old HSCs based on scRNA-seq. Clear separation of young and old HSCs indicates transcriptional changes of HSCs during aging. As known in the art, UMAP (Uniform Manifold Approximation and Projection for Dimension Reduction) is a dimension reduction technique and algorithm known in the art for general non-linear dimension reduction. UMAP is based on manifold learning techniques generated from topological data analysis and allows for the display of many-dimensional data in two or three dimensions (2D or 3D space). Using UMAP, gene expression can be compared between cells. (Becht, E. et al., (2018), *Nature Biotech.*, 37, 38-44). **FIG. 2C:** Cell cycle phase analysis of young and old HSCs based on scRNA-seq. The S-phase and G2/M-phase marker genes are from Seurat package (V4.0.2). The cell cycle phase was determined by the relative expression levels of these marker genes. If neither S-phase nor G2/M phase genes were expressed, they were classified as G0/G1 phase. **FIG. 2D:** UMAP plot showing unsupervised clustering of young and old HSCs. In total, 6 clusters were identified with two clusters (a1 and a2) representing active, and four clusters (q1-q4) representing quiescent cells. **FIG. 2E:** Heatmap showing the cluster-specific marker genes expression across the 6 clusters and their enriched GO terms. Differentially expressed genes with min.pct = 0.25, logfc.threshold = 0.25 among the 6 clusters were used to generate the heatmap. Well-known HSC aging related genes in clusters q1 and q2 are highlighted. Commonly identified marker genes in clusters q3 and q4 were also highlighted. **FIG. 2F:** Box plot showing relative expression of marker genes of q1, q2 and q3 in young and old HSCs via analysis of bulk RNA-seq. The expression level was normalized to the average expression level of the old. Plot shows the mean and 5-95 percentile. Two-sided unpaired Wilcoxon test. **FIG. 2G:** UMAP presentation of well-known HSC aging marker genes, *Sbspon*, *Gpr183*, *Clu* and *Ramp2* in each of the single cells. **FIG. 2H:** UMAP plot and violin plot showing the calculated aging score of single cells of different

clusters based on the HSC aging genes identified in bulk RNA-seq. In total, 332 up-regulated genes in old HSCs were used for aging score calculation. Two-sided unpaired Wilcoxon test. ** $p < 0.01$, **** $p < 0.0001$.

FIGs. 3A-3I provide schematic diagrams, FACS analysis plots, heat maps, bar and line graphs, and line plot data related to the identification of CD150 as a hematopoietic stem cell (HSC) aging heterogeneity marker. **FIG. 3A:** Workflow for identifying heterogeneity marker genes in old HSCs. **FIG. 3B:** FACS plot showing expression level of CD150 in young (3 months) and old (22-24 months) HSCs. **FIG. 3C:** HSCs from old mice were separated into four subgroups (25% for each) based on their CD150 protein levels and subjected to bulk RNA-seq, $n=3$. **FIG. 3D:** Dot plot showing Pearson correlation between CD150 signature score and aging score based on scRNA-seq data, $R=0.78$. **FIG. 3E:** Heatmap showing changes in expression of aging-related genes with ascending level of CD150 based on bulk RNA-seq in **FIG. 3C**. **FIG. 3F:** Bar graph plot showing the epigenetic age of CD150^{low} and CD150^{high} HSCs from 22-24 months old mice, $n=4$, paired t test, the HSC subsets from the same mice were paired with dash line. **FIG. 3G:** Line plot and heatmap showing ATAC-seq signal difference between CD150^{low} and CD150^{high} HSCs in aging-related open and closed regions, respectively. **FIG. 3H:** Diagram of competitive transplantation to evaluate the repopulation capacity of CD150^{low} and CD150^{high} HSCs from old mice. **FIG. 3I:** Whole blood chimerism of CD150^{low} and CD150^{high} HSCs from 22-24 months old donor mice at different times after the 1st and 2nd transplantation. $n=6$ for the first transplantation, and $n=3$ for the second transplantation. Mean $\pm$ SD, student t test, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

FIGs. 4A-4H provide schematic depictions, FACS analysis maps, bar graphs, and UMAP data and results showing that differentiation, but not self-renewal, is a major defect of old CD150^{high} HSCs. **FIG. 4A:** Diagram illustration of the transplantation experiment comparing old CD150^{low} and CD150^{high} HSCs. Donor HSCs derived HSPCs from the bone marrow were analyzed on days 7 and 14 after transplantation. **FIG. 4B:** Representative FACS analysis of donor HSCs derived HSPCs (left) and quantification of different cell populations 7 days after transplantation (right). LT-HSC (CD45.2 LSK (i.e., Lin-Sca1+c-Kit+), CD34⁻CD48⁻CD150⁺), ST-HSC (CD45.2 LSK, CD34⁺/CD48⁺CD150⁺) and MPPs (CD45.2 LSK, CD34⁺/CD48⁺CD150⁻) were analyzed, $n=3$. **FIG. 4C:** Diagram illustration of the competitive transplantation for evaluating the long-term differentiation of CD150^{high} HSCs. Both peripheral blood and bone marrow were analyzed five months after

transplantation. **FIG. 4D:** Representative FACS analysis of donor HSC-derived HSPCs (left) and quantification of different cell populations (right) 5 months after transplantation, n=3.

FIG. 4E: Bar graph showing the chimerism of donor HSCs in LT-HSCs, ST-HSCs, MPPs

and PB 5 months after transplantation, n=3. **FIG. 4F:** UMAP plot showing cell distribution

of HSPCs derived from CD150^{low} and CD150^{high} HSCs in scRNA-seq 14 days after

transplantation. **FIG. 4G:** UMAP presentation of cell types predicted based on HSPC

marker gene expression (left). In total, 7 different cell types were identified in HSPCs.

FIG. 4H: Bar graph showing relative abundance of predicted cell types in HSPCs from mice received old CD150^{low} or CD150^{high} HSCs. Mean $\pm$ SD, student t test, *p<0.05, **p<0.01,

***p<0.001.

FIGs. 5A-5G provide schematic depictions, bar graphs, FACS analysis, and survival curve data and results demonstrating that transplantation of a “youthful” subset of old HSCs alleviates aging phenotypes of old or aged mice. **FIG. 5A:** Diagram showing the

experimental design of individual transplantation of 2,000 old CD150^{low} (25% lowest),

whole-HSCs (un-selected) and CD150^{high} (25% highest) HSCs into middle-aged recipients

(13-month-old), n=8. Five months after transplantation, a series of hematopoietic and

physical tests were performed. **FIG. 5B:** Bar graph showing the percentage of B cells, T

cells, and myeloid cells in the PB of recipient mice of the three groups, n=8. **FIG. 5C:** Bar

graph showing absolute numbers of blood cells in PB of recipient mice from different

transplanted groups, n=8. **FIG. 5D:** Representative FACS analysis of naïve T cells, central

memory T cells (Tcm) and effector T cells (Tem) ratio in CD8 positive (CD8+) T cells and

their quantification in recipient mice of the three groups, n=8. **FIG. 5E:** Physical tests of

recipient mice that had received CD150^{low}, whole-HSCs and CD150^{high} HSCs. Muscle

strength, motor coordination and endurance were assessed, n=8. **FIG. 5F:** Bar graph

showing the epigenetic age of recipient mice from different groups, n=4. **FIG. 5G:** Survival

curve showing the lifespan differences among recipient mice that had received old CD150^{low},

whole-HSCs and CD150^{high} HSCs. Each mouse received 2,000 HSCs. Log-rank (Mantel-

Cox) test, n=17. For **FIGs. 5B-5E**, Mean $\pm$ SD, one-way ANOVA, *p<0.05, **p<0.01,

p<0.001, *p<0.0001.

FIGs. 6A-6E provide schematic depictions, bar graphs, and FACS analysis data and results illustrating that the reduction of dysfunctional HSCs ameliorates aging phenotypes in old mice after transplantation. **FIG. 6A:** Diagram showing the experimental design.

Individual transplantation of 500 old CD150^{low} HSCs with: 2,500 CD150^{high} HSCs (1:5

group); 1,000 CD150^{high} HSCs (1:2 group), and 0 CD150^{high} HSCs (1:0 group) into middle-aged recipients (13-months-old). Five months after transplantation, a series hematopoietic and physical tests were performed. **FIG. 6B:** Bar graph showing the percentage of B cells, T cells, and myeloid cells in the PB of recipient mice of the different groups. **FIG. 6C:** Bar graph showing absolute number of blood cells in PB of recipient mice from different transplanted groups. **FIG. 6D:** Representative FACS analysis of naïve T cells and effector T cells (Tem) ratio in CD8 positive T cells and their quantification in recipient mice of different groups. **FIG. 6E:** Bar plot showing the results of physical functional tests of recipient mice of different groups. Muscle strength, motor coordination, endurance and locomotor activity were assessed. n=6 for 1:5 and 1:2 group, n=7 for 1:0 group, Mean $\pm$ SD, one-way ANOVA, *p<0.05, **p<0.01.

FIGs. 7A-7F show FACS analysis data, bar graphs, line graphs and results illustrating age-related functional decline of HSCs in mice (related to above **FIGs. 1A-1H**). **FIG. 7A:** The gating strategy for sorting LT-HSCs. Cells of the lineage Sca-1⁺c-Kit⁺(LSK) were first gated, and LT-HSCs (CD48⁺CD34⁺CD150⁺) were gated in the LSK population. **FIG. 7B:** Bar graph showing the percentage of LT-HSCs in LSK HSPCs from young (2-3 months) and old (22-24 months) mice, n=10 for young, n=9 for old. **FIG. 7C and FIG. 7D:** Peripheral blood chimerism of donor HSCs at different times after the first (**FIG. 7C**) and second (**FIG. 7D**) transplantation, n=6 for the first and n=3 for the second transplantation. **FIG. 7E:** Diagram illustration of individual transplantation of young and old HSCs into young recipient mice for assessing differentiation bias. **FIG. 7F:** Analysis of HSC differentiation toward T cells, B cells, and myeloid cells at different times after transplantation, n=3. Mean $\pm$ SD, student t test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

FIGs. 8A and 8B provide FACS analysis data and bar graph results demonstrating the immune profile alterations during aging in mice (related to above **FIGs. 1A-1H**). **FIG. 8A and FIG. 8B:** Representative FACS analysis and bar graph plots showing the change in percentage of naïve, Tcm and Tem with aging in PB of mice in CD8⁺ T cells (**FIG. 8A**) and CD4⁺ T cells (**FIG. 8B**). Mean $\pm$ SD, student t test, n=4, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

FIGs. 9A-9D provide FACS analysis data, bar graphs and pictorial guides demonstrating that **transplantation of young HSCs rejuvenates old mice (related to FIGs. 1A-1H)**. **FIG. 9A:** Representative FACS analysis and bar plot showing the percentage of naïve T cells in CD4⁺ T cells from recipient mice, n=8. **FIG. 9B:** Bar plots showing the body

weights of recipient mice that had received young or old HSCs, n=8. FIG. 9C: Bar plots showing the total moving distance and the ratio of duration in novel arm to familiar arm in Y maze test of recipient mice, n=8. FIG. 9D: Bar plot showing the results of the fear conditioning test. Compared to acclimation, the increased freezing time was calculated, n=8.

5 Mean $\pm$ SD, student t test, **p<0.01, ns, not significant.

FIGs. 10A-10D provide a heat map, bar graph data, UMAP data, and violin plot data and results demonstrating that scRNA-seq reveals increased heterogeneity of old HSCs (related to FIGs. 2A-2H). FIG. 10A: Heatmap showing HSC aging related genes that were identified through bulk RNA-seq analysis. Well-known HSC marker genes in public datasets are bolded. FIG. 10B: Bar graph showing the enriched GO terms in HSC aging related up and down genes identified in (FIG. 10A). FIG. 10C: UMAP showing the expression of the young HSC marker genes (*Rnase6* and *Arhgap30*) in single cells. FIG. 10D: UMAP (left) showing the calculated aging score based on public HSC aging marker genes in each single cell. Violin plot (right) showing the aging score of cells from different clusters. Two-sided
15 unpaired Wilcoxon test, **** p<0.0001.

FIGs. 11A-11H provide dot plots, violin plots, heat map, UMAP, and bar graph data and results showing that CD150 can serve as an aging heterogeneity marker of old HSCs (related to FIGs. 3A-3I). FIG. 11A: Dot plot showing ranking of genes based on the correlation of their expression and aging score in scRNA-seq. The top 150 highly ranked
20 genes were highlighted. FIG. 11B: Violin plot showing expression level of potential marker genes in cluster q1, q2 and q3 by scRNA-seq. FIG. 11C: Dot plot showing the ranking of genes based on their correlation with CD150 levels in expression of the four cell groups in FIG. 3C measured by bulk RNA-seq. A total of 131 positively correlated genes (R>0.8) and 103 negatively correlated genes (R<-0.75) were identified as CD150 related genes. FIG.
25 11D: Heatmap showing expression changes of CD150-related genes with ascending CD150 level based on bulk RNA-seq. For positive correlated genes, previously identified HSC aging marker genes are in bold. For negative correlated genes, genes that are highly expressed in young HSCs are in bold. FIGs. 11E and 11F: Feature plot (FIG. 11E) and violin plot (FIG. 11F) showing CD150 signature scores of the six cell clusters in scRNA-seq. FIG. 11G:
30 Representative images and bar plot showing the level of γ H2AX in old CD150^{low} and CD150^{high} HSCs, n=3. FIG. 11H: Left, representative FACS plot showing the percentage of old CD150^{low} and CD150^{high} HSCs in different cell cycle phases. Right, bar graph showing the percentage of old CD150^{low} and CD150^{high} HSCs in active cell cycle (G1 and G2/S/M),

n=3. For **FIG. 11G** and **FIG. 11H**, Mean $\pm$ SD, student t test, * $p < 0.05$, ** $p < 0.01$. For (F), Two-sided unpaired Wilcoxon test, **** $p < 0.0001$.

FIGs. 12A and 12B provide graphs of transplantation results showing that old CD150^{low} HSCs are functionally superior to CD150^{high} HSCs *in vivo* (related to **FIGs. 3A-3I**). **FIGs. 12A and 12B**: The peripheral blood chimerism of donor HSCs at different times after transplantation in the first (**FIG. 12A**) and second (**FIG. 12B**) competitive transplantation. B cells, T cells, and myeloid cells were analyzed individually. Mean $\pm$ SD, student t test, n=6 for the first and n=3 for the second transplantation, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

FIGs. 13A-13C provide FACS analysis plots, bar graphs, schematic diagrams, and line graphs illustrating the comparable function of CD150^{low} and CD150^{high} HSCs from young mice (related to **FIGs. 3A-3I**). **FIG. 13A**: Left, representative FACS plots showing the percentage of young CD150^{low} (25% lowest) and CD150^{high} (25% highest) HSCs in different cell cycle phases. Right, bar graph showing the average percentage of young CD150^{low} and CD150^{high} HSCs in active cell cycle (G1 and G2/S/M), n=3. **FIG. 13B**: Diagram illustrating the competitive transplantation for evaluating the repopulating capacity of CD150^{low} and CD150^{high} HSCs from young mice. The ratio of competitor to donor HSCs to was 1:2 (300 competitor HSCs with 600 donor HSCs). **FIG. 13C**: The peripheral blood chimerism of donor HSCs at different times after transplantation. Whole blood, T cells, B cells, and myeloid cells were analyzed. n=3, Mean $\pm$ SD, student t test, * $p < 0.05$, ** $p < 0.01$, ns, not significant.

FIGs. 14A-14K provide schematic diagrams, FACS analysis plots, Venn diagrams, bar graphs, UMAP data, and plots showing that differentiation, but not self-renewal, is a major defect of old CD150^{high} HSCs (related to **FIGs. 4A-4H**). **FIG. 14A**: Schematic diagram of individual transplantation of old HSCs to study the differentiation trajectory after transplantation. The donor HSCs derived from HSPCs in bone marrow were analyzed on days 4, 7, 10 and 14 after transplantation. **FIG. 14B**: FACS analysis results showing the differentiation trajectory of old HSCs after transplantation. On the 4th day, most donor HSCs were still maintained as LT-HSCs. From day 7 and beyond, the LT-HSCs started to differentiate toward multipotent progenitor cells (MPPs), the immediate progeny of HSCs. (See, e.g., L. Purton, 2022, Society for Hematology and Stem Cells, doi.org/10.1016/j.exphem.2022.10.005). **FIG. 14C**: Representative FACS analysis of donor HSCs derived HSPCs (left) and quantification of different cell populations after

transplantation (right) on day 14, n=3. **FIG. 14D:** Diagram of transplantation to evaluate the differences in activation between old CD150^{low} and CD150^{high} HSCs on day 4 after transplantation. The sorted donor HSCs were collected for transcriptome analysis, n=3. **FIG. 14E:** Venn diagram showing the genes that were commonly activated in old CD150^{low} and CD150^{high} HSCs compared to freshly isolated old CD150^{low} and CD150^{high} HSCs 4 days after transplantation (left). Bar graph showing enriched GO terms of the 794 commonly activated genes (right). **FIG. 14F:** GSEA analysis showing that transplanted HSCs highly expressed cell cycle related genes in both CD150^{low} (left) and CD150^{high} (right) HSCs when compared with freshly isolated HSCs. **FIG. 14G:** Schematic diagram showing the experimental design for examining HSC proliferation *in vitro*. In each well, 50 GFP+ cell and 50 old CD150^{low} or CD150^{high} HSCs were co-cultured. The percentage and absolute number of old CD150^{low} and CD150^{high} HSCs were quantified 6 days after culture. **FIG. 14H:** Bar graph showing comparable proliferation rate of old CD150^{low} and CD150^{high} HSCs 6 days after culture. The percentage of non-GFP HSCs was shown, n=10. **FIG. 14I:** Bar graph showing the absolute number of old CD150^{low} and CD150^{high} HSCs 6 days after culture, n=10. **FIG. 14J:** UMAP and bar graph showing the distribution and percentages of cells in different cell cycle phases of HSPCs from CD150^{low} and CD150^{high} HSCs 14 days after transplantation. **FIG. 14K:** Violin plot showing the expression pattern of cell type marker genes, including *Ly6a*, *Kit*, *Cd34*, *Cd48*, *Flt3* and *Slamf1* (CD150). Mean $\pm$ SD, student t test, ns, not significant.

FIGs. 15A-15B provide FACS analysis plots and bar graphs showing that transplantation of a “youthful” subset old HSCs attenuates aging phenotypes of old mice (related to **FIGs. 5A-5G**). **FIG. 15A:** Representative FACS (left) analysis of naïve T cells, Tcm and Tem ratio in CD4 positive T cells of mice from different groups and their quantification (right, bar graphs). **FIG. 15B:** Bar graph showing body weight of recipient mice from different groups. Mean $\pm$ SD, one-way ANOVA, n=8, *p<0.05, **p<0.01, ***p<0.001, ns, not significant.

FIGs. 16A and 16B provide FACS analysis plots and bar graphs demonstrating that reducing dysfunctional HSCs ameliorates aging phenotypes in old mice post transplantation (related to **FIGs. 6A-6E**). **FIG. 16A:** Representative FACS (left) analysis of naïve T cells and Tem ratio in CD4 positive (CD4+) T cells of mice from the different groups and their quantification (right, bar graphs). **FIG. 16B:** Bar graph showing body weight of recipient mice from different groups. n=6 for 1:5 and 1:2 group, n=7 for 1:0 group; Mean $\pm$ SD, one-way ANOVA, *p<0.05, **p<0.01, ns, not significant.

FIGs. 17A-17D provide a schematic diagram, and bar graphs demonstrating the targeted depletion of dysfunctional CD150^{high} HSCs *in vitro*. **FIG. 17A**: Diagram illustration of the CD150-SAP mediated killing principle. Bio-CD150, biotin-conjugated CD150 antibody; Strep-SAP, streptavidin-conjugated saporin. **FIG. 17B**: Dosage-dependent killing of cultured HSCs *in vitro* by CD150-SAP, measured using a cell titer assay. The TC15-12F12.2 clone of the CD150 antibody was used for CD150-SAP complex reconstitution. **FIG. 17C, FIG. 17D**: CD150^{high} HSC-specific elimination was achieved by careful titration of the CD150-SAP complex concentration. 300 CD150^{low} HSCs from a 17-month-old B6.SJL-*Ptprca*^a *Peprc*^b/BoyJ (CD45.1) mouse were mixed with 300 CD150^{high} HSCs from a 17-month-old C57BL/6J (CD45.2) mouse and the resulting cell mixture was treated with CD150-SAP, IgG-SAP or CD150 antibody for 3 days. The cell number (**FIG. 17C**) and percentage (**FIG. 17D**) of CD45.1⁺ and CD45.2⁺ cells were analyzed after the 3 day treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

FIGs. 18A-18D provide a schematic depiction, line and bar graphs, and FACS analysis data illustrating the targeted depletion of dysfunctional CD150^{high} HSCs *in vivo*. **FIG. 18A**: Experimental scheme for assessing the persistency and labeling efficiency of HSC in mice by the bio-CD150 antibody. At 1, 3, 6, 12, 20 days, mice were sacrificed, and the CD150 signal on HSCs in the bone marrow was measured by strep-FITC (streptavidin-conjugated FITC). Bio-CD150 antibody was added to half of the sample to label the HSCs in the bone marrow. **FIG. 18B**: The percentage of HSCs labeled by injected bio-CD150 at different time points. **FIG. 18C**: The percentage of HSCs in total bone marrow cells or in the LSK (lineage-, Sca1⁺, cKit⁺) compartment 3 weeks post CD150-SAP treatment. **FIG. 18D**: The distribution of HSCs in relation to CD150 levels in mice with or without CD150-SAP treatment *in vivo*. The expression level of CD150 on the remaining HSCs was analyzed 3 weeks after different doses of CD150-SAP injection. The percentage of CD150^{low}, CD150^{med}, and CD150^{high} HSCs was calculated 3 weeks post CD150-SAP treatment. HSCs were divided into 3 groups (CD150^{low}, CD150^{med}, and CD150^{high}) with equal numbers based on the CD150 signal in PBS-injected control mice. Quantification of the three cell groups is shown by the bar-graphs. *p<0.05, **p<0.01, ****p<0.0001.

FIGs. 19A-19E provide bar graphs and FACS analysis results showing that depletion of dysfunctional CD150^{high} HSCs *in vivo* improves hematopoiesis. **FIG. 19A**: The percentage of HSCs in total bone marrow cells or in the LSK compartment 1.5 months after treatment with CD150-SAP (1 mg/kg). **FIG. 19B**: The expression level of CD150 on the

remaining HSCs 1.5 months after treatment with CD150-SAP (1 mg/kg). The percentage of CD150^{low}, CD150^{med}, and CD150^{high} HSCs was calculated based on equal distribution of the cells in the three cell groups (CD150 low, med, and high) in PBS. **FIGs. 19C-19E**: The percentage of B cell (**FIG. 19C**), T cell (**FIG. 19D**), and myeloid cell (**FIG. 19E**) in peripheral blood analyzed at different time points after the CD150-SAP treatment. Number of mice in each group, n=6-9. In **FIGs. 19C-E**, the leftmost bar in the groups of bars on the x-axis for each of the delineated days represents PBS control; the second bar represents a CD150-SAP dose of 1 mg/kg; the third bar represents a CD150-SAP dose of 2 mg/kg; and the fourth bar (rightmost bar) represents a CD150-SAP dose of 4 mg/kg.

DETAILED DESCRIPTION OF THE EMBODIMENTS

As featured and described herein, a clear heterogeneity of old (aging) hematopoietic stem cells (HSCs) exists, and this heterogeneity can be marked by the levels of CD150 glycoprotein in or expressed by the HSCs. Comparative molecular and functional analyses revealed that HSCs from old mice with low levels of CD150 (CD150^{low} HSCs) have a younger aging clock, transcriptome, and better repopulation capacity compared to HSCs having high levels of CD150 (CD150^{high} HSCs). Mechanistically, CD150^{high} HSCs from old mice have greatly compromised differentiation capacity compared to that of the CD150^{low} HSCs. In addition, decreasing the CD150^{high} HSC ratio in old mice can alleviate aging-related functional decline. Described herein are disclosure and results showing that HSC heterogeneity contributes to aging, and providing therapeutics and methods for rejuvenation and countering aging in the body.

Characteristics of the Aging Process

The aging process is marked by a functional decline across multiple tissues and organs, including the hematopoietic system, which significantly increases the risk for a multitude of chronic diseases. Hematopoietic homeostasis declines with age, leading to deterioration in blood regeneration and the immune system response, as well as to an increase in the risk of anemia and leukemia. Interestingly, transfusion of young blood and its components into an old or aged subject's body, e.g., old or aged mice, is able to induce rejuvenation of multiple organs, including brain, muscle and liver, supporting an important role of the hematopoietic system in whole body aging. As the source of all cell types of the hematopoietic system, hematopoietic stem cells (HSCs) undergo profound functional changes

with age, including increased prevalence of clonal hematopoiesis, a shift toward myeloid-biased differentiation, and a diminished capacity for blood regeneration. Recent studies have showed that replacing HSCs of old mice with those of young ones can improve health and extend the lifespan of old recipient mice (Guest, I. et al., (2015), *Aging* (Albany NY) 7(12): 1212-1223; Das, M.M. et al., (2019), *Commun Biol*, 2: 73; Li, J. et al., (2019), *Aging Cell*, 18(6): e13026; Guderyon, M.J. et al., (2020), *Aging Cell*, 19(3): e13110), suggesting that HSCs play a central role in systematic aging. Transplanting aged HSCs into young mice can accelerate immune system aging and reduce their lifespan. Conversely, transplanting HSCs from young mice into old or aged recipient mice can improve the health and expand the lifespan of the old or aged recipient mice. The hematopoietic system, and particularly HSCs, are thus highlighted as key players in the aging process, suggesting that HSCs could serve as promising targets for aging-related intervention.

The function of HSCs is influenced by the bone marrow niche, and the bone marrow niche of old HSCs has been reported to restrict the functions of HSCs, as old HSCs transplanted into young recipients were found to show a better engraftment than those transplanted into old ones (Ergen, A.V. et al. 2012, *Blood* 119(11): 2500-2509). However, recent research studies have indicated that intrinsic alterations of HSCs with aging are associated with their functional decline, such as increased DNA damage, mitochondrial degeneration, diminished asymmetric distribution of CDC42, altered H4K16 acetylation during mitosis, and epigenetic modifications. The HSC population exhibits intrinsic heterogeneity in both young and old mice, distinguishable by lymphoid-biased, balanced, and myeloid-biased subtypes, based on surface markers and lineage predispositions. Notably, a fraction of HSCs can preserve long-term dormancy and functionality during aging. The changes of HSC heterogeneity during aging and the contribution of HSCs to systemic aging are comprehensively characterized as described herein.

As described and demonstrated herein, the transplantation of young HSCs can rejuvenate old mice. Single cell RNA sequencing (scRNA-seq) has revealed an increased aging heterogeneity in old HSCs. Systematic molecular and functional characterization indicated the presence of “youthful” and functionally defective “old” subsets of HSCs in old mice, marked by differential expression levels of CD150. Mechanistically, old CD150^{high} HSCs exhibit differentiation defects when compared to CD150^{low} HSCs. Notably, transplantation of the “youthful” subset of HSCs, e.g., CD150^{low} HSCs, into old recipient mice can attenuate aging phenotypes, including decreased epigenetic age and extended

lifespan of old recipient mice when compared with old recipient mice that received old unselected HSCs or CD150^{high} HSCs. Based on FACS analysis of LT-HSCs to determine CD150 surface expression level, the lowest 25% subpopulation (lower level of CD150) is defined as CD150^{low} HSCs, which shows youthful signatures and exhibits good function. The highest 25% subpopulation (higher level of CD150) is defined as CD150^{high} HSCs, which show aging signatures and phenotypes when administered, e.g., via transplantation, into recipient mice. In an embodiment, the LT-HSCs, which comprise a source of CD150^{low} and CD150^{high} HSCs, express the following biomarker signature on their cell surface: Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺. In an embodiment, the LT-HSCs are derived from bone marrow. In an embodiment, HSCs are isolated and/or purified from bone marrow. Importantly, reducing the dysfunctional CD150^{high} HSCs was found to attenuate aging phenotypes in old recipient mice, thus, highlighting that the removal of defective CD150^{high} HSCs from old mice could be a potential strategy for rejuvenation.

A key role of HSCs in systematic aging was demonstrated by transplantation, as described and exemplified herein (**FIGs. 1A-1H**). By integrating scRNA-seq and functional evaluation, a “youthful” subset of HSCs was identified in old / aged mice that is marked by a low level of CD150 (**FIGs. 2A-2H and FIGs. 3A-3I**). Comparative analyses demonstrated that CD150^{high} HSCs from old mice are defective in Long Term-Hematopoietic Stem Cell (LT-HSC) to Short Term-Hematopoietic Stem Cell (ST-HSC) differentiation when compared with the CD150^{low} HSCs (**FIGs. 4A-4H**). Importantly, transplantation of the “youthful” CD150^{low} HSCs was able to alleviate aging phenotypes and extend lifespan in old mice (**FIGs. 5A-5G**). As further described and demonstrated herein, the hematopoietic system and physical performance of old recipient mice was improved by reducing the ratio of functionally defective CD150^{high} HSCs in these mice (**FIGs. 6A-6E**), supporting the feature that rejuvenation can be achieved by removing the dysfunctional HSCs in the elderly.

In an embodiment, young hematopoietic stem cells (HSCs) can rejuvenate an old or aging subject when transplanted into the old or aging subject as described and exemplified herein (**FIGs. 1C-1H and FIGs. 9A-9D**). In an embodiment, CD150 is identified as a marker of HSC aging heterogeneity as described and exemplified herein (**FIG. 3G and FIGs. 11G and 11H**). In an embodiment, transplantation of the “youthful” subset of old HSCs can rejuvenate an old recipient subject as described and exemplified herein (**FIG. 5G**). In an embodiment, the removal, elimination, deletion, depletion, or reduction of dysfunctional

HSCs rejuvenates old recipient subjects in transplantation as described and exemplified herein (**FIGs. 6A-6D** and **FIGs. 15A and 15B**).

CD150 level as an indicator of aging heterogeneity in old HSCs

Single cell RNA sequencing (scRNA-seq) revealed that while quiescent HSCs in young mice are relatively homogeneous, the quiescent HSC counterpart in old mice are heterogenous (**FIG. 2D** and **FIG. 2H**). By analyzing the genes highly expressed in “older” subpopulations of aged HSCs, the CD150 glycoprotein (encoded by the *Slamf1* gene) was identified as a cell surface marker expressed on HSCs that can serve as an indicator of HSC aging in transcriptome, epigenome and function in old HSCs (**FIGs. 2A-2H** and **FIGs. 3A-3I**). Originally characterized as a surface marker for LT-HSCs (Kiel, M.J. et al., (2005), *Cell* 121, 1109-1121. 10.1016/j.cell.2005.05.026), CD150 has also been used to reflect differentiation bias of HSCs in young and old mice. In addition, CD150^{low} HSCs from aged mice have been shown to retain normal lymphoid differentiation capacity when removed from the aged microenvironment (Montecino-Rodriguez, E., et al., (2019). *Stem Cell Reports*, 12, 584-596. 10.1016/j.stemcr.2019.01.016). These previous reports suggest a potential link between CD150 levels and HSC function during aging and are in line with the aspects and embodiments described herein, which further support CD150 as a marker of HSC aging heterogeneity.

In addition to CD150, other cell surface proteins, such as *Lpar6*, *Ehd3* and *Tm4sf1*, were identified that can also mark the “older” populations in old HSCs. *Clca3a1* can also serve as a marker to capture the HSC heterogeneity in old mice. In particular, *Clca3a1*^{high} HSCs from aged mice have a decreased repopulation capacity in primary transplantation and exhibit a myeloid-biased differentiation compared to *Clca3a1*^{low} HSCs (Kim, K.M. et al., (2022), *Nat Commun*, 13, 5187. 10.1038/s41467-022-32970-1). However, the functional difference between *Clca3a1*^{high} and *Clca3a1*^{low} old HSCs was not maintained in a second transplantation. In contrast, the functional difference between CD150^{high} and CD150^{low} old HSCs persisted in a second transplantation (**FIG. 3I**), indicating that CD150 is a more robust indicator of the functional heterogeneity of aged HSCs. *Clca3a1* was not in the potential marker gene list as described herein due to its minimal expression difference between q3 and q1+q2 clusters in the scRNA-seq analysis.

A “youthful” subset of old HSCs is functional in alleviating aging phenotypes

As described and demonstrated herein, transplantation of young HSCs is promising for anti-aging treatment (**FIGs. 1A-1H**). However, due to immune rejection, transplantation of young HSCs as an anti-aging therapy may be problematic. By combining scRNA-seq with functional analysis, a “youthful” subset of HSCs that is marked by a lower level of CD150 was identified in old mice (**FIGs. 2A-2H and FIGs. 3A-2I**). Based on FACS analysis of LT-HSCs, based on the CD150 level, the lowest 25% subpopulation (lower level of CD150) is defined as CD150^{low} HSCs, which shows youthful signatures and exhibit good function. The highest 25% subpopulation (higher level of CD150) is defined as CD150^{high} HSCs, which shows aging signatures and phenotypes when administered, e.g., via transplantation, into recipient mice. Comparative analyses further demonstrated that this subset of HSCs is superior to the whole-HSCs or CD150^{high} HSCs in terms of: 1) balanced differentiation (**FIG. 5B**); 2) absolute blood cells numbers (**FIG. 5C**); 3) immune system quality (**FIG. 5D**); 4) physical functions (**FIG. 5E**); 5) epigenetic age (**FIG. 5F**) and 6) lifespan (**FIG. 5G**). These results support the feature that transplantation of a “youthful” subset from old donors has anti-aging effects. Given that these “youthful” HSCs reside in aged individuals, immune rejection is not an issue. The demonstrated rejuvenation effect of this “youthful” HSC population found in old mice could serve as a potential source for anti-aging therapies in other mammals, including humans.

Removal of dysfunctional HSCs in old subjects as a means of rejuvenation

Since the discovery of the rejuvenating effects of young blood through parabiosis, great efforts have been expended searching for rejuvenation factors from young blood. Although several modulating candidate factors, such as CDC42, SELP, CCL5, PHF6, IGF1, PF4 and the Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) have been reported to have rejuvenating effects during the past years, their rejuvenating capacities are limited, and the mechanisms underlying their rejuvenating effects are not clear. In some cases, such as GDF11, conflicting results have been reported. Thus far, no universally accepted rejuvenating factors have been identified. The findings described herein reveal that old HSCs exhibit molecular and functional heterogeneity, comprising “youthful” and dysfunctional “old” subsets (**FIGs. 4A-4H and FIGs. 5A-5G**). Moreover, reducing the proportion of dysfunctional HSCs ameliorates aging phenotypes in aged mice after transplantation (**FIGs.**

6A-6E), suggesting that removal of functionally defective HSCs from old subjects offers a promising approach for rejuvenation. Consistent with this notion, depletion of the myeloid-biased HSCs (marked by NEO1) by a specific antibody cocktail was reported to restore immune functions in old mice (Ross, J.B. et al., (2024), *Nature*, 628, 162-170.

10.1038/s41586-024-07238-x), although it is not known if this approach leads to a systemic body rejuvenation. The aspects and embodiments as described herein offer approaches to achieving rejuvenation in the elderly by targeted removal of dysfunctional HSCs.

Targeted depletion of dysfunctional CD150^{high} HSCs as a strategy to achieve rejuvenation in old or aged mammalian subjects

The development of translatable rejuvenation approaches for the hematopoietic system holds great promise for mitigating age-associated blood disorders and improving immune system of aged individuals. Although various interventions have been applied to rejuvenate old or aged HSCs to restore their youthful state, many of these methods are non-specific and have been met with limited success. A significant proportion of rejuvenation methods rely on transplantation, which involve irradiation and other manipulation that do not reflect real physiological conditions. The use of an anti-CD150 antibody conjugated to saporin (CD150-SAP) provides a new strategy for HSC rejuvenation by specifically targeting and removing dysfunctional, CD150-expressing HSCs in old or aged subjects via specific binding to the CD150 cell surface antigen. As described and exemplified herein, hematopoietic rejuvenation after CD150-SAP treatment was achieved *in vivo* in mammalian subjects.

Methods of Use

Provided herein are methods that are useful for reducing, abrogating, checking, or slowing down the aging process. Methods are further provided for rejuvenating blood components of an aged or aging subject, or alleviating old or aging-related functional decline in an old, aging, or aged subject. In accordance with the aspects and embodiments described herein, removal or dilution of the defective HSCs, e.g., CD150^{high} HSCs, in an aged subject's body can improve the blood quality of the subject. In an embodiment, the methods involve administering to an aging or old subject an effective amount of a composition comprising CD150^{low} HSCs and a physiologically acceptable carrier, excipient or diluent, in particular, to increase the number of CD150^{low} HSCs in the aging or old subject, thereby improving the

composition of the old or aging subject's blood components (blood cell types/lineages) which differentiate from the CD150^{low} HSCs.

The methods described herein may be advantageous and beneficial to rejuvenate or to improve the immune system and physical performance, particularly in aging, aged, older or elderly mammalian subjects and individuals, including humans. Without intending to be limiting, an old, older, aged, or aging individual, subject, or body, including a human subject or individual, may encompass one of a variety of ages and age ranges, for example, 40, 50, 60, 70, 80, 90 years of age or older. Ages between any of the foregoing years of age are also included. In nonlimiting embodiments, an aging, aged, old, or older subject is greater than or equal to 50 years of age, or is greater than 60, 70, or 80 years of age. In an embodiment, an old or aging subject is characterized by having a significant number or amount of CD150^{high} HSCs in peripheral blood and/or bone marrow. A young individual, subject, or body, including a human individual, subject, or body, may encompass one of a young age or age range, for example, an infant (1-12 months of age), a child (1-3, 1-5, or 1-10 years of age), or a young subject prior to adulthood (e.g., 10-21 years of age). Ages between any of the foregoing are also included. In a particular embodiment, a young subject is characterized by having a significant number or amount of CD150^{low} HSCs in peripheral blood and/or bone marrow. Without wishing to be bound by theory, and for relative comparison purposes, a mouse of 3-6 months of age, e.g., young (mature) adult mouse, may correlate with a human of 20-30 years of age; a mouse of 10-15 months of age, e.g., a middle-aged mouse, may correlate with a human of 38-47 years of age; and a mouse of 18-24 months of age, e.g., an old (elderly), aged or aging mouse, may correlate with a human of 56-69 years of age. (Hagan, C., (2017), The Jackson Laboratory Blog Post, jax.org/news-and-insights/jax-blog/2027/november/when-are-mice-considered-old).

Identifying or detecting CD150^{high} HSCs and other associated markers (e.g., other cell surface proteins, such as, for example, *Ramp2*, *Gpr183*, *Ehd3* and *Tm4sf1* that can distinguish the q3 from the q1+q2 HSC populations as described herein, and can be used to distinguish the heterogeneous population of aged HSCs) in an individual or subject may be used to identify, categorize, characterize, or select an individual or subject as being old, older, aged, or aging, and in need of an increased number of CD150^{low} HSCs in accordance with the methods and results described herein. An older individual, subject, patient, body, and the like, may be identified through testing or assay as having CD150^{high} HSCs in a blood, serum, plasma, bone marrow, or other bodily sample or fluid. Such testing may involve RNAseq,

and the like, or may involve an immunoassay or other binding assay in which an antibody, e.g., an anti-CD150 antibody, e.g., a detectably labeled antibody, is used to detect expression of the CD150 glycoprotein in HSCs isolated from a sample, e.g., blood, peripheral blood (PB), serum or bone marrow, and the like. Detection of CD150 expression on cells (HSCs) and analysis thereof can be performed using flow cytometry analysis, FACS, or any other suitable detection assay used in the art.

Hematopoietic stem cells (HSCs) may be isolated from bone marrow by methods known and used in the art. CD150^{high} HSCs and CD150^{low} HSCs may be identified using labeled, detectable anti-CD150 antibodies that bind to CD150 expressed on HSCs. CD150 alone, or in combination with other surface markers, may be employed to separate HSCs from other blood cells. The identified populations of cells can be isolated via flow cytometry, for example. *See, e.g.,* Rossi, L. et al., 2011, Methods Mol Biol, 750:47-59; Celso, C.L. 2007, J Vis Exp., (2):157.

The methods provided herein involve administering to a subject having, or who is identified or selected as having, hematopoietic stem cells (HSCs) expressing a high level of the glycoprotein CD150 (i.e., CD150^{high} HSCs), an effective amount of HSCs expressing a lower level of CD150 (i.e., CD150^{low} HSCs). In an embodiment, the CD150^{low} HSCs are isolated from a donor subject. In an embodiment, the CD150^{low} HSCs are components of peripheral blood obtained from a subject having normal or higher than normal levels of CD150^{low} HSCs. In embodiments, CD150^{high} HSCs obtained, isolated, or purified from a sample of an older or aging subject may be contacted with an agent that reduces the amount or levels of CD150 in the subject's HSCs, such that the levels of CD150 in the HSCs is reduced and CD150^{high} HSCs are replaced or replenished by CD150^{low} HSCs. By way of nonlimiting example, the agent may be an antisense nucleic acid, polynucleotide, or oligonucleotide, an siRNA, or iRNA. In an embodiment, the subject may be treated so as to remove CD150^{high} HSCs from the blood or to dilute the number of CD150^{high} HSCs in the blood of a subject, e.g., by administering a blood sample containing CD150^{low} HSCs from a donor or a composition comprising an effective amount of CD150^{low} HSCs. In embodiments, the CD150^{low} HSCs may be autologous or allogeneic. In an embodiment, the administering is via transplantation, infusion, or immunotherapy. In an embodiment, the administered CD150^{low} HSCs serve to dilute the number of CD150^{high} HSCs in the subject, thereby resulting in dilution-based rejuvenation. In embodiments, the methods slow down the aging

process, or rejuvenate; or improve the immune system and physical performance of the subject to whom CD150^{low} HSCs have been administered.

Antibodies

As described herein, antibodies that specifically bind a marker (e.g., a cell surface moiety or protein receptor such as CD150) are useful in the methods described herein, including therapeutic and diagnostic methods. In embodiments, a nanoparticle or polymer having an scFv or antibody fragment that specifically binds a cell surface marker of a microglia and contains a therapeutic and/or diagnostic agent is provided.

Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. Tetramers may be naturally occurring or reconstructed from single chain antibodies or antibody fragments. As used herein, the term “antibody” means not only intact antibody molecules, but also fragments of antibody molecules that retain immunogen-binding ability. Such fragments are also well known in the art and are regularly employed both *in vitro* and *in vivo*. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, scFv antibodies, single-domain antibodies, such as camelid antibodies (Riechmann, 1999, *Journal of Immunological Methods*, 231:25-38), composed of either a light chain variable (VL) or a heavy chain variable (VH) domain which exhibit sufficient affinity for the target, and multispecific antibodies formed from antibody fragments.

The antibodies for use may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab')₂, as well as single chain antibodies (scFv), humanized antibodies, and human antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). For example, F(ab')₂, and Fab fragments that lack the Fc fragment of an intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)). Thus, the antibodies may comprise, without limitation, whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain V region fragments (scFv), fusion polypeptides, and unconventional antibodies.

Unconventional antibodies include, but are not limited to, nanobodies, linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062,1995), single domain antibodies, single chain antibodies, and antibodies having multiple valencies (*e.g.*, diabodies, tribodies, tetrabodies, and pentabodies). Nanobodies are the smallest fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain. Nanobodies have the affinity and specificity of conventional antibodies although they are only half of the size of a single chain Fv fragment. The consequence of this unique structure, combined with their extreme stability and a high degree of homology with human antibody frameworks, is that nanobodies can bind therapeutic targets not accessible to conventional antibodies. Recombinant antibody fragments with multiple valencies provide high binding avidity and unique targeting specificity to cancer cells. These multimeric scFvs (*e.g.*, diabodies, tetrabodies) offer an improvement over the parent antibody since small molecules of ~60-100 kDa in size provide faster blood clearance and rapid tissue uptake. See Power et al., (Generation of recombinant multimeric antibody fragments for tumor diagnosis and therapy. Methods Mol Biol, 207, 335-50, 2003); and Wu et al. (Anti-carcinoembryonic antigen (CEA) diabody for rapid tumor targeting and imaging. Tumor Targeting, 4, 47-58, 1999).

Various techniques for making and using unconventional antibodies have been described. Bispecific antibodies produced using leucine zippers are described by Kostelny et al. (J. Immunol. 148(5):1547-1553, 1992). Diabody technology is described by Hollinger et al. (Proc. Natl. Acad. Sci. USA 90:6444-6448, 1993). Another strategy for making bispecific antibody fragments using single-chain Fv (scFv) dimers is described by Gruber et al. (J. Immunol. 152:5368, 1994). Trispecific antibodies are described by Tutt et al. (J. Immunol. 147:60, 1991). Single chain Fv polypeptide antibodies include a covalently linked VH::VL heterodimer which can be expressed from a nucleic acid including VH- and VL-encoding sequences either joined directly or joined by a peptide-encoding linker as described by Huston, et al. (Proc. Nat. Acad. Sci. USA, 85:5879-5883, 1988). See, also, U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754.

In various embodiments, an antibody is monoclonal. Alternatively, the antibody is a polyclonal antibody. The preparation and use of polyclonal antibodies are also known by the skilled artisan. Hybrid antibodies are also encompassed, in which one pair of heavy and light chains is obtained from a first antibody, while the other pair of heavy and light chains is

obtained from a different second antibody. Such hybrids may also be formed using humanized heavy and light chains. Such antibodies are often referred to as “chimeric” antibodies.

In general, intact antibodies are said to contain “Fc” and “Fab” regions. The Fc regions are involved in complement activation and are not involved in antigen binding. An antibody from which the Fc’ region has been enzymatically cleaved, or which has been produced without the Fc’ region, designated an “F(ab’)₂” fragment, retains both of the antigen binding sites of the intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an “Fab” fragment, retains one of the antigen binding sites of the intact antibody. Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain, denoted “Fd.” The Fd fragments are the major determinants of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity). Isolated Fd fragments retain the ability to specifically bind to immunogenic epitopes.

Methods of preparing antibodies are well known to those of ordinary skill in the science of immunology. Antibodies can be made by any of the methods known in the art utilizing a soluble polypeptide, or immunogenic fragment thereof, as an immunogen. One method of obtaining antibodies is to immunize suitable host animals with an immunogen and to follow standard procedures for polyclonal or monoclonal antibody production. The immunogen will facilitate presentation of the immunogen on the cell surface. Immunization of a suitable host can be carried out in a number of ways. Nucleic acid sequences encoding polypeptides or immunogenic fragments thereof, can be provided to the host in a delivery vehicle that is taken up by immune cells of the host. The cells will in turn express the polypeptide thereby generating an immunogenic response in the host. Alternatively, nucleic acid sequences encoding human polypeptides or immunogenic fragments thereof, can be expressed in cells *in vitro*, followed by isolation of the polypeptide and administration of the polypeptide to a suitable host in which antibodies are raised.

Alternatively, antibodies may, if desired, be derived from an antibody phage display library. A bacteriophage is capable of infecting and reproducing within bacteria, which can be engineered, when combined with human antibody genes, to display human antibody proteins. Phage display is the process by which the phage is made to 'display' the human antibody proteins on its surface. Genes from the human antibody gene libraries are inserted

into a population of phage. Each phage carries the genes for a different antibody and thus displays a different antibody on its surface.

Antibodies made by any method known in the art can then be purified from the host. Antibody purification methods may include salt precipitation (for example, with ammonium sulfate), ion exchange chromatography (for example, on a cationic or anionic exchange
5 column preferably run at neutral pH and eluted with step gradients of increasing ionic strength), gel filtration chromatography (including gel filtration HPLC), and chromatography on affinity resins such as protein A, protein G, hydroxyapatite, and anti-immunoglobulin.

Monoclonal antibodies can be conveniently produced from hybridoma cells
10 engineered to express the antibody. Methods of making hybridomas are well known in the art. The hybridoma cells can be cultured in a suitable medium, and spent medium can be used as an antibody source. Polynucleotides encoding the antibody of interest can in turn be obtained from the hybridoma that produces the antibody, and then the antibody may be produced synthetically or recombinantly from these DNA sequences. For the production of
15 large amounts of antibody, it is generally more convenient to obtain an ascites fluid. The method of raising ascites generally comprises injecting hybridoma cells into an immunologically naive histocompatible or immunotolerant mammal, especially a mouse. The mammal may be primed for ascites production by prior administration of a suitable composition (*e.g.*, Pristane).

20 Monoclonal antibodies (Mabs) produced by the described and commonly used methods can be "humanized" by methods known in the art. "Humanized" antibodies are antibodies in which at least part of the sequence has been altered from its initial form to render it more like human immunoglobulins. Techniques to humanize antibodies are particularly useful when non-human animal (*e.g.*, murine) antibodies are generated.

25 Examples of methods for humanizing a murine antibody are provided in U.S. patents 4,816,567, 5,530,101, 5,225,539, 5,585,089, 5,693,762 and 5,859,205.

In embodiments, an antibody, such as an anti-CD150 antibody, is conjugated to saporin (SAP) to provide an anti-CD150 antibody-saporin (CD150-SAP) conjugate by methods known and used by those in the art and as described herein. Such a CD150-SAP
30 conjugate is suitable for use in depleting or removing from a sample hematopoietic stem cells (HSCs) that express CD150, such as HSCs that express high levels of CD150 (CD150^{high} HSCs). In an embodiment, the anti-CD150 antibody is a monoclonal antibody that binds to/reacts with CD150 (signaling lymphocyte activation molecule or SLAM), which is an ~75

kDa type I transmembrane glycoprotein. In an embodiment, the anti-CD150 antibody is TC15-12F12.2 (Stemcell Technologies, Cambridge, MA). In an embodiment, the saporin protein is conjugated to streptavidin.

Pharmaceutical Compositions and Methods of Delivery

5 Provided in another aspect are compositions, particularly, pharmaceutically acceptable compositions or formulations, comprising the described and exemplified CD150^{low} HSCs or physiological preparations containing the CD150^{high} HSCs for use as therapeutics and for the treatment of aging, for removing or reducing defective HSCs (e.g., CD150^{high} HSCs) in an old, elderly, aging, or aged subject, sample, or body, for improving
10 physical performance, and improving hematopoietic stem cell function, as described herein, or a symptom or side effect thereof. In a particular embodiment, a pharmaceutical composition is provided that contains CD150^{low} HSCs, isolated in a physiologically acceptable solution and/or contained in a blood preparation, as described herein.

 The compositions described herein comprise a pharmaceutically-acceptable diluent,
15 carrier, or excipient. In an embodiment, the pharmaceutically acceptable compositions are in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the admixed components to aging or older patients. Administration may also begin before a patient is symptomatic. The compositions as described herein containing excipients can be prepared by any technique
20 routinely or typically used by the practitioner in pharmacy, pharmaceuticals, drug delivery, pharmacokinetics, medicine, or other related discipline in which an excipient is admixed with other components or reagents, e.g., a drug, medicament, cell population, or therapeutic agent.

 Any appropriate route of administration may be employed. In general, the described methods may be practiced using any mode of administration that is medically acceptable and
25 practicable, i.e., any mode or route that produces effective levels of the active ingredients, such as CD150^{low} HSCs disclosed herein, without causing clinically unacceptable, adverse effects. By way of nonlimiting example, modes and routes of administration include parenteral, intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intracapsular, intrathecal, intracisternal, intranasal, intratracheal, aerosol, topical, transdermal, intravaginal,
30 rectal (suppository), oral administration, transplantation, or within/on implants, e.g., fibers such as collagen, or osmotic pumps, etc. Therapeutic compositions may be in the form of liquid solutions or suspensions or emulsions.

Methods well known in the art for making formulations and compositions are found, for example, in "Remington: *The Science and Practice of Pharmacy*" Ed. A. R. Gennaro, Lippincourt Williams & Wilkins, Philadelphia, Pa., 2000, and updated editions thereof. Formulations suitable for administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful delivery systems for the compositions may include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The formulations may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate. In embodiments, the formulations or compositions comprise the active and inactive ingredients and components, for example, as described in Examples 1-7 herein. In embodiments, the excipients may include, without limitation, pharmaceutically acceptable components such as solvents, thickening agents, or wetting agents.

The compositions and formulations can be administered to human patients in therapeutically effective amounts (e.g., amounts which reduce, improve, or eliminate aging or an age-related condition, e.g., one that is associated with CD150^{high} HSCs) to provide therapy and treatment for aging or reducing the effects of aging, e.g., physical decline, loss of integrity of bone marrow and blood components (e.g., loss of healthy hematopoietic cells of different lineages). The preferred dosage or administered amount of a composition as described herein is likely to depend on such variables as the type and extent of the disease, disorder, or condition, the overall health status and condition of the particular patient, the formulation of the excipients, and the route of administration. In the event that a response in a subject is insufficient at the initial doses, amounts, or cell numbers administered, higher doses (or effectively higher doses by a different, more localized delivery route), or a larger number of doses, may be employed to the extent that patient tolerance permits. A single dose or multiple doses per day are contemplated to achieve appropriate systemic levels of the active ingredients, e.g., CD150^{low} HSCs, and maintenance thereof over time in the body. Buffers used herein may include acidic buffers, neutral pH buffers, and buffers meant to simulate or mimic biological pH levels.

Kits or Articles of Manufacture

Also provided are kits or articles of manufacture for use in the described methods of counteracting the effects of aging of an old, aged, or aging subject or body, or rejuvenation of blood cell components of an old, aged, or aging patient (recipient) following CD150^{low} HSC transplantation, improving physical health, and the like. In one embodiment, the kit includes a therapeutic agent, e.g., composition comprising CD150^{low} HSCs or a sample or physiological solution containing the CD150^{low} HSCs. In an embodiment, the kit contains a composition comprising an effective amount of hematopoietic stem cells (HSCs) expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs), wherein said CD150^{low} HSCs have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell based on flow cytometry or fluorescence activated cell sorting (FACS) analysis and/or wherein CD150^{low} HSCs express one or more genes selected from *Serpina1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*, which correlate with CD150^{low} expressing HSCs. In an embodiment, CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature and CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis. In an embodiment, the composition contains a physiologically acceptable carrier, excipient or diluent. In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic composition; such containers can be boxes, ampoules, bottles, vials, tubes, culture plates, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments or biological therapeutics. In an embodiment, the kit contains the composition in the form of a solution for intravenous administration.

If desired, instructions for administering the therapeutic to a subject to improve physical performance of the subject by increasing the numbers, levels, or amounts of CD150^{low} HSCs or decreasing the numbers, levels, or amounts of CD150^{high} HSCs in the subject as described herein. The instructions, if provided, will generally include information about the use of the composition. In other embodiments, the instructions include at least one of the following: description of the compositions and components; methods for using the

enclosed materials for the treatment of a disease; precautions; warnings; indications; clinical or research studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

5 The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the aspects and embodiments as described herein, and are not intended to limit the scope of the disclosure or the embodiments as described herein.

10 **EXAMPLES**

Example 1: Transplantation of young HSCs rejuvenates old recipient mice

To directly evaluate the functional differences between young and old HSCs *in vivo*, long-term (LT)-HSCs [lineage⁻c-Kit⁺Sca-1⁺ (LSK) CD48⁻CD34⁺CD150⁺] (referred to as HSCs) were sorted from young (2-3 months old) and old (22-24 months old) mice (**FIG. 7A**).

15 Consistent with previous reports (Sudo, et al. 2000, *Ibid.*, Rossi et al. 2005. *Ibid.*), a significant increase in the number of HSCs in old mice was observed when compared with their young counterparts (**FIG. 7B**). Serial transplantation of both young and old HSCs into lethally irradiated young recipient mice (**FIG. 1A**), followed by examination of the donor HSC chimerism in the peripheral blood (PB), revealed a notable decline in the repopulation
20 capacity and biased differentiation of old HSCs compared to their young counterparts (**FIG. 1B and FIGs. 7C-7F**). These results are consistent with previous studies (Rossi, et al. 2005, *Ibid.*; Leins, et al. 2018, Blood **132**(6): 565-576), confirm the age-related intrinsic functional decline of HSCs.

To analyze the contribution of HSCs to systemic aging, young or old HSCs were
25 independently transplanted into irradiated middle-aged mice (13 months). After a stable engraftment of the transplanted HSCs in the peripheral blood (5 months post-transplantation), the aging status of the recipient mice was evaluated by performing blood analyses and physical tests (**FIG. 1C**). Previous studies have shown that the blood cell composition changes with aging, characterized by an increase in myeloid cells and a corresponding
30 decrease in lymphoid cells. Notably, mice transplanted with young HSCs exhibited a more youthful blood cell composition, characterized by increased B cell and reduced T cell and myeloid cell proportions, as well as an increase in overall white blood cells and lymphoid

counts, compared to the recipients received old HSCs (**FIG. 1D and FIG. 1E**). These results suggest a crucial role of HSCs in systematic hematopoietic aging.

The ratio of naïve T cells serves as a critical indicator of immune function, which declines during the aging process. Conversely, central memory T cells (T_{cm}) and effector memory T cells (T_{em}) exhibit an increase with aging, as confirmed by the experiments herein (**FIG. 8A and FIG. 8B**). Notably, the old mice that received young HSCs had more naïve T cells in both the CD4 and CD8 T cell populations, indicating a rejuvenated immune system after young HSCs transplantation (**FIG. 1F and FIG. 9A**).

The DNA methylation clock is widely used as an indicator of aging. Importantly, old mice that had been transplanted with young HSCs had a significantly younger epigenetic age compared to those transplanted with old HSCs (**FIG. 1G**). Consistently, old mice that had received young HSCs exhibited improved physical functions when compared to those that had received aged HSCs, including muscle strength (grip strength), motor coordination (pole test, beam balance and rotarod), locomotor activity (open field test) and cognitive functions (fear conditioning test), without significant difference in body weight of the recipients (**FIG. 1H and FIGS. 9B-9D**). Collectively, these results demonstrate that transplantation of young HSCs into old or aged mice can lead to whole body rejuvenation.

Example 2: Single cell RNA-seq reveals a “youthful” HSC subpopulation in old mice

The results described in Example 1 suggested that HSCs from old mice are functionally defective compared to those from young mice. To understand the defects, both bulk RNA-seq and scRNA-seq were performed on young and old HSCs (**FIG. 2A**). The results revealed a clear transcriptomic difference between young and old HSCs (**FIG. 2B and 10A**). Bulk RNA-seq experiments revealed 332 up-regulated genes in old HSCs, including previously annotated HSC aging marker genes such as *Clu*, *Selp*, *Mt1*, *Ramp2* and *Gpr183* (**Figure 10A; Tables 1A and 1B**). These genes were defined as HSC aging marker genes for downstream analysis. Interestingly, unsupervised clustering analysis of scRNA-seq revealed that while the quiescent young HSCs are largely uniform (only form a single cluster q4), the quiescent old HSCs could be further divided into 3 clusters (q1-3) (**FIG. 2C and Fig. 2D**). This result indicates that quiescent old HSCs are transcriptionally more heterogeneous compared to young HSCs.

Marker genes for each of the clusters were identified (**FIG. 2E**). Although q1-q3 were all from old HSCs, the known aging marker genes, such as *Mt1*, *Prtn3*, *Nupr1*, *Plscr2*,

Cavin2, *Sult1a1*, *Clu*, *Ramp2*, *Alcam*, *Selp* and *Gpr183*, were highly expressed in q1 and q2 clusters, but not in q3 cluster (**FIG. 2E**). In contrast, both q3 clusters and q4 (young HSCs) clusters shared highly expressed genes, even though the q3 cluster is from old mice. GO analysis of q3 marker genes revealed an enrichment of cell proliferation related pathways (**FIG. 2E**), which were also enriched in the young HSCs compared to old HSCs based on bulk RNA-seq (**Figure 10B**, aging-down). The relative expression levels of q1, q2, and q3 marker genes in old and young HSCs were calculated, and it was found that the q1 and q2 marker genes have significantly higher expression in old HSCs, while expression of q3 marker genes is higher in young HSCs (**FIG. 2F**), indicating that q3 HSCs have a younger transcriptome than those of q1 and q2 HSCs.

To further investigate whether a subset of HSCs from old mice are “youthful” in their transcriptome, the UMAP distribution of some well-known HSC aging marker genes (*Sbspon*, *Gpr183*, *Clu*, *Ramp2*) was analyzed. It was found that these marker genes are expressed at a higher level in q1 and q2 compared to their expression in q3 (**FIG. 2G**). In contrast, the young HSC marker genes, such as *Rnase6* and *Arhgap30*, exhibited an opposite expression pattern (**FIG. 10C**). To quantify the aging heterogeneity of old HSCs, the aging score, a measure of aging-related transcriptome, was calculated for each single cell using the 332 aging marker genes identified in the bulk RNA-seq (**Tables 1A and 1B**) or the top 100 HSCs aging genes as previously identified (see, e.g. Flohr Svendsen, A., et al., (2021), *Blood*, 138, 439-451. 10.1182/blood.2020009729.). Both analyses indicated that cluster q3 had a lower aging score than q1 and q2 despite that all cells were from the same old mice (**FIG. 2H and FIG. 10D**). Collectively, comparative scRNA-seq revealed increased aging heterogeneity in old HSCs and a subset of old HSCs have a youthful transcriptome.

Example 3: CD150 can serve as an aging heterogeneity marker for old HSCs

To better understand the aging heterogeneity of old HSCs and to assess its contribution to overall body aging, a unique cell surface marker that facilitates the separation of “youthful” from “old” cells in aged HSCs was needed. To this end, the top 150 genes showing strong correlation with aging scores in scRNA-seq were identified (**FIG. 11A**) and compared with the 332 genes upregulated in old HSCs (**FIG. 10A**), resulting in 54 shared genes (**FIG. 3A; Table 2**). For sorting purposes, membrane location was included, which reduced the candidate gene list to 26 (**FIG. 3A**). Furthermore, because the potential marker genes should have lower expression levels in q3 than in q1 and q2, the candidate gene list

was reduced to 7, namely, *Slamf1*, *Ehd*, *Efnal*, *Gpr183*, *Jam2*, *Ramp2*, and *Tm4sf1* (**FIG. 11B**). Considering the availability and specificity of the antibodies, CD150 (*Slamf1*) was selected as the marker for aging heterogeneity of old HSCs. FACS analysis of HSCs from young and old mice indicated that the population of HSCs with higher CD150 levels was significantly increased with aging (**FIG. 3B**), thus supporting the use of CD150 level to serve as an indicator of aging heterogeneity of old HSCs. By way of example, the relative fluorescent staining of CD150 is 5-fold higher in HSCs in old animals (e.g., mice) versus that in HSCs of young animals (e.g., mice). (**FIG. 3B**).

To further confirm that the CD150 level could serve as a marker of aging heterogeneity of old HSCs, old HSCs were separated into 4 groups (G1 to G4) by FACS, based on CD150 levels, and their transcriptomes were profiled (**FIG. 3C**). The expression correlation between CD150 and each gene was calculated. Based on this, 131 and 103 genes were identified that exhibited strong positive or negative correlation with CD150 level in aged HSCs, respectively (**FIG. 11C; Table 3A**). Notably, the genes that positively correlated with CD150 included many known HSC aging markers, such as *Sbspon*, *Ehd3*, *Chu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2* and *Enpp5*. In contrast, the negatively correlated genes included genes that are highly expressed in young HSCs, such as *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2* and *Kif15* (**FIG. 11D**). These results suggested that CD150 level is a potential indicator of HSC transcriptome age.

Using the 131 genes that positively correlated with the CD150 level (referred to as CD150 feature genes), the CD150 feature score was calculated for each cell. It was found that q3 HSCs had a significantly lower CD150 feature score than q1 and q2 HSCs (**FIG. 11E and FIG. 11F**). Notably, a strong correlation between the CD150 feature score and aging score was observed (**FIG. 3D**). This indicates that the aging heterogeneity of old HSCs can be well reflected by CD150 feature genes. Consistently, HSC aging-related up- and down-regulated genes in the 4 groups of HSCs from G1 to G4 exhibited a trend that is similar to the aging process (**FIG. 3E**), confirming the positive correlation between CD150 level and HSC aging status in old mice. Other studies indicated that CD150 level can mark HSC subsets with different differentiation bias in both young and old mice (Morita, Y., et al., (2010), *J Exp Med*, 207, 1173-1182. 10.1084/jem.20091318; Beerman, I. et al., (2010), *Proc Natl Acad Sci USA*, 107, 5465-5470. 10.1073/pnas.1000834107), provided support for the selection of CD150 to mark the aging heterogeneity of old HSCs.

Example 4: CD150^{low} HSCs have a younger epigenome with superior functions in old mice

Epigenetic changes are believed to be one of the drivers of the aging process. To further determine whether the CD150 level can reflect epigenetic aging status in old HSCs, the epigenetic age of old CD150^{high} and CD150^{low} HSCs was compared by analyzing their DNA methylation profiles. Notably, CD150^{low} HSCs had a significantly lower epigenetic age than that of CD150^{high} HSCs, despite that they are derived from the same old mice (**FIG. 3F**). Given that stem cell aging is accompanied by chromatin accessibility changes, the chromatin accessibility landscapes were compared between old CD150^{low} and CD150^{high} HSCs, as well as young and old HSCs by performing ATAC-seq analyses. It was found that 4,694 peaks displayed increased chromatin accessibility and 3,842 peaks showed decreased accessibility in aged HSCs relative to young HSCs. Interestingly, a similar change between CD150^{low} and CD150^{high} old HSCs was also observed around the aging-related open and closed ATAC-seq peaks (**FIG. 3G**). These results indicate that CD150 can serve as a marker reflecting both transcriptional and epigenetic aging in aged HSCs.

Previous studies have shown that increased DNA damage and decreased percentage of cells in proliferation are associated with functional decline of old HSCs. Interestingly, an increase of double stranded breaks (indicated by gH2AX) as well as a decrease in the proportion of cells in proliferation for CD150^{high} was observed in the studies conducted in the described experiments compared to CD150^{low} old HSCs (**FIG. 11G and FIG. 11H**). This indicates that CD150^{low} HSCs may have better function than CD150^{high} HSCs in old mice.

To directly compare the function between old CD150^{low} and CD150^{high} HSCs, serial competitive transplantation was performed (**FIG. 3H**). It was found that CD150^{low} old HSCs had significantly better engraftment capacity compared to their CD150^{high} counterparts in both the first and second transplantation (**FIG. 3I and FIGs. 12A and 12B**). These results demonstrate that CD150 marks transcriptome and epigenome heterogeneity; also shown is that the functional heterogeneity of old HSCs with CD150^{low} HSCs have better function than CD150^{high} HSCs.

Although young HSCs exhibit a homogeneous transcriptome (**FIG. 2D**) and aging score (**FIG. 2H**), their CD150 levels are variable (**FIG. 3B**). This raised the question whether CD150^{low} HSCs are also functionally superior to CD150^{high} HSCs in young mice. To address this question, cell cycle was investigated and similar HSC transplantation experiments were performed using HSCs from young mice (**FIG. 13A and FIG. 13B**). Consistent with their

transcriptome and aging score homogeneity, no significant functional difference was observed between CD150^{low} and CD150^{high} young HSCs for cell cycle analysis or peripheral blood (PB) chimerism during the period of 120 days after transplantation (**FIG. 13A and FIG. 13C**). However, a significantly higher contribution of CD150^{high} HSCs to myeloid cells was noted, along with an upward trend in PB chimerism when compared to CD150^{low} young HSCs. These results indicate that CD150^{high} HSCs from young mice have comparable or even better function than CD150^{low} HSCs, which is in contrast with that in old mice.

Example 5: Old CD150^{high} HSCs are defective in differentiation, but not in self-renewal or activation

Experiments were conducted to dissect the mechanism underlying the differential repopulation capacity of CD150^{low} and CD150^{high} HSCs in old mice. Considering that the capabilities of self-renewal and differentiation are the two major features of stem cells, both features were compared after their transplantation. The differentiation dynamics were determined by analyzing the differentiation state of old HSCs at different time points after transplantation (**FIG. 14A**). It was found that transplanted old HSCs started to differentiate on day 7, and a progenitor subpopulation became apparent on day 10 (**FIG. 14B**). Based on this information, old CD150^{low} and CD150^{high} HSCs were separately transplanted, and the donor-derived hematopoietic stem and progenitor cells (HSPCs) were analyzed in bone marrow on days 7 and 14 after transplantation (**FIG. 4A**). Notably, it was found that CD150^{high} HSCs showed a significantly lower ratio in short term HSCs (ST-HSCs) and multipotent progenitors (MPPs), but a higher ratio in LT-HSCs compared to CD150^{low} HSCs (**FIG. 4B and FIG. 14C**), indicating that old CD150^{high} HSCs might have differentiation and/or activation (quiescent to activated state) defects compared to CD150^{low} HSCs.

To determine whether old CD150^{high} HSCs exhibited an activation defect, transcriptomes of CD150^{low} and CD150^{high} HSCs were profiled 4 days after transplantation, when the HSCs had yet to initiate differentiation, but were under stimulation (**FIG. 14D**). By comparing with freshly isolated HSCs (without activation), 794 commonly up-regulated genes in both CD150^{low} and CD150^{high} HSCs were identified after transplantation (**FIG. 14E**). The commonly up-regulated genes are highly enriched in terms related to cellular division. Consistently, the G2M checkpoint related genes were similarly upregulated in both CD150^{low} and CD150^{high} HSCs upon transplantation (**FIG. 14F**). These results indicate that old CD150^{high} HSCs can be effectively activated as CD150^{low} HSCs following

transplantation. The proliferation capacity of old CD150^{low} and CD150^{high} HSCs in culture were also compared (**FIG. 14G**). It was found that old CD150^{low} and CD150^{high} HSCs exhibited comparable proliferation rates (**FIG. 14H and FIG. 14I**). Taken together, these results indicate that impaired repopulation capacity of old CD150^{high} HSCs is caused by defective differentiation, but not activation or proliferation.

To rule out a possible delay in differentiation of old CD150^{high} HSCs after transplantation, the persistence of the differentiation and self-renewal capacity of old CD150^{high} HSCs were analyzed over an extended period of 5 months. Old CD150^{low} or CD150^{high} HSCs (GFP negative) were co-transplanted with competitors (GFP positive) and the differentiation and chimerism of donor HSCs were analyzed in both bone marrow and PB 5 months later (**FIG. 4C**). A consistent differentiation defect of old CD150^{high} HSCs was observed when compared to their CD150^{low} counterparts when analyzing donor derived HSPCs in the bone marrow (**FIG. 4D**). Additionally, the donor chimerism of old CD150^{high} HSCs exhibited a clear trend of decline from LT-HSCs through ST-HSCs, MPPs, to PB, while the donor chimerism of old CD150^{low} HSCs remained relatively stable (**FIG. 4E**), supporting a long-term differentiation defect of old CD150^{high} HSCs. Collectively, these results indicate a long-lasting impairment in differentiation, but not in activation or self-renewal of old CD150^{high} HSCs, which explains the diminished repopulation capacity of this subpopulation.

Example 6: Old CD150^{high} HSCs are defective in the LT-HSCs to ST-HSCs transition

To determine the specific stage when the differentiation defects of old CD150^{high} HSCs occur, comparative scRNA-seq analysis of the HSPCs derived from old CD150^{low} and CD150^{high} HSCs was performed 14 days after transplantation. 2,406 and 1,925 high quality HSPCs derived from transplanted CD150^{low} and CD150^{high} HSCs, respectively were obtained (**FIG. 4F**). Cell cycle phase analysis indicated that similar proportions of HSPCs derived from CD150^{low} and CD150^{high} HSCs were actively cycling (**FIG. 14J**), consistent with the conclusion that CD150^{low} and CD150^{high} HSCs can be similarly activated by transplantation (**FIG. 14E and FIG. 14F**).

Based on the expression of the HSPC marker genes and the HSPC sorting markers (*Ly6a*, *Kit*, *Cd34*, *Cd48* and *Slamf1*), the 4,331 HSPC were divided into 7 subclusters that include LT-HSC-like, ST-HSC-like, MPP2-like, MPP3-like, MPP4-like, GMP-like and MEP-like cells (**FIG. 4G and FIG. 14K**). When HSPCs derived from CD150^{low} and

CD150^{high} HSCs were compared, it was found that HSPCs derived from CD150^{high} HSCs were significantly enriched in LT-HSC-like cells compared to those derived from CD150^{low} HSCs (**FIG. 4G and FIG. 4H**), consistent with the FACS analysis (**FIG. 4B and 14C**). Since the proportions of CD150^{low} and CD150^{high} HSCs derived ST-HSCs were comparable, the data indicated that the differentiation defects of CD150^{high} HSCs were mainly at the stages of LT-HSC to ST-HSC, and ST-HSC to MPP4 transitions. Given that MPP4 are the major progenitor cells of the lymphoid lineage, the substantial decrease in the MPP4 population might be a major cause of the biased differentiation of CD150^{high} HSCs. Collectively, comparative bone marrow scRNA-seq analysis of transplanted old HSCs indicated that the differentiation defects of old CD150^{high} HSCs is caused by a block of LT-HSCs to ST-HSCs transition.

Example 7: The “youthful” subset of old HSCs can alleviate aging phenotypes and extend lifespan

Transplantation of young HSCs as an anti-aging therapy is impractical for humans due to immune incompatibility among different individuals. The demonstration that HSCs in old mice have molecularly and functionally “youthful” CD150^{low} HSCs prompted an investigation of whether this subset of HSCs can help to attenuate aging phenotypes of old mice. To this end, CD150^{low}, whole HSCs (un-selected), or CD150^{high} HSCs from old mice were transplanted into 13-month-old recipients and aging phenotypes were analyzed 5 months later (**FIG. 5A**). Compared to recipients that received whole-HSCs and CD150^{high} HSCs, mice transplanted with CD150^{low} HSCs showed a higher percentage of B cells, and a lower percentage of T cells and myeloid cells (**FIG. 5B**), as well as increased blood cell numbers, including lymphoid cells, neutrophils, eosinophils and red blood cells (**FIG. 5C**). Importantly, a significant increase in the naïve T cell ratio and a decrease in Tcm and Tem ratios in the CD8⁺ (**FIG. 5D**) and CD4⁺ (**FIG. 15A**) T cells were also observed in mice that had received CD150^{low} HSCs relative to those that had received whole-HSCs or CD150^{high} HSCs. These results suggest that transplantation of old CD150^{low} HSCs can improve both hematopoiesis and immune parameters in old mice.

To determine whether improved hematopoiesis and immune parameters could benefit physical performance, a series of tests that measure muscle strength (grip strength, wire hanging), coordination (beam balance, pole test), and endurance (rotarod and treadmill) were performed. Consistently, a general trend of degrading physical performance in the order of CD150^{low}, whole-HSCs, and CD150^{high} HSCs (**FIG. 5E**) was found, without significant

differences in their body weight (**FIG. 15B**). In almost all cases, mice transplanted with old CD150^{low} HSCs performed significantly better than those transplanted with CD150^{high} HSCs. Consistent with improved physical performance, it was found that old mice that had received CD150^{low} HSCs displayed a lower epigenetic age (**FIG. 5F**). Importantly, aged recipient mice transplanted with old CD150^{low} HSCs exhibited a substantial lifespan extension, achieving 9.2% and 11.7% increase in median lifespan compared to counterparts transplanted with whole-HSCs or CD150^{high} HSCs (**FIG. 5G**).

Taken together, these results indicate that not only transplantation of young HSCs, but the molecularly and functionally “youthful” subset of old HSCs can attenuate aging phenotypes of old mice. Considering that the functionally defective CD150^{high} HSCs increase with aging, these results are indicative that rejuvenation might be achieved by reducing or removing the functionally defective HSCs from old mice.

Example 8: Reducing dysfunctional HSCs can alleviate aging phenotypes in old recipient mice

To assess whether reducing defective HSCs in old mice can enhance hematopoiesis and mitigate aging-related phenotypes, a transplantation experiment was performed, as follows: 500 old CD150^{low} HSCs were co-transplanted into irradiated middle-aged (13 months) mice with 2,500 (1:5 ratio), 1,000 (1:2 ratio) and 0 (1:0 ratio) CD150^{high} old HSCs in order to mimic whole-HSC, partial removal, and complete removal of CD150^{high} HSCs, respectively. Five months after the transplantation, the hematopoietic system and physical capabilities of recipient mice were assessed to compare the aging status of the three groups (**FIG. 6A**). The 1:5 ratio served as a control, which was determined based on the percentage of q3 to the total HSC in old mice revealed by scRNA-seq (**FIG. 2D**).

Peripheral blood (PB) analysis indicated that compared to the 1:5 group, the 1:2 and 1:0 groups had an increased percentage of B cells and a decreased percentage of myeloid cells (**FIG. 6B**). Complete blood counting analysis also showed higher numbers of whole white blood cells, neutrophils, and lymphoid cells in the 1:0 and 1:2 groups compared to the 1:5 group (**FIG. 6C**). Importantly, increased naïve CD4⁺ and CD8⁺ T cells, as well as decreased effective memory T cells, were also detected in the 1:2 and 1:0 groups compared to the 1:5 group (**FIG. 6D and FIG. 16A**), indicating the presence of the defective CD150^{high} HSCs had adverse effects on the “youthful” functional CD150^{low} HSCs for hematopoiesis and immune function in old mice.

Next, it was examined whether reducing the CD150^{high} HSCs ratio could improve physical functions of the recipient mice by performing a multitude of physical tests. The results indicated consistent improved physical functions in the 1:2 and 1:0 groups compared to the 1:5 group, including muscle strength (grip strength), motor coordination (pole test, beam balance, and rotarod), and locomotor activity (open field test), with no notable differences in body weight (**FIG. 6E and FIG. 16B**). These findings not only confirmed the adverse effect of the defective CD150^{high} HSCs in aged mice, but also suggested that their reduction in old mice can ameliorate aging-related phenotypes. It is noted that the 1:2 group exhibited a significantly younger and healthier hematopoietic system, immune parameters, and enhanced physical functions compared to those of the 1:5 group. This observation indicates that anti-aging effects can be accomplished by partial elimination of defective HSCs in aged mice, highlighting its application potential, since partial removal is much more practical than complete removal.

Example 9: Materials and methods

The following materials and methods relate to the experimental studies described in Examples 1-8 hereinabove.

Mice

All experiments were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) of Boston Children's Hospital and Harvard Medical School. For bulk and single-cell RNA-seq, 2-3 months young and 22-24 months old C57BL/6 mice were used (Jackson Lab #00664). For HSC transplantation, 2-3 months old young (Jackson Lab #00664) or 2-3 months old GFP+ young (Jackson Lab #006567) mice were used. For competitive transplantation, competitor HSCs were collected from 3-4 months GFP+ young (Jackson Lab #006567) mice. Except as particularly mentioned, the recipient mice were B6 CD45.1 mice (Jackson Lab #002014), and the helper cells were also collected from B6 CD45.1 mice. For old recipient mice, 13 months old wild type (Jackson Lab #00664) were used. The helper cells were from age-matched wild type mice.

Long Term Hematopoietic Stem Cell (LT-HSC) sorting and transplantation

Bone marrow (BM) cells were collected by crushing tibias, femurs, pelvic bone and spin (spin cord is removed). The resulting cell suspension was filtered through 70 µm cell strainers and red blood cell were lysed with red blood cell lysis buffer (ebioscience catalog

#00-4333-57). To improve the sorting efficiency, filtered cells were further enriched by removing lineage positive cells. For detail, the filtered cells were first stained with biotin-conjugated antibodies against lineage markers (CD4, CD8, Gr-1, CD11b, CD5, B220 and Ter119). Lineage positive cells were removed by streptavidin-conjugated magnetic beads (STEMCELL catalog #19856), and the lineage negative cells were further stained with antibodies against mouse c-Kit, Sca-1, CD48, CD34, CD150. The dead cells were labeled with DAPI. The LT-HSCs were defined as Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺. To prepare helper cells, bone marrow cells were collected from long bones (tibias and femurs) of CD54.1 mice (JACKSON LAB #2014) by flushing. After lysing red blood cells, all HSPCs were removed by Sca-1 positive selection kit (STEM CELL catalog # 18756). The remaining cells were counted and used as helper cells for transplantation.

For transplantation experiments, a specific number of LT-HSCs were sorted with FACS sorter (SONY SH800). Sorted LT-HSCs were mixed with 3×10^5 helper cells and transplanted into lethally irradiated (9.5 Gy) recipient mice (Jackson lab #2014) using 1 ml syringe attached with a 27.5-gauge needle.

Cell cycle and DNA damage marker γ H2AX analysis of HSCs with FACS

The analysis of cell cycle status of HSCs was performed as previously described (Szade, K., et al., (2016), *Methods Mol Biol*, 1516, 91-99. 10.1007/7651_2016_361). Briefly, bone marrow cells were prepared, enriched, and stained with HSC marker antibodies. The enriched bone marrow cells were then fixed and permeabilized with BD Intra Kit (#641776). After washing, the fixed HSCs were stained using a monoclonal antibody against mouse KI67 (eBioscience, #11-5698-80) and Hoechst33342 (Thermo Scientific, #R37165) or using an antibody against DNA damage marker γ H2AX (Cell Signaling Technology, #9719S). The cell cycle state of the HSCs or DNA damage marker γ H2AX levels were analyzed with FACS after washing.

Bulk RNA-seq library

A bulk-RNA sequencing library was constructed based on Smart-seq2 (Picelli, Björklund Å et al. 2013). SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Takara catalog # 634890) was used and experiments were performed according to the manufacturer's instructions. Briefly, sorted HSCs were incubated in lysis buffer with RNase inhibitor on dry ice for 1 minutes, and then thawed at room temperature. First-Strand Buffer, SMART-Seq v4 Oligonucleotide, 3' SMART-Seq CDS primer II and RNase inhibitor were added into the lysed cells and incubated at 72°C for 3 min before being put on ice. Incubation was

performed after adding SMARTScribe II Reverse Transcriptase as follows: 42°C for 90 minutes, then 70°C for 10 minutes. Without purification, SeqAmp PCR Buffer, PCR Primer II A and SeqAmp DNA Polymerase were added for pre-amplification using thermal cycler for 12 cycles following the manufacturer's instructions. The amplified cDNA was then
5 purified by 1.8× volume of SPRIselect beads (Beckman Coulter catalog # B23318). Next, 200 pg cDNA was used for library preparation with Nextera XT DNA Library Preparation Kit (Illumina catalog # FC-131-1024) according to the manufacturer's instructions.

scRNA-seq library preparation

Single-cell RNA seq library was prepared with Chromium Next GEM Single Cell 3' Reagent Kits V3.1 (Dual Index) from 10X Genomics according to the manufacturer's
10 instructions. Briefly, single HSCs were sorted by FACS into PBS solution. Then microfluids were used to construct droplets containing single-cell, GEMs and reverse transcription reagents. 100 µl of droplets were incubated at 53°C for 45 minutes, then at 85°C for 5 minutes. Droplets were then released by Recovery Agent, cleaned up with Dynabeads
15 MyOne SILANE and amplified by PCR for 12 cycles. The amplified cDNA was used for library construction according to the manufacturer's instructions.

Peripheral blood analysis via FACS

For PB analysis, up to 30 µL vein blood was collected from retro orbital sinus or tail tips into EDTA-coated tubes. Red blood cells were firstly removed by red blood cell lysis
20 buffer (ebioscience catalog #00-4333-57). The remaining white blood cells were stained with mixed monoclonal conjugated antibodies (CD45.1, CD45.2, CD3, B220, Gr-1 and CD11b). After incubation in 4°C and darkness for 30 minutes, PB was analyzed by FACS (BD FICR canto-II).

HSC culture *in vitro*

Sorted LT-HSCs were cultured in STEMSPAN™ SFEM II (STEM CELL catalog #09605) with 10 ng/ml SCF (PeproTech, catalog #AF-250-03), 100 ng/ml TPO (PeproTech, catalog #315-14), 25 ng/ml Flt3L (PeproTech, catalog #250-31L) and 25 ng/ml IL3 (PeproTech, catalog # 213-13). SCF (Stem Cell Factor) is a hematopoietic growth factor that exerts its activity by signaling through the c-Kit receptor. SCF and c-Kit are essential for the
30 survival, proliferation and differentiation of hematopoietic cells committed to the melanocyte and germ cell lineages. Human SCF manifests low activity on murine cells, while murine and rat SCF are fully active on human cells. TPO (thrombopoietin) is a lineage-specific growth factor produced in the liver, kidney and skeletal muscle. It stimulates the proliferation and

maturation of megakaryocytes, and promotes increased circulating levels of platelets *in vivo*. TPO signals through the c-mpl receptor, and acts as an important regulator of circulating platelets. Human and murine TPO exhibit cross-species reactivity. Six days after culture, the number of HSCs were quantified by FACS (BD FICR canto-II).

5 Complete blood counting analysis of recipient mice

For whole mouse blood analysis, 75 μ L vein blood was collected from the retro orbital sinus and mixed with 225 μ L 5mM EDTA (Research Products International, catalog # E14000-250.0). Freshly collected whole blood was analyzed by Hematology system (ADVIA 120) 2 hours after collection.

10 Naïve T, Tcm and Tem analysis

The ratios of Naïve T cells, Tcm and Tem were analyzed based on a previous report (Pakpour, N. et al. 2008, *J Immunol*, **180**(12): 8299-8305). Briefly, PB was collected and red blood cells were removed by red blood cell lysis buffer (ebioscience catalog #00-4333-57). The remaining cells were stained with antibodies directed against CD45.1, CD45.2, CD4, CD8, CD44 and CD62L. T cell subtypes were defined as follows: naïve (CD62L^{high} CD44^{low}), Tcm (CD62L^{high} CD44^{high}) and Tem (CD62L^{low} CD44^{high}).

Reduced-representation bisulfite sequencing (RRBS) library preparation for HSCs

FACS sorted HSCs were lysed at 50°C for 3 hours and 75°C for 30 min in a 5 μ L lysis buffer (20 mM tris-EDTA (Sigma catalog # T9285-100ML), 20 mM KCl (Thermo Scientific, catalog # AM9640G), 0.3% Triton X-100 (Sigma catalog # 93443-100ML), and protease (1 mg/ml; Qiagen catalog # 19155)). The lysed cells were then subjected to Msp I digestion at 37°C for 3 hours and at 80°C for 20 minutes. Following enzymatic digestion, end-repair mix (1 μ L Klenow frag exo (5 U/ μ L), 0.2 μ L of 10 $\times$ TangoBuf, and 0.8 μ L of dNTP mix) was added and incubated at 37°C for 40 minutes and at 75°C for 15 minutes. For adaptor ligation, the reaction was incubated at 16°C for 30 minutes, 4°C overnight, and at 65°C for 20 minutes after adding the ligation mix (2.25 μ L of H₂O, 1 μ L of T4 ligase (30 U/ μ L); Thermo Scientific, catalog # EL0013), 0.5 μ L of 100 mM ATP (Thermo Scientific, catalog # R0441), and 0.75 μ M methylated adaptor). Bisulfite conversion reaction was then performed by the EpiTect Bisulfite Kit (Qiagen catalog # 59104) following the manufacturer's instructions. Adapter-tagged BS-DNA was then amplified by 16 cycles using Kapa HiFi Uracil+ ReadyMix (Kapa Biosystems, catalog # KK2801) with NEBNext Multiplex Oligos for Illumina (New England BioLabs catalog # E7500L).

ATAC-seq library construction

About 500-1000 cells were sorted by FACS in 10 μ l tagmentation buffer (33 mM Tris-Acetate, 66 mM K-Acetate, 10 mM Mg-Acetate, 16% Dimethylformamide, and 0.02% Digitonin). 0.5 μ l Tn5-Adapter complex (Diagenode) was added after FACS sorting and the samples were incubated at 37°C for 30 minutes. Then, the tagmentation reaction was stopped by adding 10 μ l stop buffer (100 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.4% SDS, 40 μ g/ml Proteinase K), and the samples were incubated at 55°C overnight to release the tagmentation fragments. SDS was then quenched by adding 5 μ l 25% Tween-20, and the samples were incubated on ice for 10 minutes. Next, 25 μ l samples were mixed with 30 μ l NEBNext High-Fidelity 2X PCR Master Mix (NEB), 2.5 μ l P5 primer (10 μ M) and 2.5 μ l P7 primer (10 μ M) for PCR amplification. The PCR products were then purified with SPRI beads with 1:1.6 ratio (96 μ l beads) before sequencing.

Physical function tests

For the grip strength test, a mouse was gently placed on the top of a grid strength meter (Bioseb, Model GT3) so that only its forepaws can grasp the grid. The tail of the mouse was pulled three times, and then the animal was returned to the cage for at least a 1 minute rest. Two (2) series of pulls were performed; the top 3 animals' maximum grip strength were averaged, and body weight was normalized for each mouse.

For the beam balance test, home-made equipment was used. Briefly, a 1-meter-long smooth wood strip was elevated to 50 cm tall from the table. A black box (20×20×20 cm) was put on the plate at one end and the mouse was put at the other end. For training day, a thicker pole was used (28 mm diameter). Each mouse was trained until it was able to go through the pole and to the box without dropping. The next day, the mice were tested with a thicker pole or a thinner pole (17 mm diameter), 3 times per mouse. Before being used for another test, the pole and box were cleaned with 70% ethanol. The latent time for the mice to go through the pole was recorded.

For the pole test, mice were placed at the top of a 50 cm grooved metal pole with a diameter of 1 cm with the head of the mice pointing down. The time from initial placement on the top of the pole to the time a mouse reached the base of the pole (forelimb contact with platform) was recorded with a stopwatch. Following a 30-minute rest in the home cage, the trial was repeated another 2 times. The average time was calculated as the latent time to go down for each mouse.

The rotarod test was used to evaluate motor coordination and balance. Mice were placed in separate lanes on the rod rotating at an initial speed of 4 rpm/min in an accelerating Rotarod system (Ugo Basile Apparatus). On the first day, each mouse finished a training program at speed of 4 rpm/min for 5 min and repeated another 2 times with a 20 min interval.

5 For the test day, the apparatus was set to accelerate from 4 to 40 rpm/min in 5 min. The time and speed were recorded when the mice dropped or after two consecutive rounds of passive rotation. A test was performed one- or two-times per day for each mouse with an interval of at least 30 min. The test was continued on the next day, and last for 3 tests in total. The apparatus was cleaned between tests of the mice to make sure it was dry and clean. Results
10 were the average over 3 tests.

For the wire hanging test, the mice were placed on a metal salamander with a 1-cm square mesh 50 cm above a cushioned surface covered with approximately 3-cm thick bedding. First, the mice were put on the salamander and then the salamander was inverted slowly. The stopwatch was started when the mouse was attached upside down. The latent
15 time for the mouse to fall was recorded. Within a 30 min interval, each mouse was tested for 3 times and the results were the average over 3 trials.

For the treadmill fatigue test, the protocol was adapted and modified from a previous publication (Dougherty, J.P. et al., (2016), *J Vis Exp.*, 10.3791/54052). Briefly, mice were acclimatized to the treadmill (Columbus Instruments Exer 3/6 Treadmill) for 2 consecutive
20 days prior to testing. The fatigue zone was set as one mouse body-length from the shock grid. On day 1, the mice were placed on the treadmill and allowed to explore for 5 minutes (min) with the shock grid turned off. After 5 min, the shock grid was powered on at 1.5 mA, 3 Hz and the speed was set to 6 m/min for 5 min. After 5 min, the speed of 2 m/min was increased for every 2 min. The treadmill was stopped when the mice had run at 10 m/min for 5 min. On
25 day2, the same training program was performed, except that the top speed was 12 m/min for 5 min. On the test day, the speed was increased stepwise over time, up to maximum of 26 m/min. The fatigue standard was met when mice were present in the fatigue zone for more than 5 seconds in three separate instances. The latency time for fatigue of each mouse was recorded for analysis.

30 For the fear conditioning test, contextual and cued fear conditioning (CCFC) was implemented. In brief, mice underwent the following training protocol on the first day: 2 minutes of acclimation, followed by a 30-second tone, then a 2-second foot shock (0.5 mA). This was followed by a 2-minute intertrial interval (ITI). Subsequently, another 30-second

tone was paired with a 2-second footshock (0.5 mA), and then there was a 1-minute period before lights out. The following day, a context test was conducted where mice were placed in the same context for 5 minutes without any shock or tone. On the third day, mice were assessed in a modified context: 3 minutes of acclimation without tone, followed by 3 minutes with tone. Video was captured using Noduls MediaRecorder (version 4.0), and freezing behavior was analyzed using Noldus EthoVision XT17.

For the Novel Y-Maze test, the protocol was adapted from previous study (Chen, R., et al. (2022), *Sci Adv*, 8, eabm1077. 10.1126/sciadv.abm1077). Briefly, mice were pre-acclimated to a separate holding room for 30 minutes before testing. The Y-maze procedure consisted of a 3-minute habituation phase, followed by a 3-minute test phase, with a strict 2-minute ITI. In the habituation phase, one arm (left or right) was obstructed, and the start arm remained consistent for each mouse. After habituation, mice were briefly returned to their holding cage during the ITI. The obstruction was then removed, and the maze was cleaned before the test phase. Then, the mouse was placed back into the start arm for the test trial and returned to the home cage after the trial was completed. Distance and time traveled in the maze were recorded and analyzed with Noldus EthoVision XT17.

For the open field test, the protocol was adapted from previous study (Chen, R. et al., (2022), *Sci Adv*, 8, eabm1077. 10.1126/sciadv.abm1077). In the open-field test, the apparatus was a transparent enclosure with a base measuring 27.3 cm by 27.3 cm and walls with a height of 20.3 cm. The central zone accounted for half of the total area. Prior to testing, mice were acclimated to the test environment for a minimum of 20 minutes. Each mouse was then positioned in the center of the arena to begin the assessment. Movements were tracked for a duration of 20 minutes and data were collected in intervals of 5 minutes using a tracking system (Med Associates, St. Albans, VT, ENV-510). Analysis included standard locomotor activity parameters, such as total distance moved, average speed, active and inactive periods, as well as vertical events in the first 10 minutes.

RNA-seq data analysis

For bulk RNA-seq datasets, adaptor of all sequenced reads was first trimmed by Trim Galore with parameter “-illumina -paired”. The filtered reads were then aligned to the mm10 reference genome by Hisat2 (version 2.0.0-b)⁷³ with parameters “--no-mixed --no-discordant --dta-cufflinks --no-unal”. The aligned SAM files were then converted to BAM files with Samtools (version 1.14) for further analysis. Next, HTSeq-count (V0.12.4) was used to calculate the count of reads for each gene, with the main parameters set as: -f bam -s no -r

pos. The reference genome was downloaded from the UCSC Table Browser. To quantify gene expression, the read count for each gene was normalized using DESeq2 (V1.22.2). The differentially expressed genes from each group were filtered with following criteria: adjusted P-value < 0.05 and fold-change >2 or <0.5.

5 ATAC-seq analysis

For ATAC-seq datasets, sequencing adaptors and low-quality reads were removed by Trim Galore with parameters: -q 20 --length 30 --paired. Then the clean reads were mapped to mouse reference genome (mm10) by bowtie2 (Version 2.5.1) with parameter: “-N 1 -L 25 -X 2000 -no-discordant” (ATAC-seq). After converting aligned SAM to BAM files using Samtools (Version 1.13), Picard (Version 2.26.4) was used for removing PCR duplications. Next, read counts were normalized by Reads Per Kilobase per Million mapped reads (RPKM) and converted to bigwig via bamCoverage (Version 3.5.1). The peaks were called by MACS2 (Version 2.2.7.1) with parameter “-t file -f BED -g mm -outdir OUTPUT -n NAME --nomodel --nolambda” (ATAC-seq). The “narrowpeak” files were used for downstream analysis. The peaks that overlap in more than 60% of the replicate samples in each group were kept for feature counts and subsequent analysis using DiffBind (V3.0.15). The read counts for each peak were normalized using DESeq2 (V1.30.1). Peaks that differed significantly between groups were identified using DESeq2 with the following criteria: P-value < 0.05 and fold-change > 1.5 or < 0.67. Deeptools (Version 3.5.1) was used to visualize the read coverages at peak regions.

scRNA-seq data analysis

Paired reads from single-cell RNA seq were first aligned to mm10 reference genome with Cellranger --count (version 3.0.2) from 10X Genomics. Transcriptome annotation composed of protein coding genes was download and processed from Ensembl released-102. To eliminate low-quality single cells, single cells were filtered out with feature numbers below 800 for freshly isolated HSCs (**FIGs. 2A-2H**) and below 2,000 for short-term transplanted HSCs (**FIGs. 4A-4H**). Additionally, cells with mitochondrial transcripts accounting for more than 5 percent were also removed. Then, the remaining single cells were processed and analyzed with Seurat (V4.0.2) (Hao, Y. et al., (2021), *Cell*, 184(13): 3573-3587.e3529). The young and old single cell data was first merged and then normalized and scaled. Two thousand variable feature genes were identified and used for UMAP presentation.

Epigenetic age calculation

Paired fastq files from RRBS seq were first merged and processed as a single file. Adaptors of all sequenced reads were trimmed by Trim Galore with parameters --Illumina. Adaptor trimmed reads were mapped using Bismark (v0.20.0) (Krueger F. et al. 2011, *Bioinformatics* 27(11): 1571-1572) using parameters --fastq -L 30 -N 1 --non_directional. Methylated CpGs were interpreted by bismark_methylation_extractor with parameters -s --no_overlap --report --bedGraph. For base resolution 5mC level calculation, a cutoff of minimal 3× coverage was required for each CpG site. The 5mC level for certain CpG was calculated using the number of methylated CpGs to divide the number of total CpGs detected on the CpG site. Epigenetic age was calculated based on the identified sites from a previous study (Meer, M.V. et al. 2018, *Elife*, 7).

Data accession

All data have been deposited to GEO with the accession number GSE233879. Access to the data by the reviewers can be achieved with the link below using token “ynadskqcxcvmdbal”: [worldwideweb.ncbi.nlm.nih.gov / geo / query / acc.cgi?acc=GSE233879](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE233879).

Example 10: Targeted depletion of dysfunctional CD150^{high} HSCs *in vitro*

To specially remove dysfunctional CD150^{high} HSCs from a sample, an antibody-toxin conjugation method was employed according to previous methods (Palchaudhuri R. et al., (2016), *Nature biotechnology* 2016; 34(7): 738-45; Czechowicz A. et al., (2019), *Nat Commun*; 10(1): 617), (FIGs. 17A-17D). In this system, through biotin and streptavidin binding, CD150 antibody is conjugated with saporin, a specific toxin that inhibits translocation of the nascent protein chain during protein synthesis. (Polito L., et al., (2013), *Toxins (Basel)*, 5(10): 1698-722). When the biotin-conjugated CD150 antibody is complexed with streptavidin-conjugated saporin (CD150-SAP), it is expected to induce cell death upon endocytosis into the targeted cells. The killing effect of the CD150-SAP was assessed using HSCs *in vitro*. To this end, purified old HSCs (e.g., obtained from old mice (about 24 months) were treated with different concentrations of CD150-SAP, IgG-SAP, or CD150 Ab for 3 days, and live cells were quantified by the ATP concentration using a titer assay. The results showed that CD150-SAP treatment resulted in HSC killing in a dose-dependent manner (FIG. 17B). Old or aged mice exhibit HSC heterogeneity as they contain CD150^{high}

HSCs and CD150^{low} HSCs, which have functional differences as described herein. Based on the FACS profile of HSCs analyzed in the sample used in the experiments described in this example, the CD150^{high} HSCs represented the top 15% of HSCs and the CD150^{low} HSCs represented the bottom 15% of HSCs.

5 It was found that the IgG-SAP control reduced the cell number compared to a PBS control, which indicated a certain level of off-target effect. Thus, the killing specificity of CD150-SAP under a defined concentration should be carefully evaluated. To this end, CD150^{low} HSCs from a 17-month-old CD45.1 mouse were mixed with the same number of CD150^{high} HSCs from an age-matched CD45.2 mouse, and the cell numbers and percentages
10 of CD150^{low} HSCs (CD45.1⁺) and CD150^{high} HSCs (CD45.2⁺) were analyzed 3 days after the treatment with different concentrations of CD150-SAP.

It was found that with increasing CD150-SAP concentration, the numbers of both CD150^{low} and CD150^{high} HSCs were decreased when compared to the PBS treated control (FIG. 17C). However, at the lowest concentration of 0.01 nM, CD150-SAP significantly
15 reduced the number of CD150^{high} HSCs, while no obvious effect was observed for the CD150^{low} HSCs (FIG. 17C). These data indicate that at a concentration of 0.01 nM, the CD150-SAP antibody conjugate exhibited a specific killing effect on CD150^{high} HSCs. In addition, the decreased ratio of CD150^{high} to CD150^{low} HSCs in response to increased CD150-SAP also supports that the CD150^{high} HSCs were preferentially targeted by CD150-
20 SAP (FIG. 17D).

Example 11: Targeted depletion of the dysfunctional CD150^{high} HSCs *in vivo*

Having demonstrated the preferential killing effect of CD150-SAP toward CD150^{high} HSCs *in vitro*, it was investigated whether the effect was also observed *in vivo*. Because
25 CD150 is one of the surface markers of HSCs for FACS analysis, the injected CD150-SAP complex could hinder the flow detection of CD150⁺ cells after CD150-SAP treatment. To avoid such a potential masking effect, a time course experiment was performed to measure the persistence of the CD150 antibody after injection of the CD150-SAP complex (FIG. 18A). To this end, mice were injected with bio-CD150 antibody, and bone marrow was
30 collected on different days and divided into two parts. One part was used to determine the percentage of total HSCs that can be labeled by added bio-CD150 antibody, while the other part received no additional bio-CD150 antibody to reflect the labeling of HSCs by the injected bio-CD150 antibody. The labeling efficiency of HSCs by bio-CD150 antibody was

calculated by comparing these two percentages at different time points. The results indicated that the percentage of labeled HSCs gradually decreased, reaching zero by day 20 (**FIG. 18B**), indicating that the injected bio-CD150 antibody was totally removed and absent by this time point. Consequently, 3 weeks was established as the minimum time needed to use FACS to measure the CD150-SAP killing effect.

To examine whether CD150-SAP can deplete CD150^{high} HSCs *in vivo*, two doses of CD150-SAP were injected into 17-month-old C57BL/6J mice intravenously compared with PBS injection as a control. The percentage of HSCs in the total bone marrow cells or LSK was calculated 3 weeks after the treatment, which revealed a dose-dependent decrease when compared with the PBS control (**FIG. 18C**), indicating that HSCs were at least partially depleted by the treatment. To assess whether CD150^{high} HSCs were preferentially depleted by the treatment, the distribution of HSCs in relation to their CD150 level was analyzed 3 weeks after the treatment. The results indicated a dose-dependent shift of the CD150^{high} to the CD150^{low} population (**FIG. 18D**), peak shift to the left). Quantification of the distribution of the HSCs indicated that the treatment resulted in a decrease in the percentage of CD150^{high} HSCs concomitant with an increase in the CD150^{low} HSCs when compared with the control (**FIG. 18D**). These data support the finding that CD150-SAP preferentially depleted CD150^{high} HSCs *in vivo*.

Example 12: Depletion of dysfunctional CD150^{high} HSCs *in vivo* improves hematopoiesis

To avoid potential off-target depletion, a lower dosage (1.0 mg/kg) of CD150-SAP was also evaluated. Forty-five (45) days after the injection, the percentage of HSCs was determined in bone marrow and LSK. It was found that at a dosage of 1.0 mg/kg, CD150-SAP treatment was still able to reduce about 60% of the HSCs compared with control (**FIG. 19A**). Importantly, compared to the PBS-injected control, the distribution pattern of CD150 expression level on HSCs shifted to the left side (**FIG. 19B, left**), leading to a decreased ratio of CD150^{high} to CD150^{low} HSCs in old mice (**FIG. 19B, right**).

Because doses of CD150-SAP from 1 to 5 mg/kg all were able to decrease the CD150^{high} to CD150^{low} HSC ratio, experiments were performed to assess a dose that has a beneficial effect. *In vivo* experiments were performed by injecting three different doses (1, 2, 4 mg/kg) of CD150-SAP into 15-months old mice with PBS as a control. To monitor the effect of these treatments on hematopoiesis, peripheral blood analyses were performed at

different time points after the treatment. It was found that treatment at 1.0 mg/kg CD150-SAP significantly increased the B cell percentage at days 45, 90, and 120 after the treatment, and decreased the myeloid cell percentage at days 90 and 120 after the treatment (**FIG. 19C**, **FIG. 19E**). The decreased T cell percentage at 30 and 45 days after the treatment might be
5 due to the depletion of T cells in the blood, as some T cells also express CD150 (**FIG. 19D**). These results indicate that depletion of the dysfunctional CD150^{high} HSCs can at least partly correct the myeloid biased differentiation observed in old mice.

Consistent with the experimental results described herein, depletion of myeloid-biased HSCs (marked by NEO1 in most cases) using an antibody cocktail was reported to restore
10 immune function in old mice (Ross J.B. et al., (2024), *Nature*; 628(8006): 162-70). This approach of simultaneous targeting both myeloid-biased HSCs and c-Kit positive progenitor cells may cause serious side-effects and may not lead to a systemic body rejuvenation. It is noted that the killing mechanism as described and exemplified by experimental results herein is distinct and differs from the approaches of others. By way of example, according to the
15 report of Ross et al., the myeloid biased HSCs are eliminated by phagocytosis facilitated by blocking CD47 signaling by continuous injection of high-dose CD47 antibodies into old mice. In contrast, the examples as described herein employ the ribosome toxin saporin to inhibit protein synthesis, thus providing an approach that preferentially eliminates the dysfunctional CD150^{high} HSCs in old mice and leaves the functional CD150^{low} HSCs largely
20 unaffected when the appropriate concentration of the CD150-SAP complex is used. Notably, the amount of CD150 antibody used in the approach as described and exemplified herein is at least ten times lower than the amount of antibody reported by Ross et al. Given the conserved expansion of the dysfunctional HSCs in both mice and humans as subjects, the results of the studies described and exemplified herein provide an advantageous foundation
25 for translational applicability in humans.

Example 13: Material and methods related to Examples 10-12

Mice

All experiments were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal
30 Care and Use Committee (IACUC) of Boston Children's Hospital and Harvard Medical School. For *in vitro* CD150-SAP experiments, 18-20-month-old C57BL/6 mice (Jackson Lab #000664) and B6.SJL-*Ptprc*^a *Pepc*^b/BoyJ mice (Jackson Lab #002014) were used. For bio-

CD150 labeling experiment, 8-week-old C57BL/6 mice (Jackson Lab #000664) were used. For *in vivo* CD150-SAP depletion experiments, 15-month-old C57BL/6 mice (Jackson Lab #000664) were used.

Antibody-saporin conjugation and administration

5 Bio-CD150 (Biolegend, clone TC15-12F12.2, catalog # B115908) or bio-IgG (BioLegend, clone RTK2758, catalog # 400504) were desalted using ZEBATM Spin Desalting Columns (Thermo Scientific, catalog # 89882). Bio-CD150 or bio-IgG antibody was mixed with strep-SAP (Advanced Targeting Systems, catalog # IT-27-1000) in a 1:1 molar ratio and then diluted in PBS to the desired concentration. CD150-SAP was added to HSC culture
10 medium or administered in 300 µL via tail vein intravenous injection.

***In vitro* cell viability assay**

HSCs were purified and cultured as previously described (Wilkinson A.C. et al., (2020), *Nat Protoc*; 15(2): 628-48). HSCs were plated in 96-well microtiter plates with 300-500 cells/well in a 100 µL volume of cell culture medium containing various concentrations
15 of antibody-saporin-conjugate. After 72 hours, cell viability was determined using a Cell Titer assay (Promega, catalog # G9241).

HSC analysis

Bone marrow (BM) cells were collected by crushing tibias, femurs, pelvic bone and spine (spinal cord was removed). The resulting cell suspension was filtered through 70 µm
20 cell strainers, and red blood cells were lysed with red blood cell lysis buffer (ebioscience, catalog #00-4333-57). To improve the sorting efficiency, filtered cells were further enriched by removing lineage positive cells. In particular, the filtered cells were first stained with biotin-conjugated antibodies against lineage markers (CD4, CD8, Gr-1, CD11b, CD5, B220 and Ter119). Lineage positive cells were removed by streptavidin-conjugated magnetic
25 beads (STEMCELL, catalog #19856), and the lineage negative cells were further stained with antibodies against mouse c-Kit, Sca-1, CD48, CD34, CD150. The dead cells were labeled with DAPI. The samples were analyzed by FACS (BD FICR canto-II). HSCs are defined cells that express biomarkers as follows: Lin-Sca-1+c-Kit+CD48-CD34-CD150+.

Peripheral blood analysis

30 Up to 30 µL vein blood was collected from retro-orbital sinus or tail tips into EDTA-coated tubes. Red blood cells were first removed by red blood cell lysis buffer (ebioscience, catalog #00-4333-57). The remaining white blood cells were stained with mixed monoclonal conjugated antibodies (antibodies directed against biomarkers CD45.1, CD45.2, CD3, B220,

Gr-1 and CD11b) in FACS staining buffer (PBS containing 1% FBS and 1mM EDTA). After incubation at 4°C in the dark for 30 minutes, samples were washed with FACS staining buffer and resuspended with staining buffer containing 1 ug/ml DAPI. Peripheral blood samples were analyzed by FACS (pBD FICR canto-II).

5

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the aspects and embodiments described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

10

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

15

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

TABLES 1A, 1B, 2, 3A, 3B

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq						
Up-regulated genes in old HSCs when compared with young HSCs						
Genes	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Ntf3	91.46564939	9.937234709	1.323283145	7.509530175	5.93399E-14	1.56581E-11
Mab21l2	159.909083	9.779494394	1.264906102	7.73139949	1.06371E-14	3.09321E-12
Gabra4	70.48625962	9.560717973	1.380415948	6.925968936	4.33001E-12	7.7123E-10
Meiob	68.47190939	9.518853724	1.41227983	6.740062076	1.58319E-11	2.50654E-09
Ccbe1	62.01845392	9.376771121	1.404547913	6.676006587	2.45541E-11	3.68721E-09
Osmr	119.1878792	9.355106288	1.313307689	7.123316468	1.05361E-12	2.14469E-10
Rbpjl	58.81657363	9.299888891	1.382530217	6.726716549	1.73534E-11	2.68771E-09
Scgb3a1	46.69332853	8.966792973	1.526451009	5.874274981	4.24698E-09	3.83008E-07
Tacr1	90.22608732	8.953093419	1.442760128	6.205531498	5.45124E-10	5.92936E-08
Shisa7	44.47811575	8.896761389	1.466516304	6.066595622	1.3065E-09	1.32974E-07
Klf15	43.62208825	8.868516729	1.689836489	5.248150803	1.53634E-07	9.55949E-06
Cntn1	42.66670909	8.837519537	1.546864181	5.713183902	1.10882E-08	9.02831E-07
Kcnb2	40.07510205	8.746624563	1.487788833	5.878942204	4.12896E-09	3.77139E-07
Zkscan2	35.32711577	8.564166961	1.632782904	5.245135126	1.56168E-07	9.67494E-06
Rassf10	28.25878766	8.242779172	1.804236973	4.56856793	4.91068E-06	0.00020107
Antxr1	28.01025854	8.230769419	1.889902449	4.355129242	1.32988E-05	0.000480952
C4b	27.7848005	8.217839019	1.620800974	5.070233269	3.97328E-07	2.13643E-05
Aplp1	27.07843828	8.180310689	1.676692656	4.87883731	1.06713E-06	5.31662E-05
Nap1l2	23.73857492	7.990686805	1.66972541	4.785629277	1.70452E-06	7.86012E-05
Panct2	23.35209493	7.967526953	1.735632944	4.590559876	4.42059E-06	0.000184336
Zfp345	22.17833778	7.892454878	2.164280894	3.646686944	0.000265643	0.005970269
Elovl2	19.95213298	7.739313481	1.846665283	4.19096712	2.77768E-05	0.000893435
Gm20554	18.7309319	7.649583657	1.76612224	4.33128777	1.4824E-05	0.000530721
Efemp1	17.20355042	7.526701843	2.109965546	3.56721552	0.000360795	0.007696054
Nol3	16.56916686	7.471243946	1.888335997	3.956522546	7.60487E-05	0.002128916
Spint1	171.7996551	7.421555267	0.815748624	9.097845889	9.21411E-20	4.23522E-17
Cyt1l	15.79960413	7.403110688	2.333458524	3.172591504	0.001510849	0.024161721
Scara3	15.31217438	7.357453901	2.034912209	3.615612442	0.000299638	0.006640042
Susd2	15.16120317	7.345046038	2.364004475	3.10703559	0.001889736	0.028433849
Marveld3	14.22269444	7.250662887	2.007407325	3.611953985	0.000303898	0.006692813
Popdc2	12.69919537	7.088579901	1.984593392	3.571804647	0.00035453	0.007585128
Ptprz1	12.4505377	7.061062056	2.139796949	3.299874813	0.00096728	0.016973852
1700030C10Rik	11.55865212	6.953624108	2.05872316	3.37763923	0.000731109	0.013564553
Rcvrn	42.4273902	6.947493739	1.382445803	5.025508939	5.02099E-07	2.64978E-05
Rasgrf2	10.63015867	6.831450317	2.0760447	3.290608491	0.000999709	0.017499828
Dpyd	9.371599887	6.649621966	2.297963368	2.893702337	0.003807289	0.047629553
Ripk4	48.83604089	6.572096075	1.412201123	4.653796099	3.25879E-06	0.000139863

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Nos1	8.831672842	6.563495614	2.243801856	2.925167209	0.003442712	0.044154096
Pcdhb20	16.01287153	6.446575836	1.749200077	3.685442232	0.000228306	0.005281056
Ces1g	15.23467516	6.373675726	1.898429736	3.357340862	0.00078696	0.014427979
Ccdc79	14.55849374	6.30703989	1.987862697	3.172774406	0.001509898	0.024161721
Sbspon	2209.626159	6.256992979	1.083817445	5.773105989	7.78235E-09	6.65839E-07
Mt2	561.5331389	6.212775857	1.028586502	6.040110232	1.54009E-09	1.50306E-07
Slc16a5	13.45274983	6.191490243	1.999505065	3.096511408	0.001958123	0.029154962
Gabrb1	12.94385163	6.136273397	2.100802816	2.920918304	0.003490013	0.044480501
Zg16	298.9344361	6.009510418	1.163308512	5.165878489	2.39312E-07	1.38616E-05
Aass	11.66444494	5.982302897	2.007524345	2.979940399	0.002883045	0.039012825
Gprc5c	42.57996683	5.951853739	1.175441485	5.063504917	4.11618E-07	2.19668E-05
Elane	10.92796593	5.887995831	2.006034515	2.935141837	0.003333953	0.043147584
Gpr183	2118.133864	5.64790482	0.511302524	11.04611175	2.28916E-28	1.71675E-25
Khdrbs2	14.95337051	5.411666354	1.709481032	3.165677918	0.00154722	0.024660334
Gdf9	21.32829529	5.314276486	1.509685317	3.520121994	0.000431348	0.008894765
Sntg2	68.58254983	5.015122829	0.957672771	5.236781271	1.63401E-07	9.99272E-06
Dazl	39.18817441	4.870517422	1.368130702	3.559979623	0.000370884	0.007875887
Lox	40.70503403	4.799642751	1.336986666	3.58989575	0.00033081	0.007185541
Gipc2	137.1109726	4.745613271	0.768018148	6.179037935	6.44934E-10	6.90952E-08
Cited4	101.7377166	4.732356198	0.824155103	5.742069885	9.35262E-09	7.67745E-07
Trpc1	50.63749273	4.72775904	1.013486391	4.664847089	3.08847E-06	0.000132953
Tram1l1	28.27692328	4.688871786	1.394715013	3.361885218	0.000774123	0.014251263
Abi3bp	66.11220416	4.649653482	1.181657261	3.934857962	8.32459E-05	0.002298781
Pcdhgb2	16.61404557	4.615377064	1.593622129	2.89615523	0.003777655	0.047341956
Agtr1a	207.4272647	4.585023351	0.71678725	6.396630731	1.58843E-10	2.02085E-08
Eya4	56.25897218	4.579187496	1.213025276	3.77501408	0.000159999	0.003910498
Bmpr1a	461.7613894	4.563698155	1.040603621	4.385625863	1.15653E-05	0.000422548
Tmem215	130.6477024	4.388401721	0.761214331	5.765001446	8.16571E-09	6.88481E-07
Chrm3	154.7646229	4.346969016	0.450311163	9.653256183	4.76192E-22	2.82719E-19
Prom2	26.04563719	4.338472594	1.455187609	2.981383684	0.00286949	0.038866319
Selp	1964.535113	4.326139958	0.292661624	14.7820541	1.91244E-49	3.40629E-46
Zswim5	79.4037549	4.21212101	0.936985614	4.495395604	6.94407E-06	0.000276386
Ndst3	28.56177676	4.178353384	1.100605719	3.796412567	0.000146805	0.00367632
Matn4	139.881751	4.149168181	0.788143065	5.26448606	1.40582E-07	8.86351E-06
Tmod2	38.56117103	4.144377404	1.296829058	3.195777716	0.001394545	0.022683644
Plscr2	2941.794427	3.980629266	0.238127736	16.71636127	9.96189E-63	7.09735E-59
Pak7	49.40237256	3.966376505	1.250146632	3.172729025	0.001510134	0.024161721
Gm6623	36.7734087	3.960463102	0.965541286	4.101806064	4.09938E-05	0.001253477
Rprm	63.22168367	3.847683953	0.906936676	4.24250563	2.21038E-05	0.000739336
Wwtr1	334.3193101	3.831360447	1.228846664	3.117850712	0.001821751	0.027762699

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Muc1	119.5108139	3.719253074	0.536874997	6.927595981	4.28052E-12	7.7123E-10
Zcchc14	91.52915615	3.634185369	0.952280478	3.81629725	0.000135469	0.0034286
Clca3a1	2354.527995	3.585999029	0.53716125	6.675833428	2.45831E-11	3.68721E-09
Tgfb3	39.98164187	3.539768859	1.188753298	2.977715278	0.002904056	0.039222651
Gm5833	887.1450602	3.53623638	0.792522062	4.462003707	8.11968E-06	0.000315252
H2-T3	117.155829	3.521013135	1.013663316	3.473552885	0.000513616	0.010293272
Sept5	57.24441598	3.500302015	0.76252972	4.590381101	4.42437E-06	0.000184336
Cldn5	934.1189281	3.490798354	0.314785311	11.08945757	1.41141E-28	1.11729E-25
Rnf43	26.45057737	3.474169986	1.138333687	3.051978542	0.002273384	0.032490915
Tc2n	457.9572126	3.457237028	0.365879014	9.449126334	3.41671E-21	1.80313E-18
Rgn	92.9313366	3.447709595	1.143654008	3.014643913	0.002572811	0.035941164
Nupr1	10482.14513	3.417296032	0.356258021	9.592193939	8.62315E-22	4.91485E-19
Zmynd15	35.45487971	3.371030339	1.136264327	2.966765971	0.003009499	0.040094556
Isl1	76.02738624	3.327229314	0.923278538	3.603711316	0.000313705	0.006890605
Abca4	130.2816158	3.313847987	0.614397341	5.393656131	6.90383E-08	4.72945E-06
Prkcz	59.67562094	3.305327719	0.807143309	4.095093996	4.21997E-05	0.00127937
Mapk11	37.40353211	3.302538628	1.036557212	3.186064975	0.001442222	0.023352525
Abat	42.45657187	3.287683371	0.900915734	3.649268459	0.000262988	0.005919934
Cd200r4	166.5237405	3.279623928	0.541546352	6.056035493	1.39517E-09	1.3902E-07
Rorb	144.7247585	3.247226541	0.59302324	5.475715493	4.35747E-08	3.12008E-06
Klrb1c	1564.768926	3.204059021	0.210056903	15.25329076	1.56541E-52	5.57637E-49
Map3k9	39.58896636	3.175428263	0.921039531	3.447656865	0.000565472	0.011159847
Tm4sf1	1233.287685	3.156924232	0.258870386	12.19499951	3.30506E-34	3.36384E-31
Dnm3	235.5541925	3.156140506	0.597574972	5.281580814	1.28074E-07	8.14699E-06
Shc4	61.54022948	3.116417751	1.058412695	2.944425899	0.003235544	0.042143359
Clu	5958.622838	3.110391186	0.153257047	20.29525719	1.41612E-91	2.01783E-87
Hoxb7	92.22943988	3.01677178	0.957969279	3.149132073	0.001637562	0.025868754
Emp2	151.9479279	2.976461838	0.613086897	4.854877594	1.20461E-06	5.85819E-05
Ramp2	1620.602415	2.973265913	0.264698945	11.23263228	2.81951E-29	2.36325E-26
Aldh3a1	124.0845943	2.942449807	0.907948192	3.240768398	0.00119208	0.020084505
Dnm3os	64.71856405	2.924831938	0.94088965	3.108581264	0.001879879	0.028405516
Gpx3	596.6232668	2.890003416	0.493166505	5.860096714	4.62598E-09	4.14563E-07
Art2b	60.86923766	2.859107703	0.983813727	2.906147398	0.00365909	0.046017987
Rorc	1154.269933	2.853768782	0.245403556	11.62888113	2.93925E-31	2.61758E-28
1700012111Rik	41.45014504	2.831650044	0.915756989	3.09214134	0.001987182	0.029395106
Cd14	87.00634429	2.812782289	0.96460009	2.916008736	0.003545406	0.045025389
Cystm1	69.23607369	2.786550757	0.708454648	3.933280363	8.37944E-05	0.002309452
Npas3	65.20275156	2.77857915	0.898449908	3.09263669	0.001983868	0.029384763
Ehd3	1493.489026	2.76375836	0.297883892	9.277971845	1.72735E-20	8.79037E-18
Afap1	38.68189885	2.756166446	0.916295803	3.007943983	0.002630216	0.036563854

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Ncam1	81.93444718	2.721419596	0.778329726	3.496486778	0.000471428	0.009596251
Gm8909	101.0675199	2.713569568	0.713466478	3.803359586	0.000142747	0.003593641
Pgr	332.3886423	2.705802706	0.541023665	5.001264978	5.69554E-07	2.98367E-05
Fhdc1	289.1652518	2.698050004	0.563056542	4.7917923	1.65298E-06	7.64718E-05
Mt1	3375.166007	2.603250747	0.20758411	12.54070336	4.4701E-36	5.30788E-33
Il1rapl2	310.9879532	2.581644134	0.347351309	7.432371967	1.06667E-13	2.57611E-11
Dlec1	40.39640738	2.567510456	0.858457717	2.990840907	0.002782104	0.037986228
Gstm2	2762.498282	2.56681946	0.170182537	15.08274293	2.10346E-51	5.99443E-48
Ar	146.2542468	2.549570083	0.570186295	4.471468544	7.76843E-06	0.000303961
Pclo	159.1862323	2.543375416	0.836005611	3.042294672	0.002347819	0.033387303
Bmp4	552.782805	2.541694175	0.3773308	6.735983853	1.62824E-11	2.54954E-09
Nova1	59.52809219	2.520689259	0.876120873	2.877102163	0.004013456	0.049673439
Acpp	186.8576193	2.487800603	0.656006474	3.792341543	0.000149233	0.003711043
Crip2	85.24461602	2.481079242	0.577147634	4.298864098	1.71676E-05	0.000595184
Hsf4	35.7551283	2.478681085	0.801058787	3.094256157	0.001973071	0.029316248
Tgfb1i1	136.8643701	2.463232305	0.597876185	4.119970601	3.78921E-05	0.001173748
Ttc23	127.0645472	2.43098106	0.625942829	3.883711016	0.000102874	0.002719581
Adgrg2	579.7308357	2.418636543	0.302984303	7.982712373	1.43152E-15	4.53284E-13
Efemp2	151.1281194	2.415530893	0.429003176	5.630566463	1.79619E-08	1.38466E-06
Tmem56	269.2849068	2.413136735	0.568871569	4.241971066	2.21565E-05	0.000739364
Amotl2	276.2276402	2.412136967	0.391627274	6.159267062	7.30824E-10	7.71371E-08
Ptprk	260.0920791	2.408818664	0.501569137	4.802565559	1.56646E-06	7.36647E-05
Meis2	89.53281427	2.405345524	0.615933499	3.905203282	9.41462E-05	0.00252719
Skap1	195.1252771	2.393995376	0.375863743	6.369317124	1.89872E-10	2.31238E-08
Sfrp1	296.4104022	2.38475632	0.497031133	4.798001901	1.60256E-06	7.43968E-05
Pih1d2	94.26286577	2.334362477	0.539196922	4.329331977	1.49562E-05	0.000532778
Arhgef28	493.4673973	2.324434125	0.342694091	6.782825235	1.17848E-11	1.92236E-09
Sh3bgrl2	186.4268063	2.299884728	0.468029141	4.913977629	8.92469E-07	4.57439E-05
Ffar2	97.21888651	2.29392639	0.670241853	3.422535283	0.0006204	0.011978436
Gpx8	317.7041438	2.276047697	0.557169558	4.085018043	4.40734E-05	0.001327699
Dsg2	253.5091034	2.269181924	0.329297373	6.890980941	5.5409E-12	9.74719E-10
Gadd45g	1141.003479	2.244524882	0.246655598	9.099833546	9.04704E-20	4.23522E-17
Ackr1	156.4681092	2.242192193	0.458177084	4.893724004	9.89456E-07	4.96435E-05
Fap	243.492957	2.238776017	0.52989908	4.224910175	2.39036E-05	0.000792099
Map1b	502.239146	2.237588814	0.292200324	7.657721866	1.8926E-14	5.39354E-12
Phf11d	930.6250662	2.225903771	0.411227853	5.412823463	6.20386E-08	4.35462E-06
Il17rc	61.9580252	2.209830457	0.644765447	3.427340078	0.000609525	0.011800441
Gm9199	703.4807629	2.176205307	0.284485236	7.649624779	2.01567E-14	5.63161E-12
Neo1	409.4788928	2.175868193	0.449467782	4.840988123	1.29195E-06	6.19832E-05
Hspb1	117.3333249	2.175441304	0.602039091	3.613455233	0.000302144	0.006664463

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Hoxb6	146.9088445	2.172419095	0.511591113	4.246397248	2.17235E-05	0.00073177
Kif5a	76.9148023	2.162441556	0.653551349	3.308755401	0.000937117	0.016543317
Ehd2	96.87920061	2.155685364	0.622264247	3.464260361	0.000531692	0.010581109
Sdpr	4678.231742	2.145958511	0.135637697	15.82125435	2.22025E-56	1.05454E-52
Cyp26b1	416.9828785	2.137081514	0.49320246	4.333071477	1.47043E-05	0.000527763
Clec1a	2327.664152	2.125985506	0.154969167	13.71876453	7.83898E-43	1.24108E-39
Exoc3l4	117.574951	2.125161517	0.548833327	3.872143714	0.000107882	0.00282925
Aspa	871.923836	2.12428811	0.258618108	8.213996021	2.13947E-16	7.43543E-14
Tmem231	104.7110459	2.122416524	0.625395267	3.393720159	0.000689501	0.012995637
Lgmn	98.59854649	2.099685428	0.671672717	3.126054365	0.001771688	0.027208451
Nrg4	571.4584358	2.080683364	0.232172679	8.961792474	3.19443E-19	1.42242E-16
9530026P05Rik	90.93901094	2.077276716	0.56407567	3.682620661	0.000230849	0.00532259
Nxph1	51.44064617	2.074395414	0.698067609	2.971625365	0.002962279	0.039808186
Zfp92	104.8548233	2.048156544	0.659800989	3.104203507	0.00190792	0.028616799
C130026I21Rik	81.50112212	2.044142622	0.680617975	3.003362673	0.00267014	0.036781666
Slc16a12	320.2163372	2.038738555	0.447833339	4.552449266	5.3025E-06	0.000216491
Zfp57	266.4061294	2.014185439	0.364014881	5.533250278	3.1435E-08	2.32082E-06
Jam2	1571.492923	2.006805471	0.216483473	9.270016966	1.86116E-20	9.14473E-18
Maf	200.4607638	1.999750584	0.394866401	5.064372606	4.09748E-07	2.19492E-05
Nr1h3	148.1243537	1.994293763	0.462252065	4.314299309	1.6011E-05	0.000559168
Gpr4	203.7004868	1.971689736	0.58732708	3.357055725	0.000787772	0.014427979
Rab34	263.7825431	1.966396711	0.507870953	3.871843226	0.000108015	0.00282925
Irf2bpl	149.5842055	1.955404745	0.45448723	4.302441556	1.68926E-05	0.00058708
Enpp5	5616.301704	1.939852694	0.162939676	11.90534276	1.11006E-32	1.05449E-29
Cd38	1824.085275	1.929889453	0.155046573	12.44715968	1.44911E-35	1.58834E-32
Cxcl16	181.6418571	1.926281258	0.609534194	3.160251346	0.001576331	0.025096245
Serpinb8	443.5450853	1.918276179	0.40402831	4.747875663	2.05564E-06	9.3283E-05
Ptafr	87.28442725	1.915605822	0.607618722	3.152644502	0.001617987	0.025587902
Meis3	315.6727297	1.901135964	0.324478942	5.859042657	4.65543E-09	4.14595E-07
Sult1a1	14975.4053	1.892094378	0.181488807	10.42540532	1.89848E-25	1.28816E-22
Kdr	192.289582	1.889692799	0.547628074	3.450686497	0.000559163	0.011065985
Dhrs3	343.3786633	1.887622502	0.428468189	4.405513759	1.05533E-05	0.000396767
Rbm19	499.1169208	1.877842088	0.325614416	5.767072947	8.06601E-09	6.84123E-07
Dpy19l2	160.2871646	1.865449377	0.473255646	3.941737185	8.08936E-05	0.002246886
Fam46c	177.4112848	1.862725999	0.466882124	3.989713688	6.61531E-05	0.001873987
Entpd2	153.1795921	1.860906802	0.594135378	3.132125897	0.001735454	0.026849609
Lpl	452.490044	1.842225613	0.28296752	6.510378342	7.49618E-11	1.02705E-08
Gm13546	270.0168318	1.837773969	0.347070326	5.295105433	1.18948E-07	7.63462E-06
Ampd3	451.025726	1.836133209	0.357850885	5.131000882	2.88206E-07	1.61679E-05
Gm9958	226.4433984	1.832890778	0.460930378	3.976502452	6.99363E-05	0.001969411

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Smtnl2	67.11615099	1.831463949	0.593218804	3.08733293	0.002019613	0.029728781
Hpgds	786.7310286	1.819413498	0.371767316	4.893957642	9.88281E-07	4.96435E-05
Dlk1	325.72222	1.797512381	0.402496432	4.465908854	7.97296E-06	0.000310401
Rundc3a	121.4751211	1.79547212	0.559509987	3.209008171	0.001331937	0.021839783
Gulo	99.14996111	1.780789772	0.60027342	2.96663106	0.00301082	0.040094556
Cysltr2	1004.187476	1.767771212	0.235020855	7.52176316	5.40424E-14	1.45293E-11
Ppap2a	132.9912743	1.728865996	0.469831457	3.679757862	0.000233456	0.005365335
C1qtnf4	141.7277682	1.711035007	0.466096542	3.670988419	0.000241614	0.00549962
Pde9a	592.8933351	1.709798627	0.215722342	7.925922809	2.26458E-15	7.01479E-13
Dcbld2	152.5912765	1.689096992	0.450305729	3.751000449	0.00017613	0.004246501
Alcam	2565.137063	1.682831782	0.127452491	13.20360057	8.36397E-40	1.19178E-36
Prtn3	8497.608822	1.676364457	0.11291761	14.84590812	7.39466E-50	1.50524E-46
Mmp14	297.4552488	1.671850886	0.492247907	3.39635956	0.000682886	0.012905095
Rab40b	242.1181753	1.633480099	0.342708281	4.766386423	1.87559E-06	8.56581E-05
Klhl13	210.1446988	1.627323978	0.481004065	3.38318134	0.000716513	0.013373814
Ctsw	921.5067028	1.622828922	0.259965165	6.242486068	4.3067E-10	4.72048E-08
Rdh10	685.6202947	1.61791673	0.316490444	5.112055548	3.18672E-07	1.77373E-05
Cpne8	2008.895172	1.613091178	0.153015927	10.54198217	5.53204E-26	3.9413E-23
Epdr1	741.3928489	1.602278689	0.284612744	5.629680062	1.80544E-08	1.38466E-06
Echdc2	144.3716492	1.602206355	0.496132669	3.229390955	0.001240542	0.020698454
1810041H14Rik	83.50564396	1.597553574	0.554926181	2.878857815	0.003991182	0.049538638
Ccdc92	242.4559404	1.593326736	0.413448175	3.853752014	0.000116321	0.003002652
Plek	1719.975563	1.593176124	0.245433314	6.491279034	8.51107E-11	1.12291E-08
Tmem150a	484.7680207	1.592738117	0.263581641	6.042674715	1.5158E-09	1.48956E-07
Lsr	370.6447433	1.590264075	0.284668812	5.586365663	2.31871E-08	1.75741E-06
Gda	2573.490874	1.589070898	0.238627925	6.659199247	2.75323E-11	4.08655E-09
Nxn12	300.9684225	1.57959984	0.306722112	5.149937936	2.60573E-07	1.49112E-05
Pigg	329.0269646	1.565775417	0.356463855	4.392522259	1.12043E-05	0.000413602
Perp	313.8383856	1.546314656	0.386050084	4.005476806	6.18925E-05	0.001774459
9230112J17Rik	271.9845636	1.540221103	0.343806566	4.479906009	7.46759E-06	0.000294864
Smtnl1	862.3831481	1.538780504	0.224867388	6.843057675	7.75204E-12	1.31499E-09
Cdkn2c	601.7928118	1.535894722	0.238884534	6.429443954	1.28072E-10	1.65899E-08
Nuak2	336.8059202	1.53514246	0.426587919	3.598654329	0.000319868	0.007010085
Gkn3	384.9752643	1.534541103	0.498981712	3.075345384	0.002102589	0.030477917
Aph1b	293.2878667	1.526396432	0.407900903	3.742076622	0.000182506	0.00437799
Epcam	403.134906	1.526352719	0.332957924	4.584221038	4.55682E-06	0.000188203
Arpin	470.4475776	1.520691029	0.336061461	4.525038435	6.03845E-06	0.000244437
Apol7e	540.2597369	1.518525566	0.359217254	4.227318011	2.36493E-05	0.0007855
Fam57a	152.9401628	1.517663506	0.480567579	3.1580647	0.001588203	0.02522888
Npdc1	2158.161937	1.515565166	0.187959616	8.063248888	7.4293E-16	2.40591E-13

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Ldhd	353.1406355	1.508397126	0.271246628	5.560980195	2.68264E-08	2.0013E-06
Rarb	1015.782841	1.491397772	0.280208798	5.322451627	1.02378E-07	6.69167E-06
Klhl4	2296.958611	1.48344917	0.199243495	7.4454083	9.6645E-14	2.3743E-11
Calml4	465.4183473	1.47125947	0.332802943	4.420812673	9.83304E-06	0.000373629
Shtn1	239.2543603	1.450565258	0.3689231	3.931890571	8.42804E-05	0.002318363
4921504A21Rik	194.9198781	1.437658457	0.439543553	3.270798645	0.001072442	0.018500281
Vldlr	775.9600936	1.436547985	0.227399587	6.317284929	2.66199E-10	3.10907E-08
Dcun1d4	305.9038175	1.429603823	0.447490247	3.194715043	0.00139969	0.022729077
Runx1t1	750.4681443	1.429343375	0.234290728	6.100725322	1.05588E-09	1.09024E-07
Srd5a1	325.1095036	1.414682256	0.3669729	3.85500471	0.000115727	0.002992909
A230065H16Rik	291.3074819	1.4076396	0.421182954	3.342109615	0.000831442	0.015115768
Plbd1	212.2854781	1.402244197	0.429028601	3.268416592	0.00108151	0.018624839
Ocln	446.3636477	1.394275465	0.483351377	2.88460017	0.003919111	0.048899655
Rasl11a	198.4245836	1.393630548	0.371622446	3.75012479	0.000176747	0.004254159
Pros1	1101.986687	1.369272907	0.327427722	4.181908904	2.89072E-05	0.000921473
Oxr1	12230.70433	1.366469367	0.10697174	12.77411557	2.28733E-37	2.96292E-34
Fhl1	3118.847314	1.365408939	0.168488667	8.103862186	5.32414E-16	1.80628E-13
Pam	823.2316177	1.365227251	0.182690955	7.472878188	7.84595E-14	1.96135E-11
Plcl1	696.8679763	1.354376181	0.21179806	6.394658093	1.60907E-10	2.02899E-08
Fam169a	250.0658072	1.349885729	0.34301512	3.935353432	8.30744E-05	0.002298498
Aldh1a1	4772.323795	1.346027241	0.129465511	10.39680166	2.56395E-25	1.66062E-22
1700029J07Rik	684.2496322	1.340968525	0.302226756	4.436961655	9.12375E-06	0.000349474
2410017I17Rik	180.5696759	1.33727344	0.44926953	2.976550491	0.002915111	0.039334672
Gliplr1l1	423.5368688	1.330467302	0.307654917	4.324544241	1.52848E-05	0.000541138
Heg1	931.8311036	1.303929106	0.202395416	6.4424834	1.17534E-10	1.53646E-08
Vwf	1720.439387	1.298342607	0.228375025	5.685133957	1.3071E-08	1.03472E-06
Eps8l2	218.6312427	1.295629127	0.415288813	3.1198267	0.001809575	0.027636259
Gstm6	152.70394	1.295561757	0.450216056	2.877644502	0.004006564	0.049643066
Neu3	299.5930327	1.286187276	0.38636244	3.328965606	0.000871692	0.015643243
Rbpms2	939.5123515	1.279368283	0.217694061	5.876909452	4.17997E-09	3.79365E-07
Gem	1700.15707	1.269384618	0.259741002	4.887116816	1.02323E-06	5.11581E-05
Itgb3	1775.814631	1.268121702	0.211566444	5.993964237	2.04786E-09	1.93245E-07
Cebpd	258.1223354	1.257124657	0.303152605	4.146837715	3.37099E-05	0.001058
Bhlhe40	433.7098779	1.247904173	0.405358055	3.078523189	0.002080293	0.030308896
Rnf208	285.083629	1.244623246	0.407370975	3.05525755	0.002248673	0.032233329
Ppargc1a	163.4026831	1.242671624	0.382800482	3.246264522	0.001169301	0.019811385
Bend7	154.7152903	1.240717929	0.431701404	2.874018751	0.004052849	0.049955922
Zfp354a	428.3012542	1.238751267	0.3990114	3.104551063	0.00190568	0.028613318
Mill2	187.893856	1.238294309	0.430017127	2.879639509	0.003981301	0.049459075
Bcl3	984.0554197	1.234338764	0.240398906	5.134544013	2.82829E-07	1.59296E-05

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Rapgef3	195.3106186	1.228752676	0.396317625	3.100424001	0.001932438	0.028863007
Ptges	213.4727335	1.221010236	0.412189499	2.962254591	0.003053952	0.040555224
Col16a1	449.8342887	1.220281809	0.263450866	4.63191421	3.623E-06	0.000154563
Fads3	512.5332386	1.212555752	0.309409317	3.918937429	8.89402E-05	0.002413922
Ptger4	1149.167556	1.208006255	0.226240541	5.339477397	9.32149E-08	6.14916E-06
Sox18	592.6998902	1.200510365	0.231828808	5.178434781	2.23755E-07	1.30147E-05
Ypel2	227.7385545	1.19858235	0.326459298	3.67146029	0.000241169	0.005498257
Ak1	658.2133615	1.193426441	0.258726809	4.61268952	3.97492E-06	0.000168067
Nhsl2	154.9016906	1.192081003	0.40956721	2.910587013	0.003607505	0.045570336
Pygm	1014.21432	1.191575824	0.167260112	7.124088409	1.04772E-12	2.14469E-10
Adamts15	380.6140161	1.183396188	0.287583429	4.114966544	3.87236E-05	0.001191732
Slamf1	3241.247711	1.17155997	0.196290389	5.968503989	2.39439E-09	2.24458E-07
B3gnt8	404.0044084	1.165901237	0.286618851	4.067775833	4.7464E-05	0.001403142
Cdc14b	306.8327056	1.163047885	0.377967098	3.077114097	0.002090153	0.030390395
Efna1	1987.294291	1.161041484	0.204377878	5.680856938	1.34022E-08	1.05507E-06
Id2	1834.410622	1.147897284	0.179869135	6.38184691	1.74965E-10	2.18691E-08
B9d1	253.4558138	1.134566175	0.368690627	3.077285105	0.002088954	0.030390395
Prune2	316.687701	1.134234725	0.349999491	3.240675356	0.001192469	0.020084505
Bbs10	560.0282357	1.133861105	0.38985272	2.908434511	0.003632432	0.045804009
H2-Eb1	2168.54257	1.131055617	0.25700746	4.400866873	1.07819E-05	0.000402177
Mir155hg	1219.819008	1.130184575	0.238792349	4.732917876	2.21315E-06	9.94801E-05
Tmem50b	1670.763049	1.124956032	0.209326837	5.37416057	7.69403E-08	5.19584E-06
Art4	4357.182182	1.121766549	0.150066643	7.47512258	7.71319E-14	1.96135E-11
Dock9	516.3348651	1.121372376	0.29928151	3.746881576	0.000179047	0.00430225
Stom	476.3033005	1.120251232	0.254918701	4.394543154	1.11006E-05	0.000410837
Fyb	3075.950362	1.116141869	0.214755296	5.197272854	2.02233E-07	1.20068E-05
Srxn1	967.176831	1.108377653	0.289122097	3.833597173	0.000126283	0.00322474
Pabpc4l	454.3778874	1.1011729	0.286795255	3.839578512	0.000123246	0.003164195
Evc2	258.6596656	1.098615627	0.321767456	3.414315547	0.000639425	0.012262669
Ppp1r16b	485.5334446	1.095197877	0.35969598	3.044787649	0.002328446	0.033144889
Smarcd3	270.1158723	1.094892409	0.31039222	3.527447975	0.000419586	0.008689947
St3gal6	849.1381399	1.092900671	0.241896402	4.51805261	6.2411E-06	0.000251495
Tfcp2	303.0388621	1.085956953	0.3669024	2.959797898	0.003078409	0.040728182
Manea	700.7700352	1.08498414	0.209520419	5.178417197	2.23776E-07	1.30147E-05
Camkk1	439.7126719	1.082982207	0.323694005	3.345697451	0.000820759	0.014955236
Chac2	660.8567318	1.080782826	0.273726652	3.94840188	7.86747E-05	0.002193806
Rbm38	1135.651954	1.074265062	0.28277047	3.799070894	0.00014524	0.003649944
Selm	2210.477056	1.069897119	0.159173071	6.721596246	1.79744E-11	2.75396E-09
Xdh	943.9429888	1.064226035	0.221759809	4.799003213	1.59457E-06	7.43968E-05
Pbx3	908.7191151	1.062373768	0.203515216	5.220119591	1.78808E-07	1.07503E-05

Table 1A - List of DEGs between young and old HSCs derived from bulk RNA-seq**Up-regulated genes in old HSCs when compared with young HSCs**

Tox	1394.402636	1.054817836	0.174015253	6.06164009	1.3474E-09	1.36164E-07
Rhoj	764.9614706	1.054613517	0.203136546	5.191648368	2.0844E-07	1.2273E-05
Fads2	393.99267	1.041974034	0.31886007	3.267809715	0.001083832	0.018629099
Stk39	198.2003864	1.026518728	0.35492905	2.892180078	0.003825786	0.047818969
Cd302	1221.920239	1.016368306	0.208442289	4.876017772	1.08249E-06	5.37435E-05
Procr	5651.121685	1.014760858	0.125459832	8.088332651	6.0487E-16	2.00437E-13
Adck3	3632.644948	1.011827675	0.216821905	4.666630319	3.06179E-06	0.000132205
Tacstd2	1930.656676	1.011676823	0.230621905	4.386733433	1.15066E-05	0.000422548
Gstt1	1716.775936	1.010172966	0.183367294	5.50901388	3.60849E-08	2.62334E-06
Lpar6	9913.552595	1.008966447	0.155100467	6.505244429	7.75672E-11	1.05262E-08
Yipf2	461.3834107	1.008625485	0.346752299	2.908778078	0.003628443	0.045794232
Rab3il1	665.5925094	1.002090491	0.228469113	4.386109248	1.15396E-05	0.000422548
Plscr1	3942.018783	1.001494809	0.12018258	8.333111218	7.87451E-17	2.87702E-14
Gchfr	830.0150468	1.001463574	0.233613674	4.286836293	1.81236E-05	0.000622467
Smagp	813.8781206	1.001178075	0.214249352	4.672957318	2.96893E-06	0.000128585

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Genes	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Spns3	285.376115	-1.003060007	0.327983065	-3.058267675	0.002226206	0.032009296
Gins2	886.039298	-1.004339064	0.229830447	-4.369913027	1.24296E-05	0.00045181
Rev3l	850.140504	-1.004615372	0.240423179	-4.178529607	2.934E-05	0.000933182
Nt5dc2	1283.12923	-1.005012181	0.192392011	-5.223772946	1.75314E-07	1.063E-05
Clspn	444.852792	-1.007286438	0.322356582	-3.124758403	0.001779511	0.027264795
Trf	917.669287	-1.013601738	0.251912428	-4.023627357	5.73086E-05	0.00166651
Myo7a	271.53045	-1.015375373	0.317632863	-3.196694962	0.001390118	0.022637484
Nmral1	1527.75713	-1.017878117	0.194496232	-5.233407912	1.66413E-07	1.01334E-05
Afp	648.742424	-1.019797917	0.232853923	-4.379560825	1.18919E-05	0.000433369
Dusp6	1769.37116	-1.022914824	0.28276837	-3.617500874	0.000297461	0.006602066
Syce2	2702.43517	-1.023777629	0.141017133	-7.259952073	3.87228E-13	8.7581E-11
Acy1	532.652951	-1.024543149	0.307800944	-3.32859002	0.000872868	0.015644648
Gpr65	766.604372	-1.027778582	0.262002848	-3.922776382	8.75344E-05	0.002387228
Prim2	965.80022	-1.030007444	0.256882557	-4.009643377	6.08105E-05	0.001746954
Atad5	830.091822	-1.033320381	0.319643394	-3.232728723	0.001226139	0.020506174
Rad54l	421.894574	-1.034349483	0.247818052	-4.173826229	2.99526E-05	0.000950546
Dnmt1	1736.75296	-1.034449438	0.196999115	-5.251035952	1.51246E-07	9.45222E-06
Gng2	1139.67928	-1.044454122	0.226935667	-4.602423824	4.17602E-06	0.000176048
4930579G24Rik	949.563667	-1.051675886	0.219192356	-4.797958775	1.60291E-06	7.43968E-05

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Arhgap30	798.207636	-1.061868841	0.229792147	-4.620997091	3.819E-06	0.000162439
Carns1	256.59195	-1.064733427	0.339337192	-3.137685619	0.001702874	0.026569378
Rgcc	800.623557	-1.069651366	0.233829443	-4.574493923	4.77372E-06	0.000196025
Cd68	312.191056	-1.072267245	0.348625846	-3.075696356	0.002100116	0.030477917
Kif20b	1833.94757	-1.073056493	0.169218413	-6.341251375	2.27906E-10	2.7062E-08
E030024N20Rik	238.62727	-1.076386046	0.3696724	-2.911729539	0.003594337	0.045448669
Dtl	1068.73982	-1.089348559	0.231795431	-4.699611878	2.60656E-06	0.000115279
Slfn9	842.518094	-1.094731781	0.295045753	-3.710379723	0.000206949	0.004874067
Cd37	1030.0848	-1.095180695	0.23219188	-4.716705406	2.39694E-06	0.000106731
Lsp1	5385.75339	-1.095788819	0.126492053	-8.662906418	4.59886E-18	1.82025E-15
Rwdd2a	408.218471	-1.097584574	0.279259564	-3.930338346	8.48264E-05	0.002319945
Gins1	636.567042	-1.100133363	0.315163523	-3.490674789	0.000481802	0.009751707
Szt2	319.937154	-1.102674737	0.349753905	-3.152716009	0.001617591	0.025587902
C330027C09Rik	448.979523	-1.104419283	0.372561073	-2.964397955	0.003032758	0.040311353
Gab2	286.424144	-1.115299043	0.327270349	-3.407882948	0.00065469	0.012471489
Tnik	372.947261	-1.117158779	0.376308145	-2.968733985	0.002990293	0.040008161
Ptgr1	457.948188	-1.127724023	0.313936062	-3.592209241	0.000327886	0.007149186
Kif23	1231.64942	-1.130434016	0.239206579	-4.725764733	2.29251E-06	0.000102401
Mad2l1	2086.50659	-1.143496374	0.190024596	-6.017622967	1.76997E-09	1.69264E-07
Cdt1	799.788125	-1.144301419	0.295212262	-3.876198813	0.000106101	0.002794516
Susd1	546.059382	-1.144611345	0.291158394	-3.931232516	8.45115E-05	0.002319945
P2ry14	567.348513	-1.149216418	0.287814591	-3.992905345	6.52686E-05	0.001853055
Fgl2	451.343696	-1.15892242	0.287211604	-4.035082158	5.45832E-05	0.001597035
Ap1s2	273.093525	-1.162770524	0.338976585	-3.430238478	0.000603051	0.011690984
Il15	2219.02575	-1.164277196	0.163296845	-7.129820504	1.005E-12	2.10592E-10
Asf1b	1519.9997	-1.167131568	0.30381606	-3.841572988	0.000122248	0.003144255
Brip1	368.256003	-1.17875801	0.38001861	-3.101842856	0.0019232	0.028797512
Rad51	1007.98302	-1.188223981	0.331273662	-3.586835041	0.000334716	0.007259311
Wdhd1	681.28342	-1.188461996	0.243956009	-4.871624192	1.10685E-06	5.4762E-05
Figl1	1311.99704	-1.189596647	0.208776239	-5.697950355	1.21256E-08	9.76148E-07
Nrm	1947.54229	-1.192721423	0.135027166	-8.83319603	1.01727E-18	4.26324E-16
Tacc3	929.450817	-1.192910131	0.187358389	-6.366996098	1.92766E-10	2.32773E-08
Exo1	601.446575	-1.200776122	0.377091757	-3.18430753	0.001451008	0.023459077
Serpinb1a	1965.1794	-1.204949315	0.169897783	-7.09220152	1.31995E-12	2.57644E-10
Col4a1	419.839151	-1.210909475	0.399951611	-3.027639951	0.002464716	0.034806474
Depdc1b	275.583411	-1.216453428	0.387652565	-3.137999174	0.001701054	0.026569378
Cenpf	902.669919	-1.221755708	0.398186596	-3.068299433	0.002152808	0.031079391
Tyms	3030.83598	-1.221969906	0.195235193	-6.258963277	3.87545E-10	4.38264E-08
Cdc6	743.564791	-1.234007593	0.256644655	-4.808234152	1.52269E-06	7.18439E-05
Slc28a2	348.525789	-1.235707346	0.348012363	-3.550757035	0.000384125	0.008108733

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Hells	1581.85658	-1.236963746	0.194476718	-6.360472133	2.01135E-10	2.40837E-08
Cd34	4235.32531	-1.238016899	0.130153537	-9.511972769	1.8708E-21	1.02527E-18
Slc22a3	341.537131	-1.238047045	0.275216501	-4.498447732	6.84514E-06	0.000273211
Tipin	4767.11823	-1.246316242	0.163331231	-7.630605829	2.33653E-14	6.40254E-12
Kctd12b	220.66266	-1.254301631	0.407852011	-3.075384199	0.002102315	0.030477917
Prim1	2874.51515	-1.265776822	0.177914893	-7.114507412	1.12313E-12	2.22271E-10
Gmnn	1965.17939	-1.269462602	0.213375002	-5.949443899	2.69055E-09	2.48946E-07
Arxes2	546.482163	-1.269551866	0.261588855	-4.853233765	1.21464E-06	5.88689E-05
Arsb	287.822852	-1.276164204	0.377764902	-3.378196851	0.000729628	0.013554724
Pole2	686.244066	-1.278945761	0.312151985	-4.097189264	4.18197E-05	0.001272158
Pidd1	161.305876	-1.280497586	0.420122425	-3.047915343	0.002304348	0.03283466
Hist1h1e	327.880975	-1.281842348	0.384024981	-3.337913969	0.000844099	0.015263406
Il4	739.947261	-1.295391167	0.242067083	-5.351372644	8.72896E-08	5.8121E-06
Cryl1	496.731527	-1.300197084	0.293144983	-4.435337998	9.19279E-06	0.000351175
Spry1	725.845669	-1.303116867	0.40925872	-3.184090655	0.001452095	0.023459077
Nkg7	1345.25536	-1.306051197	0.212819784	-6.136888071	8.41536E-10	8.75259E-08
Gpatch3	138.991251	-1.310400191	0.413245403	-3.170997627	0.001519164	0.02426745
Cdca2	323.115498	-1.318244413	0.393348159	-3.351342527	0.000804208	0.014691224
Cks1b	4394.19378	-1.335892505	0.153480564	-8.703984861	3.2043E-18	1.30452E-15
E130307A14Rik	264.953883	-1.338675837	0.341278106	-3.922536531	8.76216E-05	0.002387228
2810408A11Rik	203.964047	-1.341778119	0.450592471	-2.977808564	0.002903173	0.039222651
Myh10	216.73725	-1.343797559	0.42840337	-3.136757679	0.001708273	0.026573335
1190002F15Rik	1228.27547	-1.34884084	0.23472775	-5.746405535	9.11605E-09	7.59618E-07
Cdk5rap1	951.050335	-1.355335157	0.346557751	-3.910849358	9.19721E-05	0.002482028
Mgst1	1298.85228	-1.356084626	0.204556733	-6.629381513	3.37096E-11	4.95184E-09
Filip1l	438.713224	-1.362386392	0.39023749	-3.491172499	0.000480906	0.009747403
Rad51ap1	603.668206	-1.370712554	0.359460007	-3.813254683	0.000137149	0.003464949
Rel	147.364104	-1.371446027	0.403384072	-3.399851706	0.000674224	0.012774823
St3gal5	240.129897	-1.385848745	0.48071569	-2.882886438	0.003940495	0.049080515
Cks2	858.501933	-1.386695533	0.218859611	-6.336004754	2.358E-10	2.77678E-08
Syk	796.292299	-1.3879761	0.276813995	-5.014111014	5.32792E-07	2.80139E-05
Rad51b	342.336113	-1.390101944	0.288501115	-4.818358995	1.44744E-06	6.85201E-05
Aurka	748.045968	-1.400534823	0.334802291	-4.183169775	2.87473E-05	0.00091843
Cenph	591.420387	-1.402763895	0.480892634	-2.917000171	0.003534156	0.0449259
Cenpp	432.231269	-1.412769203	0.260241287	-5.428689733	5.67693E-08	4.00448E-06
Tnfaip8l1	134.655264	-1.434229749	0.472322165	-3.036549747	0.002393026	0.033878855
Hmgb2	3511.98076	-1.435623025	0.20931783	-6.858579728	6.95485E-12	1.19397E-09
Tpx2	767.408944	-1.435848131	0.406073764	-3.535929325	0.000406343	0.008455198
Fbxo5	793.778266	-1.436220114	0.405256547	-3.543977578	0.000394139	0.00826652
Uhrf1	674.686343	-1.437131916	0.23901629	-6.012694426	1.82465E-09	1.73329E-07

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Rgs12	307.004827	-1.459687951	0.384612828	-3.795213901	0.000147516	0.003681188
Id1	120.574074	-1.472625084	0.473505782	-3.110046676	0.001870578	0.028348279
Ppbp	314.771376	-1.478721468	0.39096551	-3.782229967	0.00015543	0.003838332
Kif20a	309.657442	-1.484165108	0.459997728	-3.226461823	0.00125331	0.020813994
Phldb2	139.379737	-1.489358103	0.479001803	-3.1092954	0.001875341	0.028367024
Ms4a6b	1391.95131	-1.49464604	0.292119425	-5.116558196	3.11161E-07	1.73872E-05
Lat2	251.575335	-1.502021246	0.442534927	-3.394130392	0.000688469	0.012993371
Rps4l	813.40242	-1.507130407	0.303837645	-4.960314935	7.0379E-07	3.65996E-05
Skint3	365.035435	-1.512077455	0.428124322	-3.531865345	0.000412639	0.008558514
Sell	195.438849	-1.512954051	0.476809601	-3.17307799	0.00150832	0.024161721
Cenpk	902.140906	-1.528067944	0.301185506	-5.073510886	3.90542E-07	2.10789E-05
Tonsl	231.793998	-1.531495593	0.324921867	-4.713427277	2.43585E-06	0.000108126
Rrm2	1316.13494	-1.56095271	0.329719521	-4.734183485	2.19939E-06	9.91743E-05
Bub1	469.412703	-1.571756244	0.261190558	-6.017661045	1.76955E-09	1.69264E-07
F2rl2	267.25412	-1.571818854	0.334642198	-4.697013299	2.63993E-06	0.0001161
Ccnb1	996.355168	-1.592392545	0.355554768	-4.478613953	7.51293E-06	0.000295723
Chaf1a	616.615661	-1.597799219	0.254382117	-6.281098831	3.36188E-10	3.86318E-08
Fgd2	469.586661	-1.600799015	0.360439553	-4.441241256	8.94414E-06	0.000343518
Lrr1	190.968489	-1.611526769	0.478717076	-3.366344861	0.000761714	0.014077386
Nfam1	397.038846	-1.621599684	0.280360487	-5.783980842	7.29533E-09	6.30007E-07
Ndc80	830.004808	-1.62833525	0.343817771	-4.73604155	2.17933E-06	9.85818E-05
Apitd1	593.382583	-1.63240114	0.306830247	-5.320209312	1.03648E-07	6.71309E-06
E2f1	322.888387	-1.634637754	0.283638036	-5.763111948	8.2577E-09	6.92141E-07
Lgals1	785.131617	-1.653771552	0.19789816	-8.356679779	6.45081E-17	2.41888E-14
Cenpm	578.925418	-1.674637719	0.257858852	-6.494396866	8.33667E-11	1.11018E-08
Akr1c12	310.766582	-1.680290787	0.489627616	-3.431772904	0.000599649	0.01165676
Dscc1	475.644523	-1.686392842	0.299564256	-5.629486189	1.80747E-08	1.38466E-06
Cenpa	1449.48008	-1.688770878	0.303580283	-5.562847688	2.65408E-08	1.99042E-06
Chek1	925.117971	-1.689415257	0.327400206	-5.160092215	2.46828E-07	1.42391E-05
Ttk	325.623539	-1.691156691	0.478289108	-3.535846132	0.000406471	0.008455198
Hist1h2ae	964.202552	-1.693421442	0.329810006	-5.134536276	2.82841E-07	1.59296E-05
Fxyd1	104.476882	-1.694206776	0.52449681	-3.230156495	0.001237225	0.02066731
Lmnbl	242.386896	-1.694336491	0.478109875	-3.54382241	0.000394371	0.00826652
Tnfrsf13c	367.563352	-1.69831795	0.337128968	-5.03759128	4.71427E-07	2.50648E-05
Nrk	206.03742	-1.719182361	0.486681928	-3.532455723	0.000411719	0.008551875
Mc5r	114.157746	-1.73023846	0.522181558	-3.313480599	0.000921425	0.016330081
Il12rb2	146.901761	-1.734990032	0.467101216	-3.714377041	0.000203705	0.004805614
Ckap2	731.108628	-1.745959849	0.278334717	-6.27287846	3.54434E-10	4.04026E-08
Fam105a	669.649301	-1.746463368	0.265438275	-6.57954609	4.71887E-11	6.68292E-09
Mcm10	187.735365	-1.747837713	0.570800162	-3.062083421	0.002198022	0.031667961

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Spp1	169.284354	-1.753828579	0.58104416	-3.018408408	0.002541062	0.035672505
Spic	216.686065	-1.759409073	0.542755966	-3.241620883	0.00118852	0.020065431
Rad54b	400.362097	-1.767009397	0.335211484	-5.27132716	1.35441E-07	8.57732E-06
Cenpn	532.212876	-1.774613113	0.410437755	-4.323708266	1.53428E-05	0.000541138
Fancd2	114.716017	-1.805265566	0.47155417	-3.828331253	0.000129015	0.00327689
Tyrbp	114.362098	-1.815964396	0.51537146	-3.52360295	0.000425722	0.008791461
Ms4a6c	473.589687	-1.833809551	0.293272439	-6.252921549	4.02845E-10	4.48448E-08
Kif22	647.788325	-1.83458771	0.385157914	-4.76320918	1.90538E-06	8.67405E-05
Ect2	456.386913	-1.836475278	0.331525061	-5.539476487	3.03377E-08	2.25147E-06
Col4a2	421.959129	-1.839550864	0.375443713	-4.899671515	9.5997E-07	4.85057E-05
Oas2	194.67636	-1.85717158	0.499329414	-3.719331422	0.000199751	0.004727989
Mfsd2b	202.515868	-1.858589216	0.450554544	-4.125114795	3.7055E-05	0.001157887
Spc25	1410.98784	-1.86019522	0.225676194	-8.242762286	1.6828E-16	5.99454E-14
Mis18bp1	547.785963	-1.862446171	0.47671473	-3.906835793	9.35127E-05	0.002518831
Polq	194.621428	-1.865204815	0.588995634	-3.166754908	0.001541502	0.024596706
Mybl2	111.365472	-1.868110217	0.479667552	-3.894593684	9.83635E-05	0.002629608
Igf1	310.276766	-1.871823957	0.444480367	-4.211263527	2.53946E-05	0.000829379
Shcbp1	759.406293	-1.879617068	0.430615108	-4.36495848	1.27147E-05	0.000460997
Ebi3	1332.19493	-1.885787615	0.255613038	-7.377509508	1.61278E-13	3.83008E-11
Kif15	325.154189	-1.899696936	0.515773523	-3.683199796	0.000230324	0.005319115
Kcnk12	143.472785	-1.908747913	0.53383705	-3.575525365	0.000349525	0.007511894
Ptn	292.223688	-1.914600669	0.564520166	-3.391554077	0.000694974	0.013064039
Cdk1	1828.8631	-1.917561096	0.292071319	-6.565386509	5.18981E-11	7.17957E-09
5430427O19Rik	523.811955	-1.939514952	0.263869854	-7.350271058	1.97805E-13	4.62054E-11
Ugt1a7c	99.8099714	-1.94275864	0.573943586	-3.384929615	0.000711965	0.013330868
Slc35d3	105.180173	-1.946697005	0.580350066	-3.354349591	0.000795518	0.014551135
Ctss	430.400784	-1.948239277	0.396665317	-4.911544306	9.03619E-07	4.61493E-05
Spc24	1183.00222	-1.951846959	0.63369982	-3.080081291	0.002069441	0.030212567
Tcf19	416.026607	-1.954834234	0.297540207	-6.569983446	5.03209E-11	7.02963E-09
Traf3ip2	89.3405019	-1.961052849	0.552371774	-3.550240875	0.000384879	0.008112631
Cenpe	913.15855	-1.966531631	0.312388527	-6.295146782	3.07109E-10	3.55772E-08
Knstrn	712.578109	-1.984545896	0.381066119	-5.207878094	1.91012E-07	1.14359E-05
Fam46a	78.5934022	-1.995618468	0.65718816	-3.03660137	0.002392616	0.033878855
Arhgef39	428.769225	-1.996565719	0.367098714	-5.438770669	5.36495E-08	3.82226E-06
Tspan2	130.586566	-2.002536546	0.447012208	-4.479825183	7.47042E-06	0.000294864
Mthfd2	175.111948	-2.027600226	0.59328927	-3.417557552	0.000631857	0.012154542
Prc1	1519.17838	-2.033436011	0.53807855	-3.779069079	0.000157416	0.00387395
Pf4	2876.08509	-2.039912637	0.203189563	-10.03945579	1.02237E-23	6.33378E-21
Sik1	132.006502	-2.040234624	0.65282859	-3.125222541	0.001776706	0.027251111
9030619P08Rik	748.889835	-2.045989377	0.259207156	-7.893259607	2.94394E-15	8.73922E-13

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Cep55	480.789247	-2.046266008	0.482077611	-4.244681687	2.18904E-05	0.000735651
Hist1h1c	387.223025	-2.047839858	0.557733619	-3.67171673	0.000240927	0.005498257
Hist1h2ab	58.3626725	-2.049988359	0.682906517	-3.001857953	0.002683374	0.036906747
E2f8	192.573953	-2.05937341	0.374646601	-5.496842643	3.86651E-08	2.79665E-06
Jakmip1	106.115218	-2.063319418	0.493199043	-4.183543031	2.87001E-05	0.00091843
Endod1	73.1491809	-2.073376957	0.667272333	-3.107242507	0.001888414	0.028433849
Cdc20	1130.90773	-2.073876595	0.305396162	-6.790774917	1.11533E-11	1.84794E-09
Gata1	246.593889	-2.090842731	0.520359702	-4.018071968	5.86763E-05	0.001692466
Eldr	252.593473	-2.103586823	0.572559867	-3.674003267	0.00023878	0.005461271
Top2a	1964.10317	-2.130786855	0.708769167	-3.006319905	0.002644306	0.036694475
Csf2rb	146.432495	-2.135381981	0.68976142	-3.095826933	0.001962649	0.02919184
Spdl1	204.723773	-2.145619548	0.420953753	-5.0970434	3.44999E-07	1.9128E-05
Ncaph	339.446634	-2.154028346	0.423984647	-5.080439498	3.76563E-07	2.04801E-05
Flt3	242.788593	-2.167128028	0.435551359	-4.975596983	6.50469E-07	3.39506E-05
Hist1h3e	189.631676	-2.201870159	0.411688345	-5.348390809	8.87397E-08	5.88117E-06
Shisa8	399.782638	-2.213397119	0.31310189	-7.069255054	1.55768E-12	2.95938E-10
Ccne2	302.494123	-2.216104628	0.335972943	-6.596080653	4.22169E-11	6.07625E-09
Cd69	926.237776	-2.321355867	0.391824911	-5.924472395	3.13302E-09	2.88015E-07
Pbk	2042.59738	-2.339678883	0.672181914	-3.480722754	0.000500063	0.010064117
Fanci	123.527916	-2.342273894	0.771400208	-3.036392614	0.002394274	0.033878855
Plekhhg5	76.174811	-2.352237975	0.609099652	-3.861827811	0.000112542	0.002931644
Cdca3	570.135189	-2.437334091	0.814813099	-2.991279956	0.002778107	0.037986228
BC051537	58.6197595	-2.465480929	0.79199045	-3.11301851	0.001851844	0.02819116
Haao	80.8791377	-2.521699054	0.627416659	-4.019177713	5.84016E-05	0.001687975
Rgs2	2422.25068	-2.525257621	0.71895024	-3.512423364	0.00044404	0.009090699
Nek2	172.678666	-2.529895668	0.682032374	-3.709348362	0.000207793	0.004885887
Depdc1a	73.2615112	-2.569613295	0.660201451	-3.892165478	9.93534E-05	0.002644342
Gm16712	181.966183	-2.591964331	0.418944852	-6.186886698	6.1364E-10	6.62406E-08
Ifi2712a	57.2077771	-2.600211161	0.802103251	-3.241741205	0.001188019	0.020065431
Mmp2	80.2781721	-2.658471281	0.766932586	-3.46636892	0.000527539	0.010527874
Srl	50.4876306	-2.698631669	0.782848164	-3.44719678	0.000566436	0.011163409
Dlgap5	321.741655	-2.724411017	0.496410972	-5.488216758	4.06012E-08	2.92185E-06
Hist2h2ac	50.5959157	-2.746506165	0.767968277	-3.576327625	0.000348455	0.007505605
Esco2	637.928813	-2.760316112	0.863193535	-3.197795163	0.001384826	0.022577102
Casc5	661.740752	-2.808703621	0.840419283	-3.342026627	0.000831691	0.015115768
Rnase6	1274.52637	-2.878566889	0.193106285	-14.90664526	2.98371E-50	7.08582E-47
Cdc25c	96.5994252	-2.965136679	0.956301634	-3.100629105	0.0019311	0.028863007
Satb1	97.4800299	-3.081374488	0.600080678	-5.13493369	2.82244E-07	1.59296E-05
C1qc	62.6282347	-3.139675229	1.044876002	-3.004830452	0.002657289	0.036781666
Cd86	229.348161	-3.168116264	0.444967209	-7.119887043	1.08016E-12	2.16777E-10

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Fam83d	147.912773	-3.205415997	0.852442789	-3.760271114	0.000169729	0.004106066
Cd52	61.5410577	-3.325556149	0.7990795	-4.16173378	3.1584E-05	0.001000091
Hist1h1b	105.746292	-3.344835581	1.033353558	-3.236874305	0.001208466	0.020271062
Sdc1	32.4342957	-3.365675127	1.151562185	-2.92270376	0.003470065	0.044345251
Bank1	107.574721	-3.459718348	0.866478636	-3.992848994	6.52841E-05	0.001853055
Ncf4	194.201903	-3.560786521	0.489531261	-7.273869526	3.49333E-13	8.02847E-11
3830403N18Rik	54.7362387	-3.578678499	1.183830287	-3.022965824	0.002503105	0.035278679
Troap	86.4361274	-3.653561412	0.965725469	-3.78322984	0.000154806	0.003829576
Peg3	18.3926769	-3.78447716	1.302541557	-2.905455983	0.003667184	0.046079108
Rgs7bp	232.876383	-3.985539216	0.555792542	-7.170911658	7.45E-13	1.60841E-10
Gm13710	34.8124987	-4.083357874	1.394474161	-2.92824205	0.003408846	0.043798594
Gpr182	24.032774	-4.233526389	1.443811511	-2.932187725	0.003365832	0.043467654
Slc4a10	32.9396399	-4.654635788	1.126548441	-4.131767101	3.59985E-05	0.001127347
C1qa	46.335912	-4.699173141	1.258301986	-3.734535266	0.000188062	0.004488605
Ccdc36	17.8534849	-4.734731238	1.569082161	-3.017516454	0.002548552	0.035706963
Cnr2	66.1318567	-4.825670538	1.079331873	-4.47097937	7.78622E-06	0.000303961
Bmp8a	19.2871825	-4.849544624	1.535937466	-3.157384158	0.001591915	0.025231583
Rhag	110.605017	-4.920372499	0.910563932	-5.403654072	6.52968E-08	4.51657E-06
Tgm5	42.1734926	-4.963606735	1.126962708	-4.404410813	1.06072E-05	0.000397741
Cd83	42.949235	-5.28884643	1.221415625	-4.330095607	1.49045E-05	0.000532265
Boll	13.8608298	-5.388852262	1.793517848	-3.004627063	0.002659067	0.036781666
Dqx1	23.4675845	-5.588004729	1.447772794	-3.859724917	0.000113515	0.00295159
Mpp3	17.9392293	-5.745460923	1.562823268	-3.676334387	0.000236609	0.005422844
Pkib	58.4985381	-5.891021615	1.065924882	-5.526676144	3.26355E-08	2.38473E-06
Ptprm	11.2774827	-6.003169777	2.088716478	-2.874095092	0.004051869	0.049955922
Tmem74	32.6451651	-6.052988958	1.348018336	-4.490286812	7.11273E-06	0.00028231
Sparc	22.0613594	-6.102444267	1.814660624	-3.362857046	0.000771403	0.014219562
Ms4a4b	24.7297865	-6.197099463	1.505506769	-4.116288012	3.85023E-05	0.001187488
Hmga2-ps1	24.2841334	-6.212827366	2.033228581	-3.055646288	0.00224576	0.032233329
Eid3	15.3514956	-6.453962659	1.81430169	-3.55727093	0.000374728	0.007933869
Omd	9.11205406	-6.675950826	2.19784526	-3.037498111	0.002385509	0.033878855
Scimp	9.13079789	-6.679448082	2.168865643	-3.07969657	0.002072116	0.030220653
Fam46d	18.2675049	-6.705815358	2.135614704	-3.139993064	0.001689518	0.026513157
Mrap	9.99586803	-6.809975134	2.105520652	-3.234342597	0.001219231	0.020414598
Pde10a	20.0288123	-6.839618642	2.094893988	-3.264899647	0.001095029	0.018798871
Ret	10.3529151	-6.859671738	2.22525686	-3.082642665	0.002051714	0.030077024
Oosp1	10.5108593	-6.881949553	2.150018671	-3.200878972	0.001370091	0.022362454
Grin3a	10.6050638	-6.895525197	2.079677439	-3.315670531	0.000914235	0.01622284
AW822252	10.7174176	-6.91039281	2.101156458	-3.28885209	0.001005969	0.01758779
Synpo2	11.0704154	-6.958326367	2.212788988	-3.144595532	0.001663166	0.026186135

Table 1B -- List of DEGs between young and old HSCs derived from bulk RNA-seq**Down-regulated genes in old HSCs when compared with young HSCs**

Map3k15	11.5848097	-7.02219576	2.059781878	-3.409193874	0.000651552	0.012444985
Dsc1	12.2572573	-7.104654469	2.46404664	-2.883327918	0.003934976	0.049054654
Tespa1	12.8590521	-7.173808356	2.027063657	-3.539014836	0.000401623	0.0083911
1700024F13Rik	13.0182382	-7.191580646	2.005836362	-3.585327688	0.000336655	0.007290274
Ccr1	13.0656105	-7.196812293	2.422482685	-2.970841583	0.002969849	0.039808186
Kcne1l	13.3263498	-7.225159302	2.415857781	-2.990722119	0.002783186	0.037986228
Alox5	13.4142311	-7.234776428	2.40822688	-3.004192208	0.00266287	0.036781666
Tagln	13.5699606	-7.250958044	1.911370325	-3.793591409	0.000148484	0.003698859
Wdr86	13.8440958	-7.280894681	2.041400847	-3.566616861	0.00036162	0.007702119
Tnfrsf11b	14.186881	-7.31486422	1.937217061	-3.775965207	0.000159389	0.003902298
Arhgdig	14.637809	-7.360486736	1.891990841	-3.890339517	0.000100104	0.002651269
Spta1	15.8280528	-7.472874745	2.333779431	-3.202048422	0.001364541	0.022297407
Hesx1	15.8995739	-7.479615413	1.830617913	-4.085841922	4.39173E-05	0.001325799
Cd209f	17.0100367	-7.576639097	2.281076125	-3.321519617	0.000895287	0.015974093
Kynu	34.4775669	-7.628089501	1.500093396	-5.085076385	3.67478E-07	2.0217E-05
Pdlim4	18.0352112	-7.661070945	1.866020799	-4.105565676	4.03327E-05	0.001235914
Tmeff1	18.3048097	-7.682784802	1.78299908	-4.308911255	1.6406E-05	0.000571563
Il1bos	19.1441133	-7.747206586	1.754351389	-4.415994785	1.00547E-05	0.000379018
D430019H16Rik	21.489606	-7.914523693	1.691932862	-4.677800088	2.89969E-06	0.000126354
Siglecg	28.6042978	-8.327026823	1.717317057	-4.848858158	1.24174E-06	5.99782E-05
Plagl1	41.7727125	-8.873759651	1.833584027	-4.839570766	1.3012E-06	6.22174E-05
Flrt3	46.3705126	-9.023212856	1.584660515	-5.69409837	1.24026E-08	9.87285E-07
Cxcl2	70.5291856	-9.629234786	1.588972462	-6.060038808	1.36089E-09	1.36558E-07

Table 2 - List of the 54 potential marker genes for old HSC heterogeneity	
Membrane located proteins	Non-membrane located proteins
Alcam	Ar
Art4	B3gnt8
Cd150	Bmp4
Cd38	Clu
Chrm3	Cyp26b1
Cldn5	Dnm3
Efna1	Fads2
Ehd3	Gadd45g
Enpp5	Gda
Epcam	Gpx3
Gpr183	Mt1
Itgb3	Mt2
Jam2	Ntf3
Kcnb2	Nupr1
Klrb1c	Oxr1
Lpar6	Pam
Neo1	Plcl1
Npdc1	Rbm19
Nrg4	Rdh10
Plscr2	Rorc
Procr	Sbspon
Prtn3	Smtnl1
Ptger4	Sox18
Ramp2	Sult1a1
Selp	Tox
Tm4sf1	Vwf
Slamf1	Xdh
	Zg16

Table 3A -List of the CD150 correlated genes based on bulk RNA-seq
CD150 positively correlated genes (CD150 feature genes)
Slamf1
Sbspon
Ehd3
Clu
Dpy19l2
Kcnb2
B3gnt8

Table 3A -List of the CD150 correlated genes based on bulk RNA-seq
CD150 positively correlated genes (CD150 feature genes)
Rbpjl
Cited2
Terf1
Fam63a
Gipc2
Enpp5
Ctsw
Ccdc92
Oaf
Dnm3os
Sdf4
Selp
Arpin
Hfe
4631405J19Rik
Sla2
Jam2
Ccnd2
Eid2
Matn4
Dhx40
Prkag2
Krt18
Slc14a1
Mycn
Bace2
Ncoa7
Epdr1
Pde9a
Ces2g
Oxr1
Krt80
Gm6654
Fam213a
Efcab14
Fez2
Trim47
Rad9b
Ppap2a

Table 3A -List of the CD150 correlated genes based on bulk RNA-seq
CD150 positively correlated genes (CD150 feature genes)
Trib3
Tacstd2
Gem
Clip3
Tacr1
Efhd1
Hpcal1
Cmas
Art4
Echdc2
Lrrc73
Fkbp10
B4galt4
Rab36
Sil1
Tc2n
Nxpe2
Gstm2
Hoxb7
Rapgef4
Fam131a
Eef2kmt
Rarb
Bcat2
Relb
Armc2
Smyd2
Phactr1
Fndc4
4930512B01Rik
Tnni3
Rae1
Eid1
Gfod1
Mboat2
Rtp4
Nufip1
Slc25a4
Slc9a3r2

Table 3A -List of the CD150 correlated genes based on bulk RNA-seq
CD150 positively correlated genes (CD150 feature genes)
Zg16
Taf11
Rbm19
Nova2
Pebp1
Npdc1
Mylk
Ctsb
4921509O07Rik
Skap1
Lrrc42
Igtp
Dnm3
Fhl1
Ryr3
Isca1
Gimap5
Veph1
Mllt3
Ttc14
Ccdc64
Erc2
Anpep
Exoc3l
Ctsh
Rnf128
Kitl
Cd38
Shisa5
Fads3
Slc24a5
Ap2m1
Dyrk3
Lmtk3
Tnfsf4
Pygm
Irgm1
Rpl3
Slamf8

Table 3A -List of the CD150 correlated genes based on bulk RNA-seq
CD150 positively correlated genes (CD150 feature genes)
Zfp622
Stx1a
Klrb1c
Ptger4
AA986860
Mtg1
Pepd

Table 3B -- List of the CD150 correlated genes based on bulk RNA-seq
CD150 negatively correlated genes
Pdik1l
Serp1b1a
Elf1
1700097N02Rik
BC016579
1110002L01Rik
Smap2
Slc38a2
Usp6nl
Ptpn22
Lrr1
Zeb1
Mlec
Heatr6
Iqgap2
Col4a2
Wfdc17
Smap
Ssh2
Fxr2
Pitpnc1
Gpr171
Runx2
Gng2
Chsy1
Smek2

Table 3B -- List of the CD150 correlated genes based on bulk RNA-seq
CD150 negatively correlated genes
Ctla2a
Ccl3
Ikzf5
Kif15
Mybpc3
Eed
Clnk
St8sia4
Dpp4
Naa50
Atad2
B3galt2
Myb
Heatr5b
Arhgap18
Akr1c12
Zcwpw1
Fabp5
Tuba8
Kdm3a
Cd1d1
Sppl2a
Syk
Reep5
Clspn
Fam105a
Ptpre
Rian
Pigu
Shisa8
Agpat1
Nkap
Celf2
Fxyd1
Slbp
Wipf1
Rnase6
Hmgb2
Rnu11

Table 3B -- List of the CD150 correlated genes based on bulk RNA-seq
CD150 negatively correlated genes
Galnt6
Ppp3cb
Dennd5a
Prss57
Camk1d
Rnase4
Cux1
Ccdc112
Tsg101
Spdl1
Hspa4l
Mybl1
Slc12a6
I730030J21Rik
Nkg7
Haus6
Golm1
Lat
Fam173a
Cdh18
Prim1
Smad6
Ppfia3
Kcnab2
Ndc80
Lrrc36
Tmem65
Zc3hc1
Cmss1
Ly96
Rbl1
Cd27
Cggbp1
Kif20b
Eef1e1
Cdca8
Bfsp2
Cmtm7

What is claimed is:

1. A method for characterizing hematopoietic stem cell (HSC) functionality, the method comprising detecting the level of CD150 polypeptide or polynucleotide in an HSC, wherein
5 an alteration in the level of CD150 relative to a reference is indicative of HSC functionality.
2. A method for selecting a hematopoietic stem cell (HSC) for engraftment in a subject in need thereof, the method comprising detecting the level of CD150 polypeptide or polynucleotide expression in an HSC, wherein detection of a reduced level of CD150 expression relative to a reference selects the HSC for engraftment in a subject.
- 10 3. The method of claim 1 or 2, wherein the HSC is present in a population marked by heterogeneity in HSC functionality.
4. The method of claim 1, wherein a reduction in the level of the CD150 polypeptide or polynucleotide relative to the reference indicates that the HSC has increased self-renewal capacity and is capable of balanced differentiation.
- 15 5. The method of claim 2, wherein a reduction in the level of CD150 polypeptide or polynucleotide relative to the reference indicates that the HSC should be selected for engraftment.
6. The method of claim 2, wherein an increase in the level of CD150 polypeptide or polynucleotide relative to the reference indicates that the HSC should not be selected for
20 engraftment.
7. The method of claim 1 or 2, wherein the method further comprises detecting the expression of one or more markers selected from the group consisting of *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2* and *Enpp5*, wherein expression of said one or more markers positively correlates with CD150 expression.
- 25 8. The method of claim 1 or 2, wherein the method further comprises detecting the expression of one or more markers selected from the group consisting of *Serp1b1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6* and *Arhgap30*, wherein expression of said one or more markers negatively correlates with CD150 expression.

9. The method of claim 1 or 2, further comprising characterizing the level of one or more of 332 HSC aging marker genes (Tables 1A, 1B), wherein detecting an increase in one or more of said genes is indicative of HSC functional decline.
10. The method of claim 1 or 2, wherein levels of CD150 polypeptide are detected using
5 FACS analysis, and wherein levels of CD150 polynucleotide are detected using RNA-seq analysis.
11. A method of rejuvenating blood components of an aging or aged subject or alleviating aging-related hematopoietic cell functional decline, the method comprising:
administering to the subject an effective amount of hematopoietic stem cells (HSCs)
10 expressing low levels of CD150 polypeptide (CD150^{low} HSCs) or a composition thereof comprising a physiologically acceptable carrier, excipient or diluent; wherein the CD150^{low} HSCs differentiate into hematopoietic cell types which rejuvenate blood components in the aged subject.
12. The method of claim 11, wherein CD150^{low} HSCs have a mean fluorescence intensity
15 of less than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.
13. The method of claim 11 or 12, wherein CD150^{low} HSCs express marker genes *Rnase6*
20 and *Arhgap30*.
14. The method of any one of claims 11-13, wherein the administration is intravenous.
15. The method of any one of claims 11-13, wherein the administration is via transplant or engraftment.
16. The method of any one of claims 11-15, wherein the cell types which differentiate
25 from the CD150^{low} HSCs to rejuvenate the blood components comprise B lymphocytes, T lymphocytes, and myeloid cells; and/or wherein the rejuvenated blood components are in an activated state.

17. The method of any one of claims 11-16, wherein the aging or aged subject is selected by detecting hematopoietic stem cells expressing high levels of CD150 polypeptide (CD150^{high} HSCs) in a sample obtained from the subject.

18. The method of claim 17, wherein the CD150^{high} HSCs have a mean fluorescence intensity of greater than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein the CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.

19. The method of claim 17 or 18, wherein the CD150^{high} HSCs express a CD150 mRNA level of between about 0.3 to about 2 RNA Units per cell; and or wherein the CD150^{high} HSCs express one or more marker genes selected from the group consisting of *Sbspon*, *Gpr183*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, and *Ramp2*.

20. The method of any one of claims 11-19, wherein the subject is greater than 50 years of age.

21. The method of claim 20, wherein the subject is greater than 70 years of age.

22. The method of any one of claims 17-21, wherein the sample is a blood, plasma, serum, or bone marrow sample.

23. The method of any one of claims 11-22, wherein, following administration of the CD150^{low} HSCs, the subject exhibits increased numbers of naïve T cells and a decrease in central memory T cell (T_{cm}) and effector memory T cell (T_{em}) ratio in the CD8⁺ and CD4⁺ subgroups of T cells.

24. A method of improving physical health and aging-related hematopoietic cell functional decline of a selected subject, the method comprising:

selecting the subject by detecting hematopoietic stem cells (HSC) expressing high levels of CD150 polypeptide (CD150^{high} HSCs) in a sample obtained from the subject; and administering to the subject an effective amount of hematopoietic stem cells (HSCs) expressing low levels of CD150 polypeptide (CD150^{low} HSCs) or a composition thereof

comprising a physiologically acceptable carrier, excipient or diluent; wherein the CD150^{low} HSCs differentiate into hematopoietic cell types which improve physical health and aging-related hematopoietic cell functional decline in the subject.

25. The method of claim 24, wherein the CD150^{high} HSCs have a mean fluorescence intensity of greater than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature in the sample based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.

26. The method of claim 24 or 25, wherein the CD150^{high} HSCs express a CD150 mRNA level of between about 0.3 to about 2 RNA Units per cell.

27. The method of any one of claims 24-26, wherein the CD150^{low} HSCs administered to the subject have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.

28. The method of any one of claims 24-27, wherein CD150^{low} HSCs express marker genes *Rhase6* and *Arhgap30*.

29. The method of any one of claims 24-28, wherein the administration is intravenous.

30. The method of any one of claims 24-28, wherein the administration is via transplant or engraftment.

31. The method of any one of claims 24-30, wherein improving physical health and improving aging-related hematopoietic cell functional decline of the subject comprises administering CD150^{low} HSCs which differentiate into activated or activatable B lymphocytes, T lymphocytes, and myeloid cells.

32. The method of any one of claims 24-31, wherein the subject is greater than 50 years of age.

33. The method of claim 32, wherein the subject is greater than 70 years of age.

34. The method of any one of claims 24-33, wherein the sample is a blood, plasma, serum, or bone marrow sample.

35. The method of any one of claims 24-34, wherein, following administration of the
5 CD150^{low} HSCs, the subject exhibits increased numbers of naïve T cells and a decrease in central memory T cell (Tcm) and effector memory T cell (Tem) ratio in the CD8⁺ and CD4⁺ subgroups of T cells.

36. The method of any one of claims 24-35, wherein the subject having CD150^{high} HSCs is selected by further detecting in cells of the sample one or more age-related genes selected
10 from *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, or *Ramp2*.

37. The method of any one of claims 11-36, wherein CD150^{low} HSCs express one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*.

38. The method of any one of claims 11-37, wherein the CD150^{low} HSCs administered to the subject dilute or reduce the numbers of CD150^{high} HSCs in the subject, thereby
15 rejuvenating the blood with CD150^{low} HSCs capable of differentiation into activated or activatable hematopoietic cells and cell types.

39. The method of claim 38, wherein the CD150^{low} HSCs administered to the subject increase the numbers of activated versus quiescent CD150-expressing HSCs in the subject.

40. A composition comprising an effective amount of hematopoietic stem cells (HSCs)
20 expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs), wherein said CD150^{low} HSCs have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population of bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or
25 fluorescence activated cell sorting (FACS) analysis.

41. The composition of claim 40, further comprising a physiologically acceptable carrier, excipient or diluent.

42. The composition of claim 40 or 41, wherein CD150^{low} HSCs express one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*.

43. The composition of claim 40 or 41, wherein CD150^{low} HSCs express marker genes *Rnase6* and *Arhgap30*.

5 44. The composition of any one of claims 40-43, wherein the composition comprises a saline solution or a buffer solution.

45. Use of a composition comprising an effective amount of hematopoietic stem cells expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs), wherein said CD150^{low} HSCs have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis and/or express marker genes *Rnase6* and *Arhgap30*, in the manufacture of a medicament for treating an aging or aged subject having hematopoietic stem cells expressing a high abundance of the CD150 glycoprotein (CD150^{high} HSCs);
 10 wherein the CD150^{high} HSCs have a mean fluorescence intensity of greater than about 4×10^3 cell surface expressed CD150 proteins per cell or wherein CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or
 15 fluorescence activated cell sorting (FACS) analysis; and/or

wherein the CD150^{high} HSCs express a CD150 mRNA level of between about 0.3 to about 2 RNA Units per cell; and/or

wherein the CD150^{high} HSCs express one or more age-related marker genes selected from the group consisting of *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*,
 25 *Enpp5*, *Gpr183*, and *Ramp2*.

46. The use of claim 45, wherein the CD150^{low} HSCs express one or more genes selected from *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*.

47. A method of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) in a subject, the method comprising:

contacting a sample containing CD150-expressing hematopoietic stem cells (HSCs) obtained from a subject in need thereof with an anti-CD150 antibody conjugated to a cellular toxin in an amount effective to bind to high CD150-expressing HSCs (CD150^{high} HSCs); wherein the CD150^{high} HSCs express one or more age-related marker genes selected from the group consisting of *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2* which correlate with high expression of CD150 on the cell surface;

culturing the sample for a time sufficient for the anti-CD150 antibody-toxin conjugate to cause cytolysis of the high CD150-expressing HSCs; and

obtaining a sample in which dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) are reduced or eliminated.

48. The method of claim 47, wherein the subject is an old, aged, or aging subject.

49. The method of claim 47 or 48, wherein the anti-CD150 antibody-toxin conjugate is used in an amount selected from 0.01 to 1 nM, 0.01 to 0.05 nM, or 0.01 nM.

50. The method of any one of claims 47-49, further comprising measuring death of cells in the culture after three days or longer.

51. The method of claim 50, further comprising measuring a decrease or reduction in the number of CD150^{high}-expressing HSCs compared to a control and/or a decreased ratio of CD150^{high} HSCs to CD150^{low} HSCs in the sample.

52. The method of claim 51, wherein the control is a saline-treated sample, a buffer-treated sample, or a phosphate buffered saline-treated sample.

53. The method of claim 47, wherein the sample is selected from blood, peripheral blood, cord blood, serum, plasma, or bone marrow.

54. The method of claim 47, wherein the method is performed *in vitro* or *ex vivo*.

55. A method of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) in an old, aged or aging subject, the method comprising:

administering to a subject in need thereof an effective amount of an anti-CD150 antibody conjugated to a cellular toxin in an amount effective to bind to high CD150-expressing HSCs (CD150^{high} HSCs) in the subject; wherein the CD150^{high} HSCs express one

or more age-related marker genes selected from the group consisting of *Sbspon*, *Ehd3*, *Clu*, *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2* which correlate with high expression of CD150 on the cell surface; thereby reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs) in the subject.

- 5 56. The method of claim 55, wherein the anti-CD150 antibody-toxin conjugate is administered in an amount of 1 mg/kg to 5 mg/kg or in an amount of 1 mg/kg.
57. The method of claim 55 or 56, further comprising measuring the percentage of high CD150-expressing HSCs remaining after administration of the anti-CD150 antibody-toxin conjugate.
- 10 58. The method of any one of claims 55-57, further comprising measuring a decreased ratio of CD150^{high} HSCs to CD150^{low} HSCs in the subject after administration of the anti-CD150 antibody-toxin conjugate.
59. The method of any one of claims 55-58, further comprising measuring a reduction of about 60% CD150^{high} HSCs in the subject compared to a control after administration of the
15 anti-CD150 antibody-toxin conjugate.
60. The method of claim 59, wherein the control is a saline-treated subject, a buffer-treated subject, or a phosphate buffered saline-treated subject.
61. The method of any one of claims 57-60, wherein measuring is performed at least three weeks following administration of the anti-CD150 antibody-toxin conjugate.
- 20 62. The method of any one of claims 47-61, wherein the CD150-expressing HSCs are characterized as Lin-Sca-1+C-Kit+CD48-CD34-CD150+.
63. The method of any one of claims 47-62, wherein the anti-CD150 antibody is TC15-12F12.2.
64. The method of any one of claims 47-63, wherein the anti-CD150 antibody is
25 conjugated to saporin.
65. The method of claim 64, wherein the saporin is conjugated to streptavidin.

66. The method of any one of claims 48-65, wherein the subject is 50 years old or older.
67. The method of any one of claims 48-66, wherein the old, aged, or aging subject has a defective or dysfunctional CD150^{high}-expressing population of HSCs associated with expression of one or more HSC age-related markers selected from *Sbspon*, *Ehd3*, *Chu*,
5 *Dpy19l2*, *Kcnb2*, *Selp*, *B3gnt8*, *Jam2*, *Enpp5*, *Gpr183*, and *Ramp2*.
68. The method of any one of claims 47-67, wherein amounts of HSCs comprising lower CD150 levels (CD150^{low} HSCs) are increased, wherein said CD150^{low} HSCs express one or more genes selected from the group consisting of *Serpinb1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*.
- 10 69. The method of any one of claims 55-68, wherein reducing or eliminating the functionally defective or dysfunctional CD150^{high} HSCs attenuates an aging phenotype and improves physical functioning in the old, aged, or aging subject.
70. The method of any one of claims 55-69, wherein the percentage of B cells is increased and the percentage of myeloid cells are decreased in the subject by about 45-90 days
15 following administration of the anti-CD150 antibody-toxin conjugate.
71. The method of any one of claims 47-54, further comprising administering to the subject the sample in which dysfunctional or defective high CD150-expressing hematopoietic stem cells (HSCs) are reduced or eliminated.
72. The method of any one of claims 55-71, wherein administration is selected from
20 parenteral, intravenous, or subcutaneous administration.
73. The method of any one of claims 47-72, wherein CD150^{low} HSCs comprise the lowest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature and CD150^{high} HSCs comprise the highest 25% subpopulation of a Long-Term-HSC population in bone marrow
25 which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.

74. A kit or article of manufacture comprising a composition comprising an effective amount of hematopoietic stem cells (HSCs) expressing a low abundance of the CD150 glycoprotein (CD150^{low} HSCs), wherein said CD150^{low} HSCs have a mean fluorescence intensity of less than about 4×10^3 cell surface expressed CD150 proteins per cell based on
5 flow cytometry or fluorescence activated cell sorting (FACS) analysis and/or wherein CD150^{low} HSCs express one or more genes selected from *Serpina1a*, *Usp6nl*, *Lrr1*, *Col4a2*, *Gng2*, *Kif15*, *Rnase6*, or *Arhgap30*, which correlate with CD150^{low} expressing HSCs, and instructions for use.

75. The kit or article of manufacture of claim 74, wherein CD150^{low} HSCs comprise the
10 lowest 25% subpopulation of a Long-Term-HSC population in bone marrow which expresses a Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD34⁻CD150⁺ cell surface biomarker signature based on flow cytometry or fluorescence activated cell sorting (FACS) analysis.

76. The kit or article of manufacture of claim 74, wherein the composition is a
15 physiological or pharmaceutically acceptable composition.

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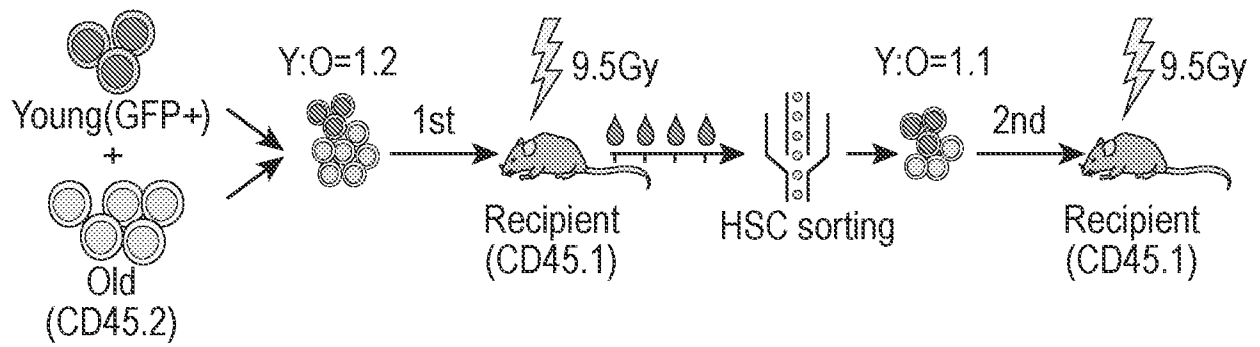


FIG. 1A

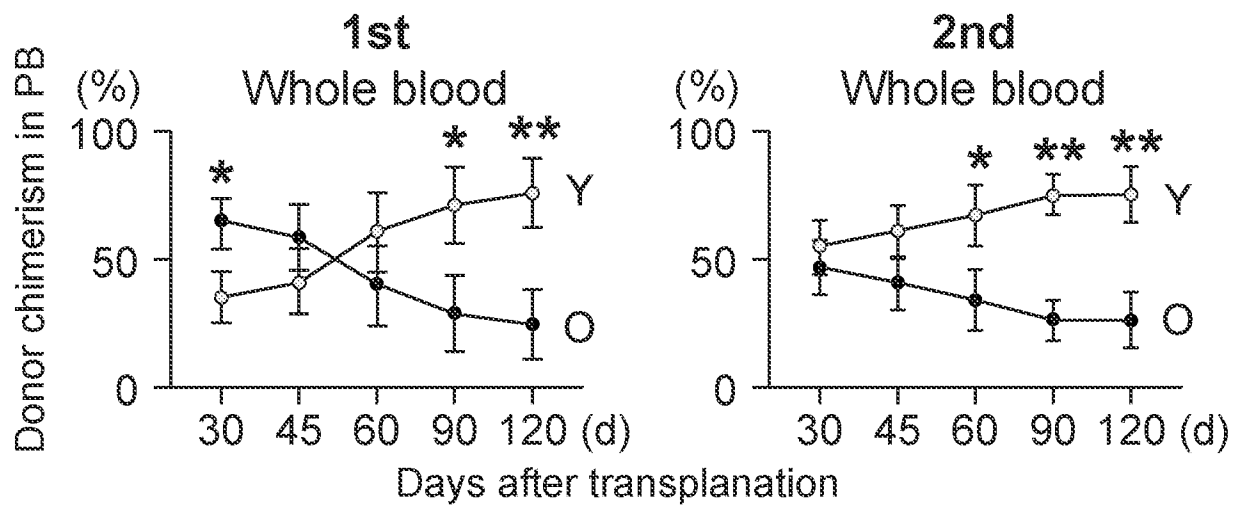


FIG. 1B

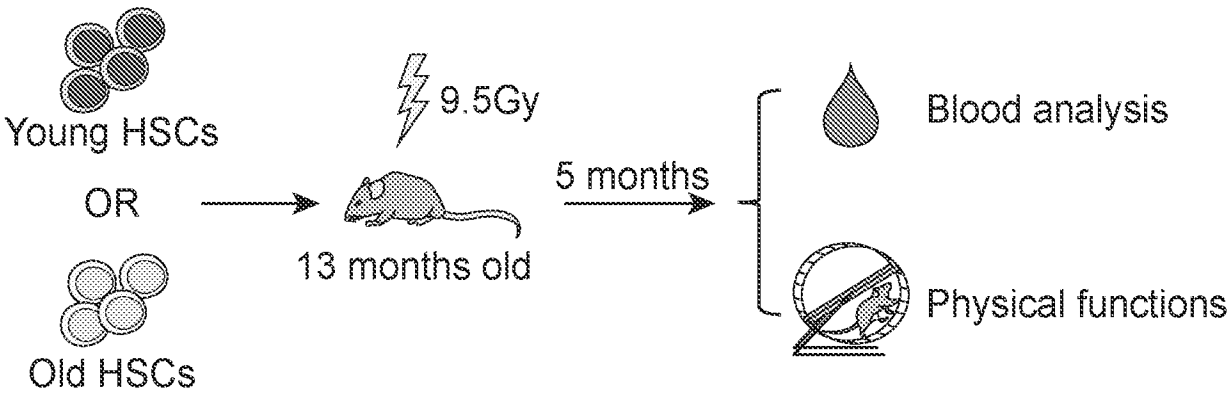


FIG. 1C

Peripheral blood analyses

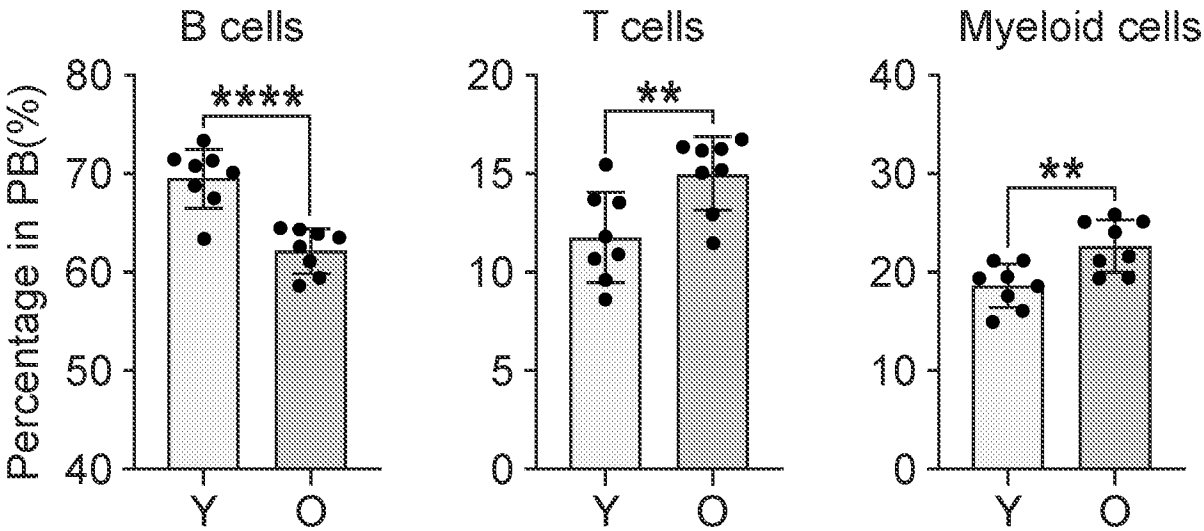


FIG. 1D

Complete blood count (CBC)

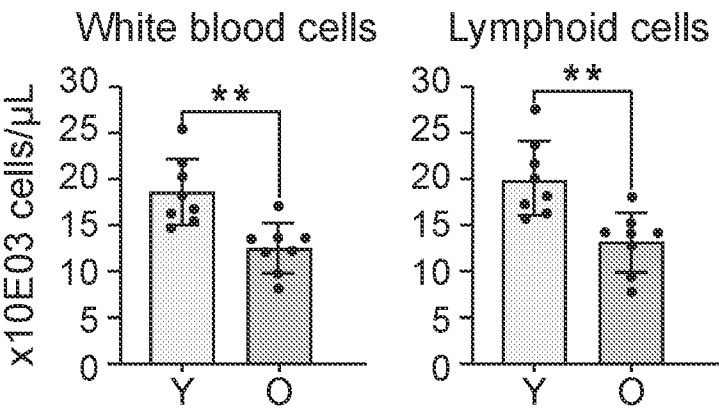


FIG. 1E

Immune cell analyses

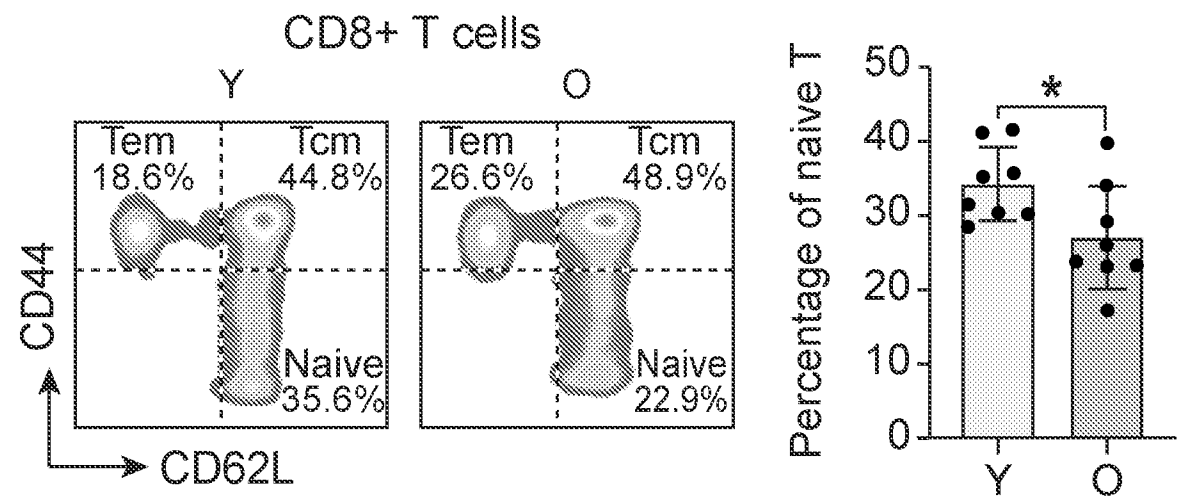


FIG. 1F

Epigenetic age

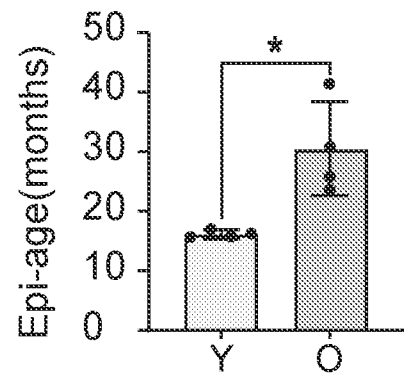
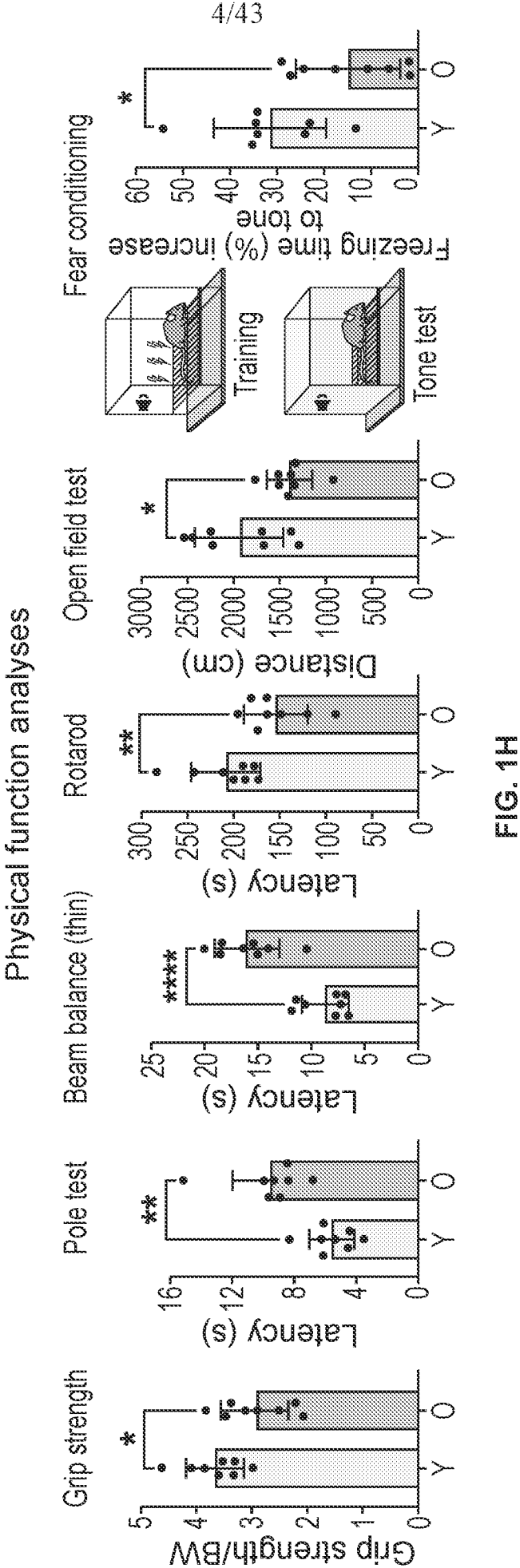


FIG. 1G



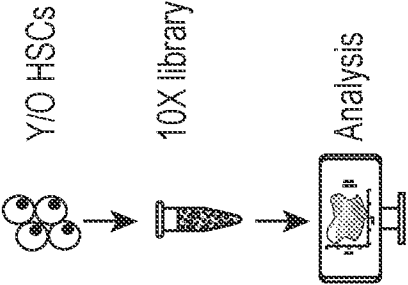


FIG. 2A

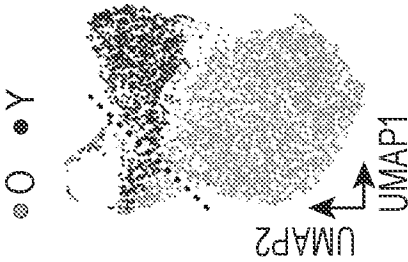


FIG. 2B

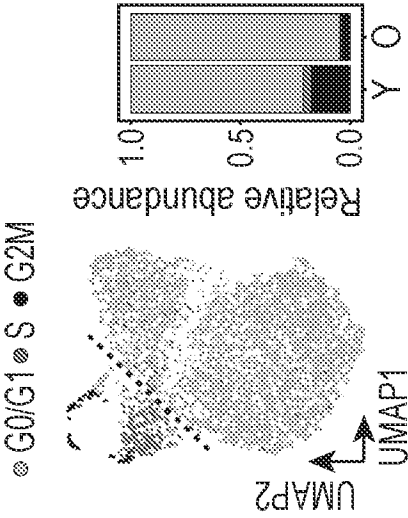


FIG. 2C

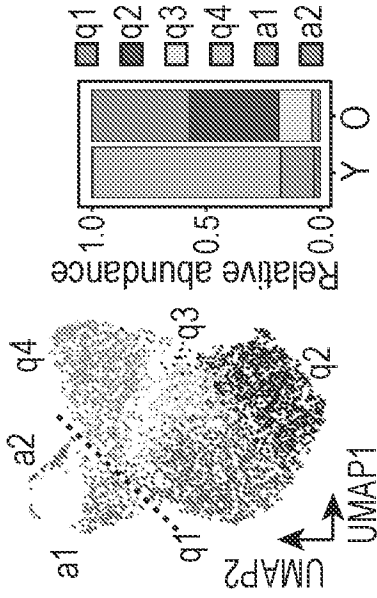
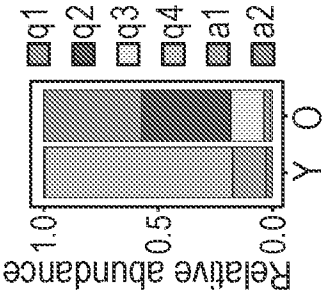
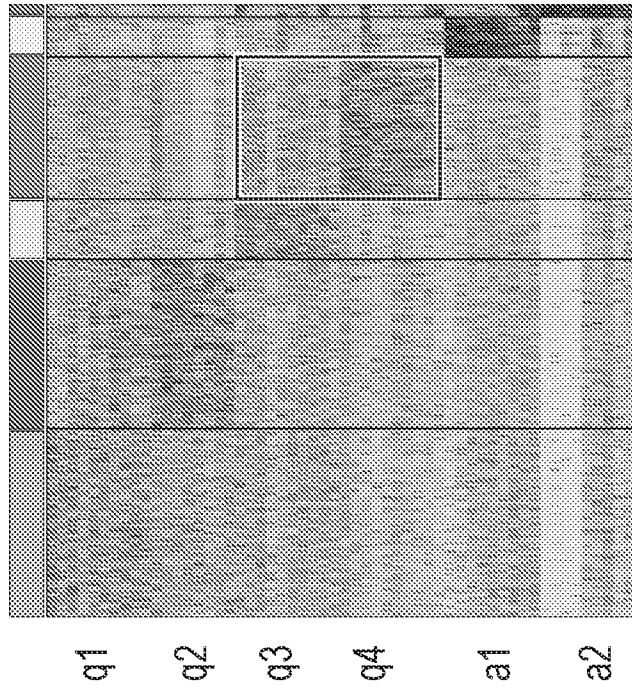


FIG. 2D



Typical genes	GO terms	P-value
<i>Mt1, Prtn3, Nupr1, Plscr2</i>	● Antigen processing and presentation of peptide antigen	2.11E-06
<i>Cavin2, Sult1a1, Clu, Ramp2</i>	● Cytoplasmic translation	4.84E-06
<i>Cavin2, Gda, Alcam, Sult1a1, Selp, Gpr183, Nupr1</i>	● Myeloid cell differentiation ● Homeostasis of number of cells	2.09E-14 9.43E-09
<i>Cmtm7, Sox4, Clla2a, Ramp1, Vim, Arhgdib, Ptpre, Msi2, Pf4</i>	● Reg. of actin cytoskeleton reorganization ● Reg. of nuclear cell cycle DNA replication	1.43E-05 9.26E-05
<i>Lst1, Socs2, Ifitm1, Ptpre, Msi2, Arhgdib, Vim, Cmtm7, Sox4</i>	● Mononuclear cell differentiation ● Reg. of leukocyte cell-cell adhesion	8.43E-10 1.53E-05
<i>Ung, Chek1, Tk1, Hells, Mcm5, Cenps, E2f1, Mcm2, Mcm4</i>	○ DNA replication ○ ATP metabolic process	4.00E-58 2.81E-25
<i>Ccnb2, Cenpe, Mki67, Spp1, Cdc43, Cdkn3, Cenpf, Birc5</i>	● Aerobic respiration ● Chromosome segregation	1.90E-32 7.26E-31

FIG. 2E



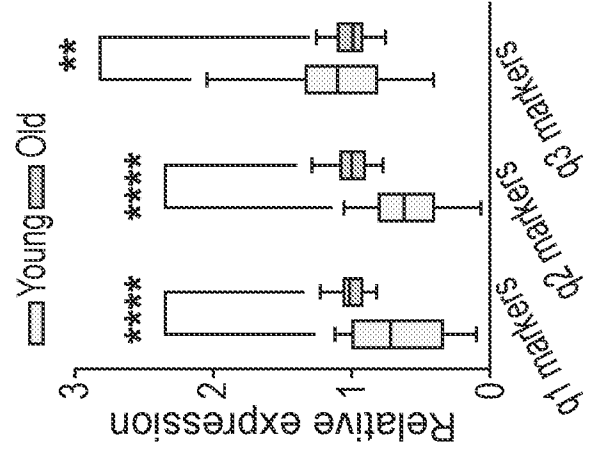


FIG. 2F

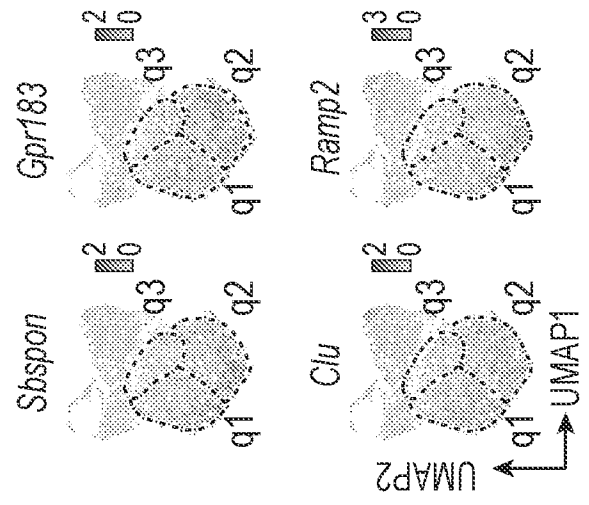


FIG. 2G

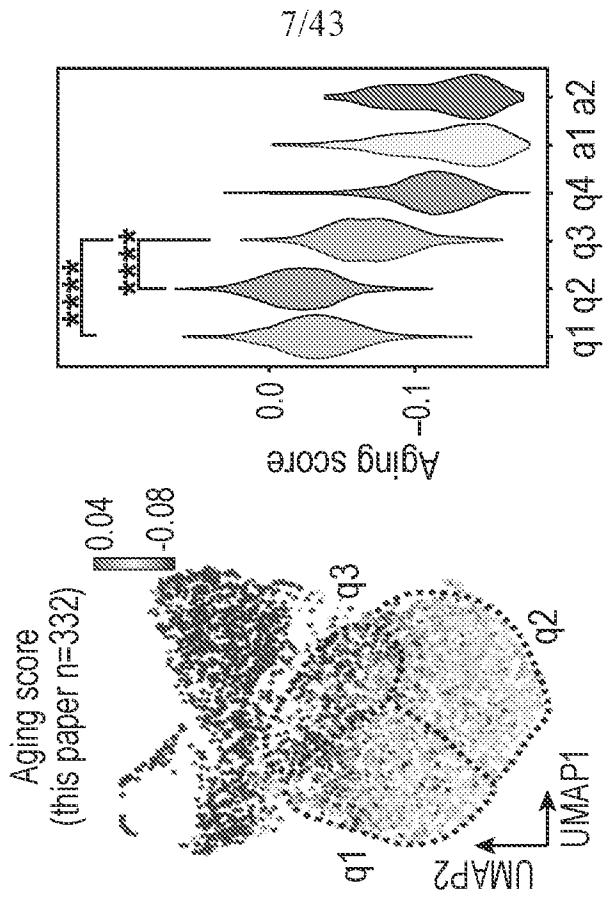


FIG. 2H

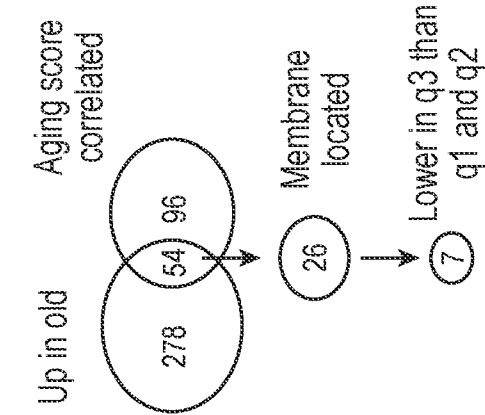


FIG. 3A

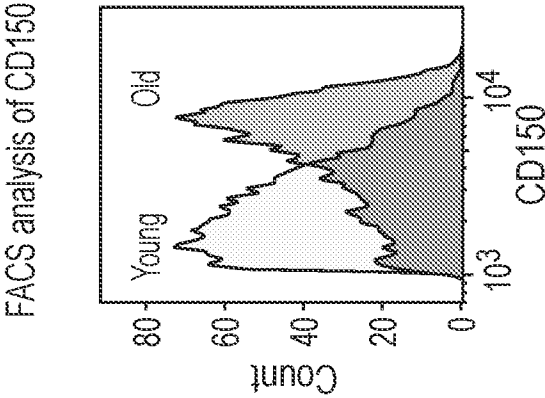


FIG. 3B

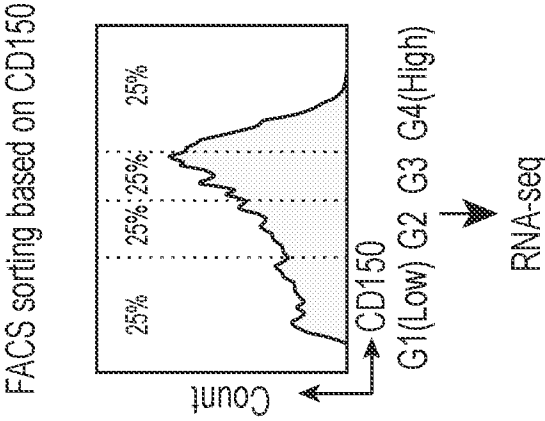


FIG. 3C

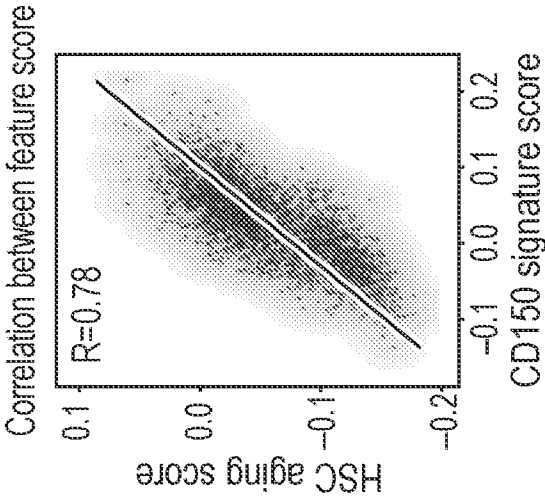


FIG. 3D

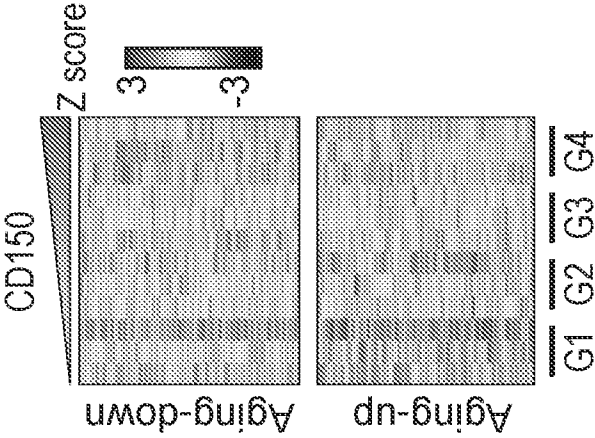


FIG. 3E

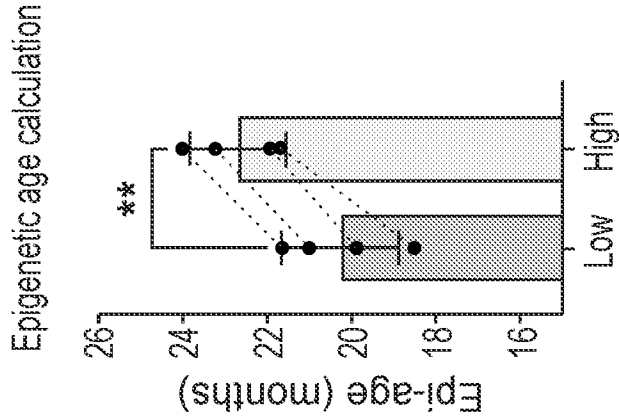


FIG. 3F

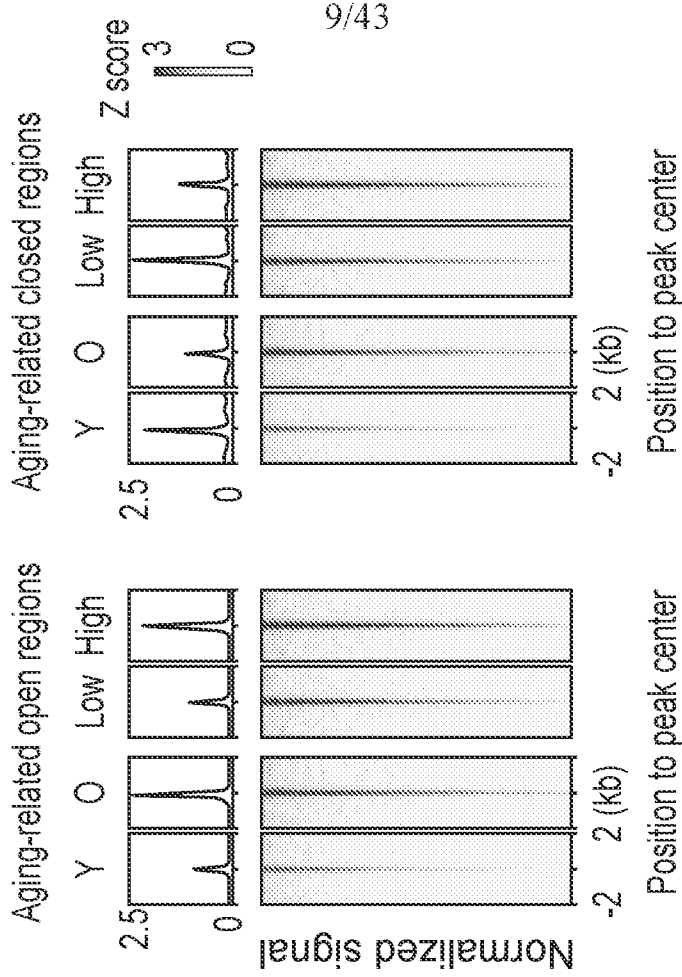


FIG. 3G

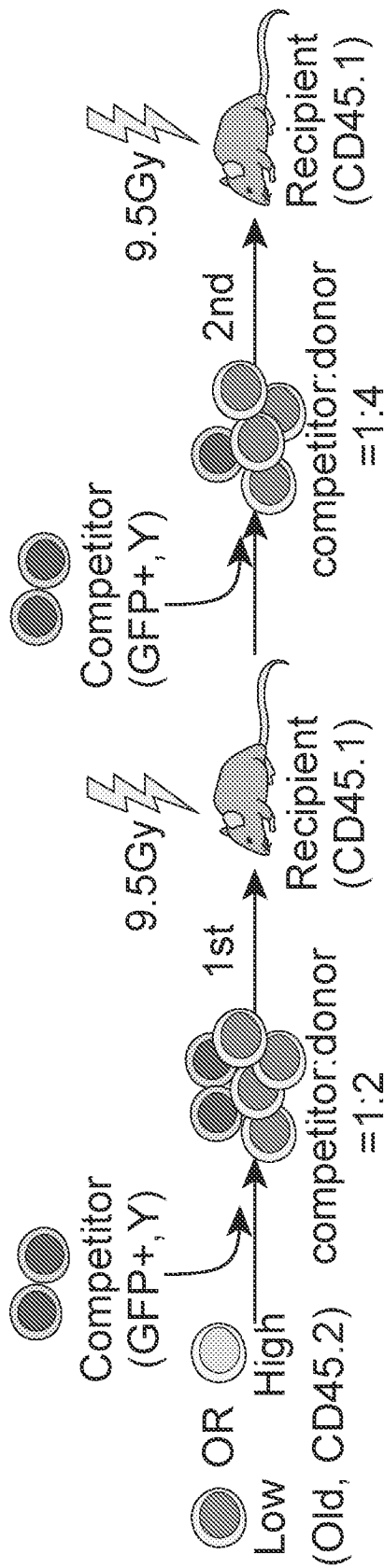


FIG. 3H

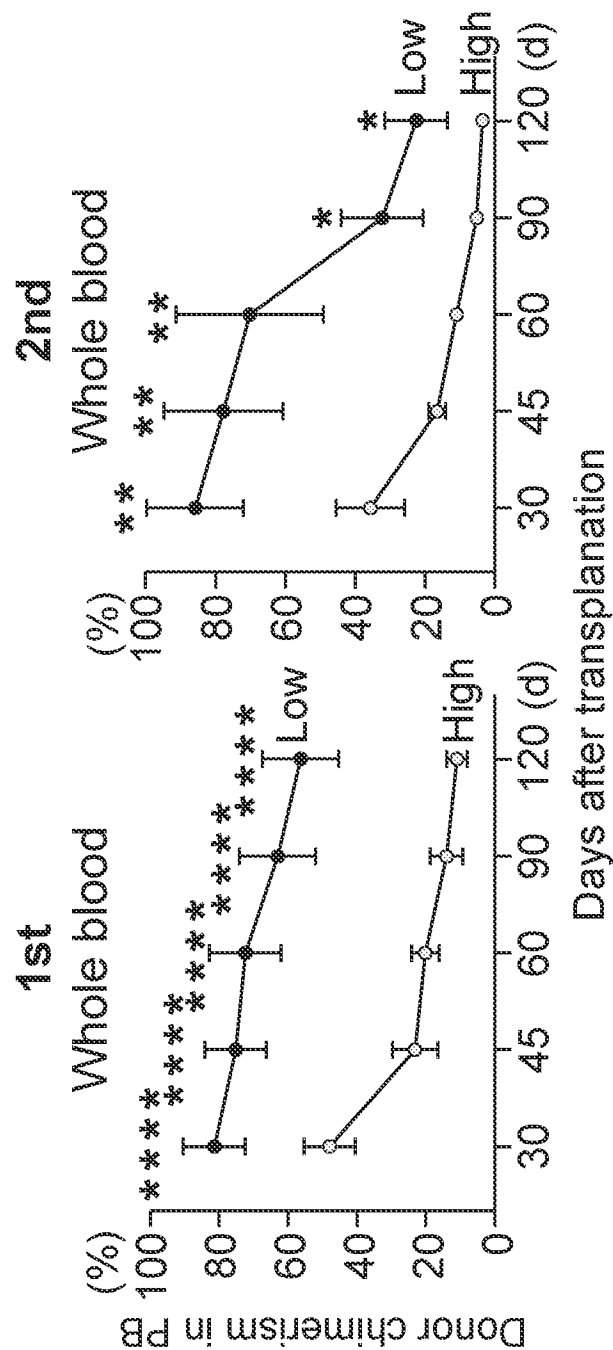


FIG. 3I

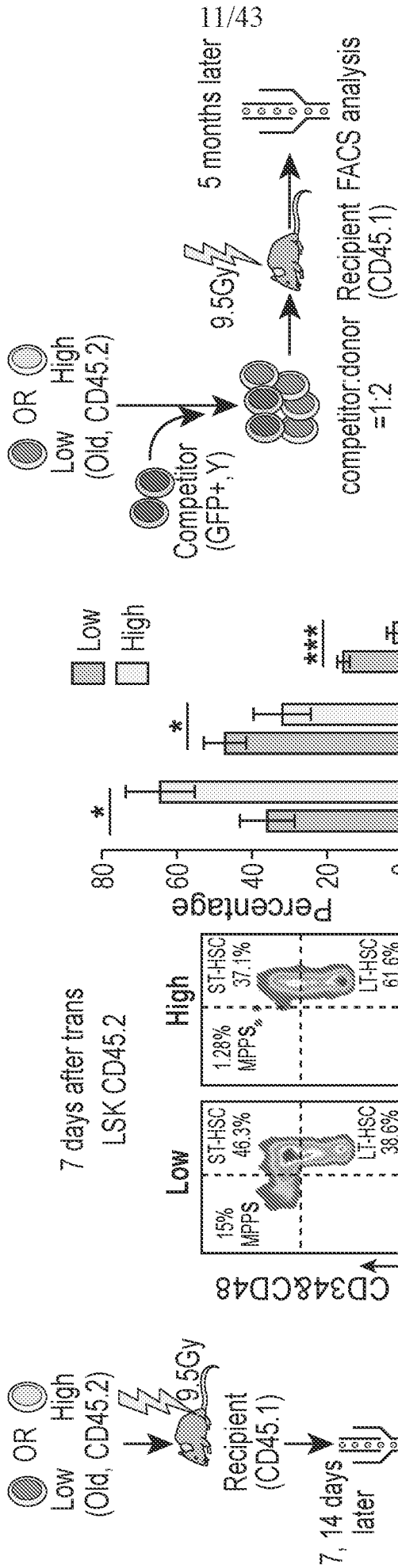


FIG. 4C

FIG. 4B

FIG. 4A

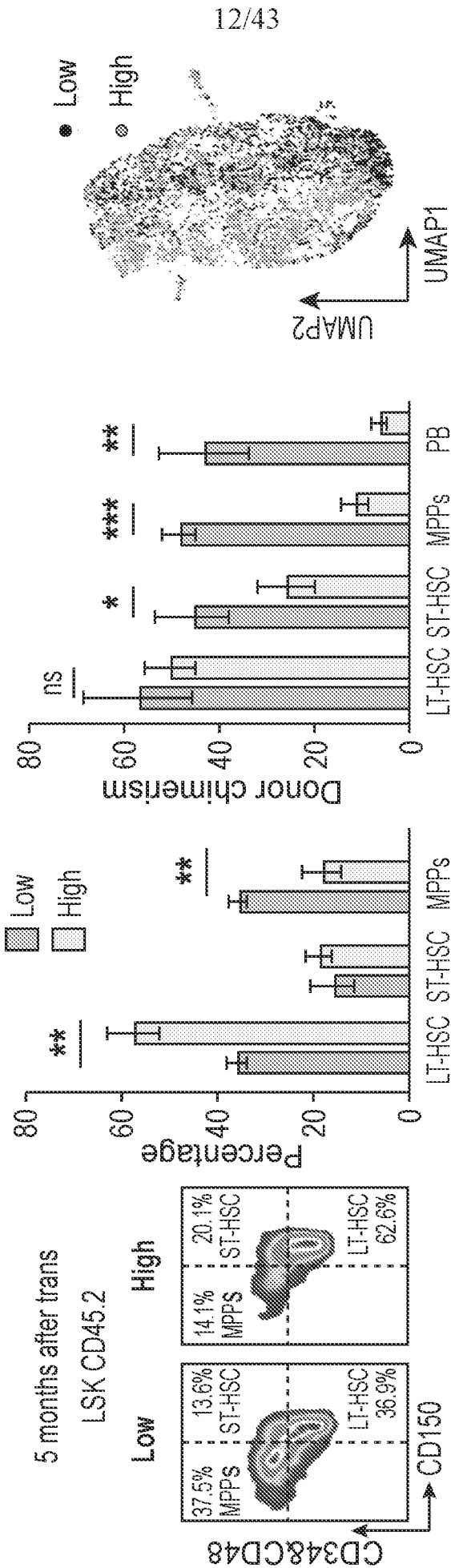


FIG. 4D

FIG. 4E

FIG. 4F

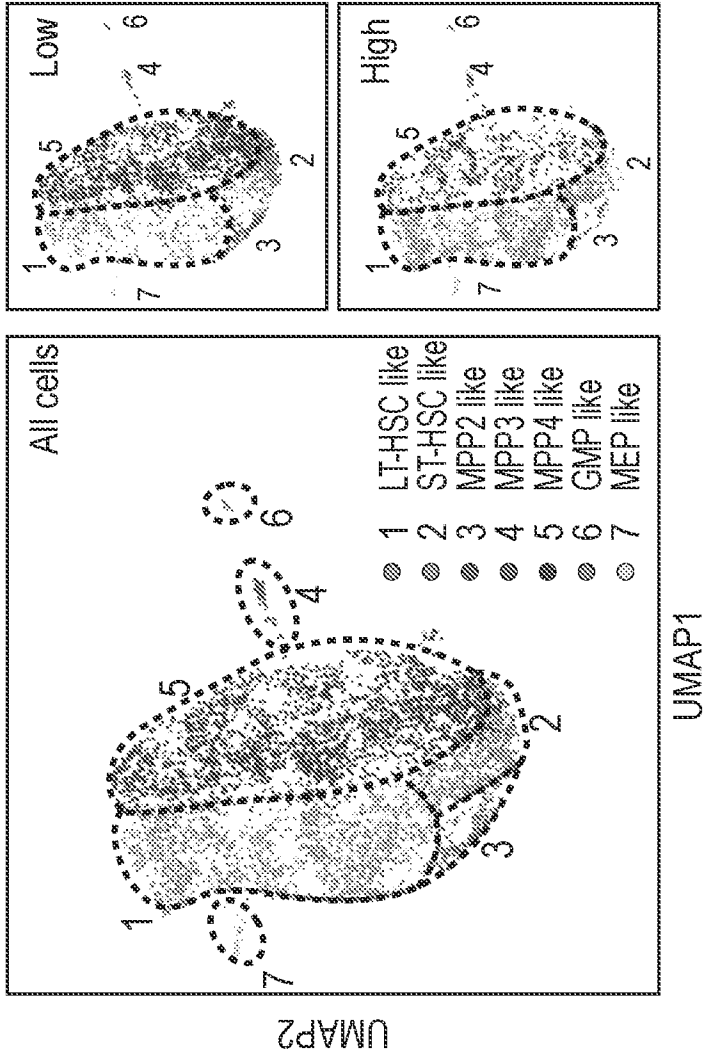


FIG. 4G

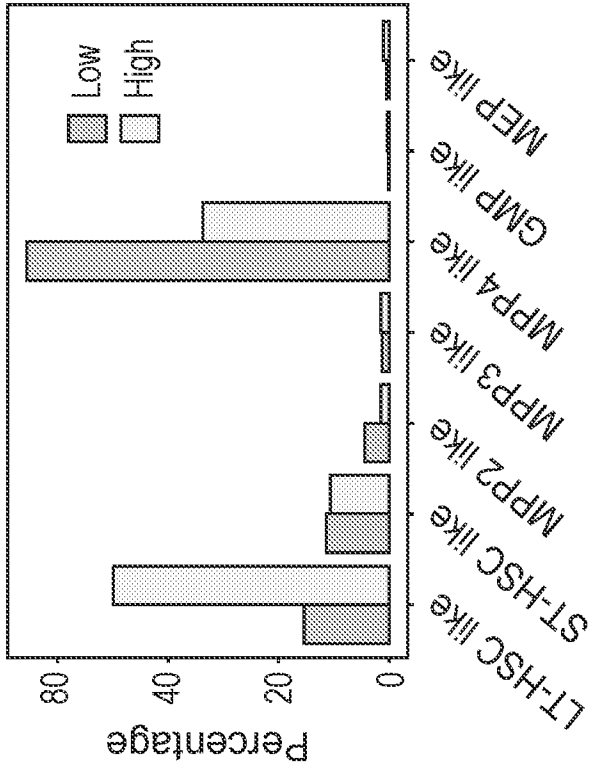


FIG. 4H

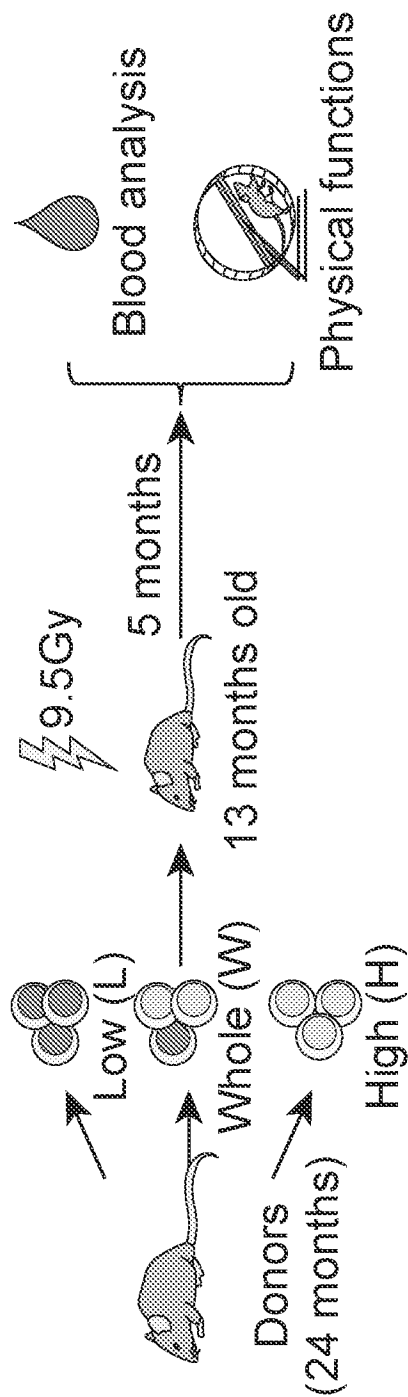


FIG. 5A

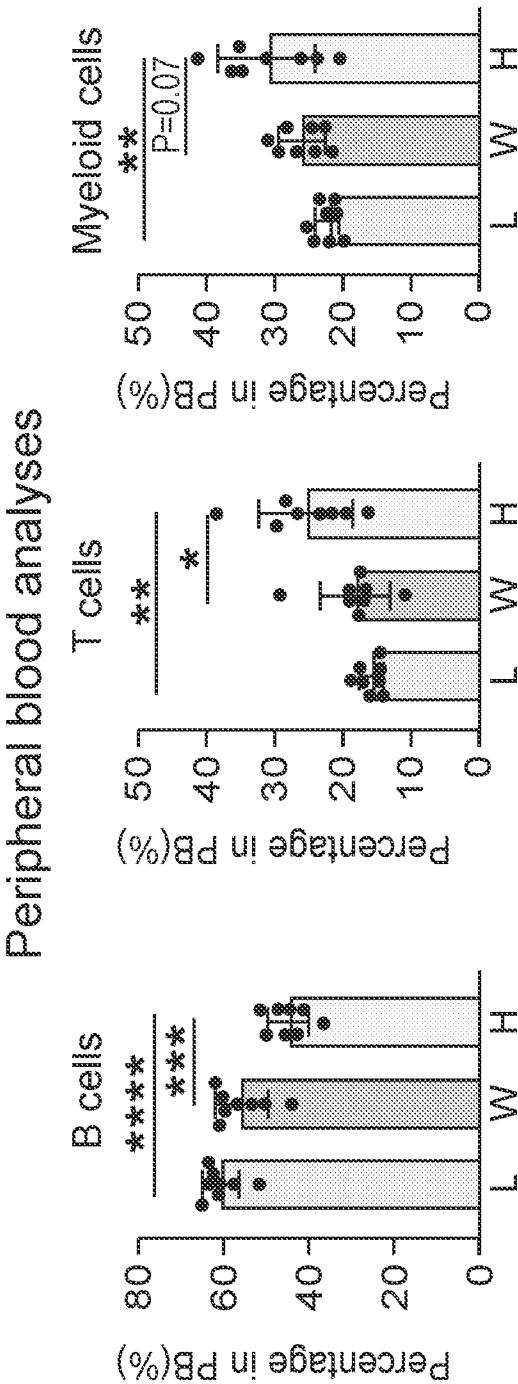


FIG. 5B

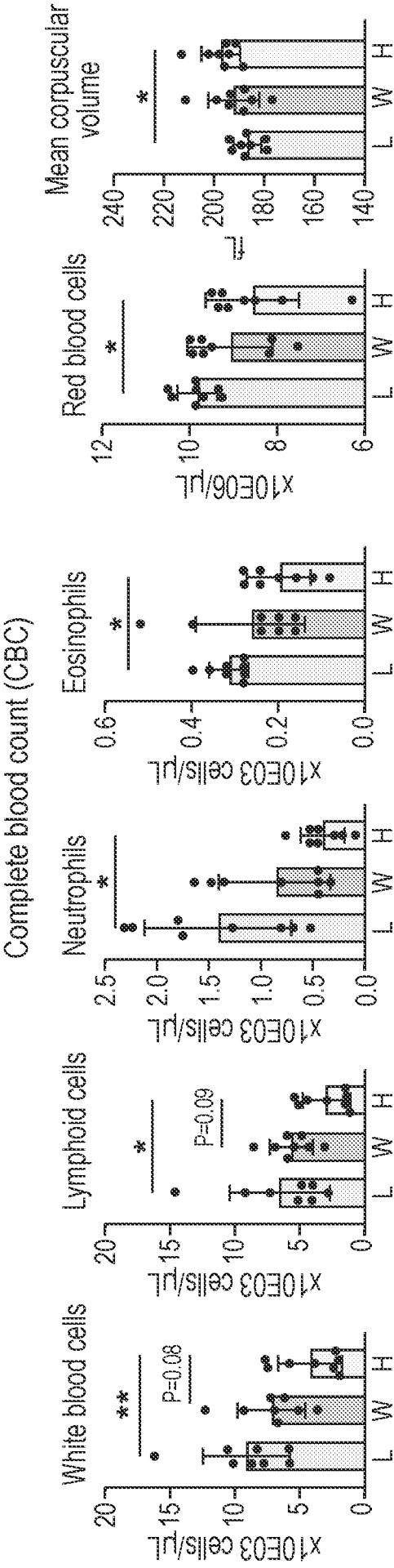


FIG. 5C

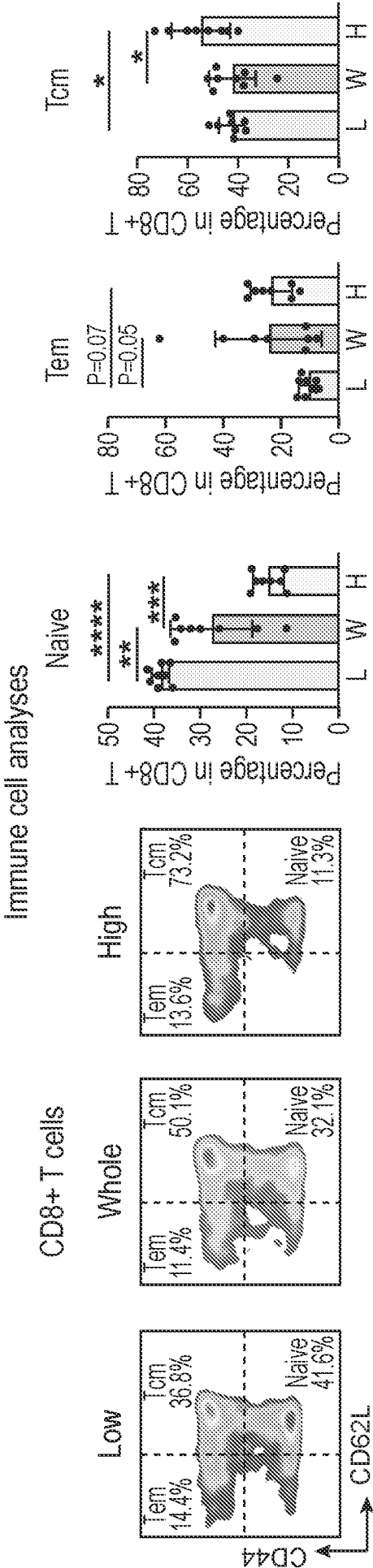


FIG. 5D

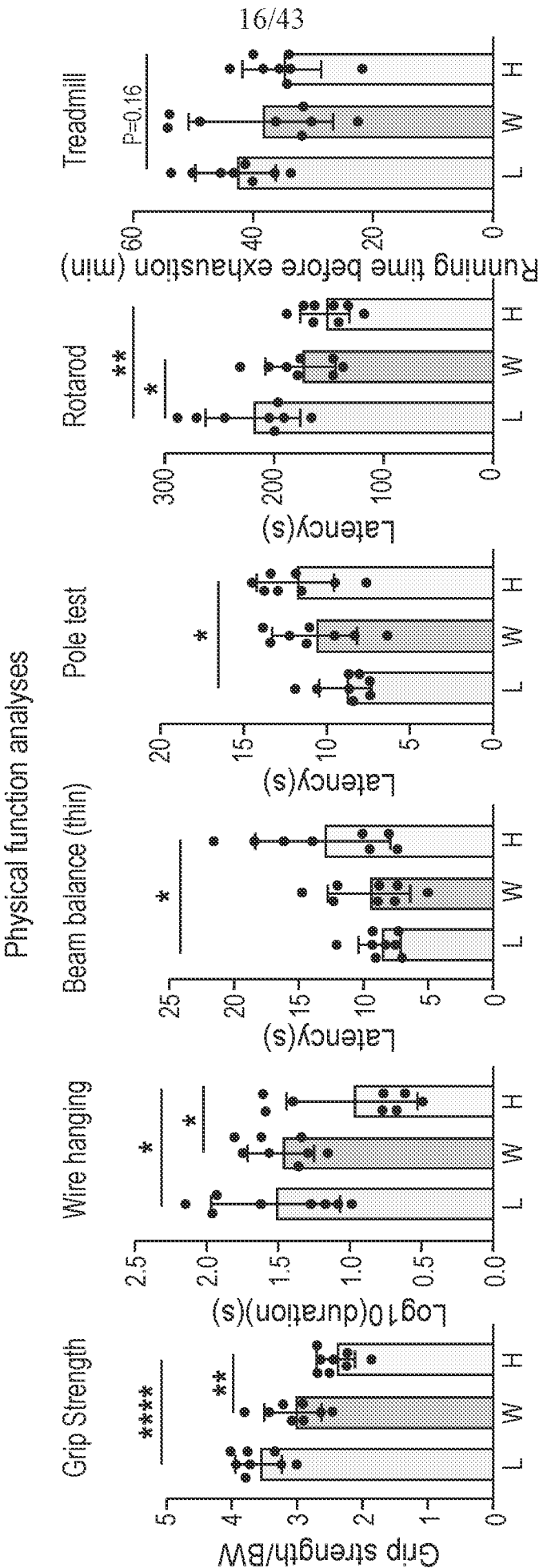


FIG. 5E

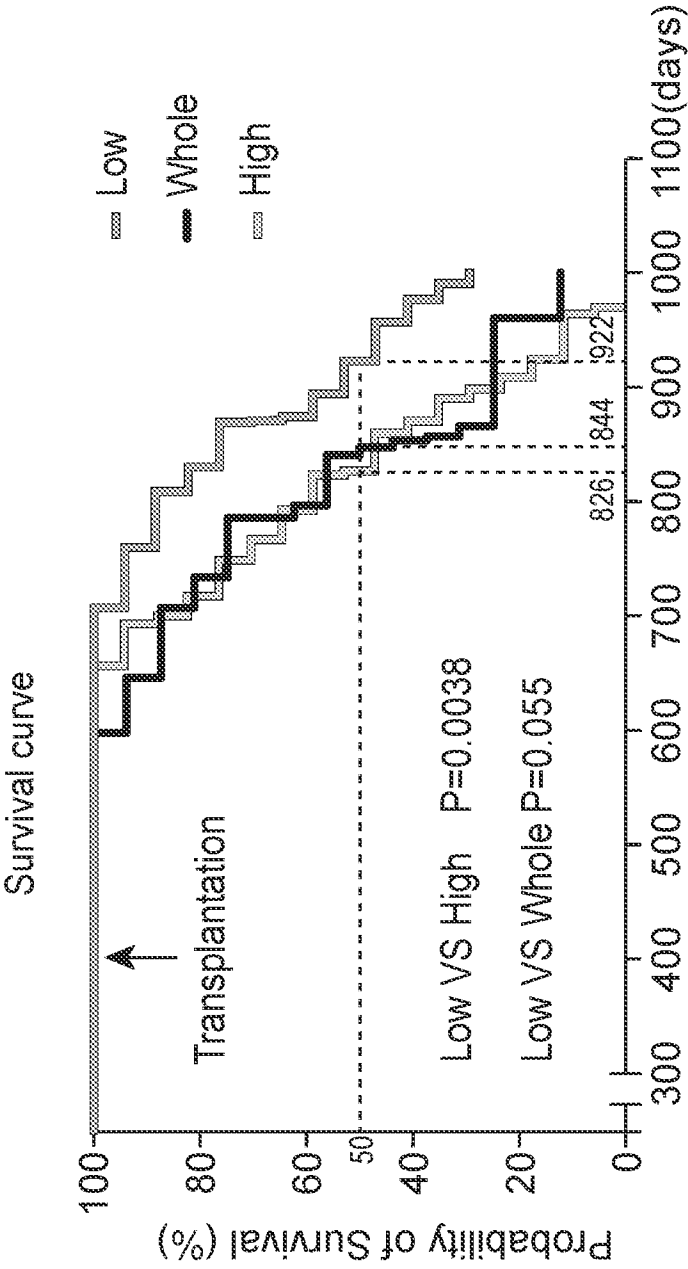


FIG. 5G

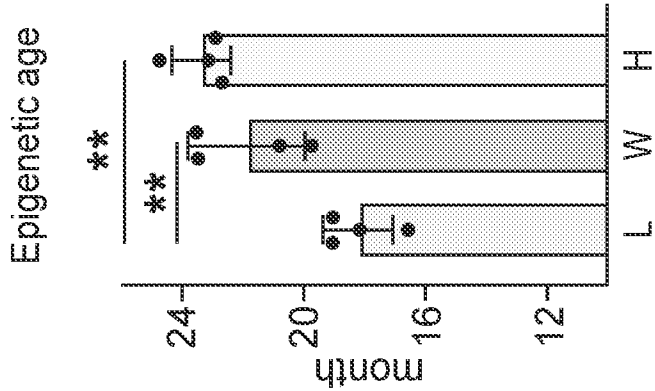


FIG. 5F

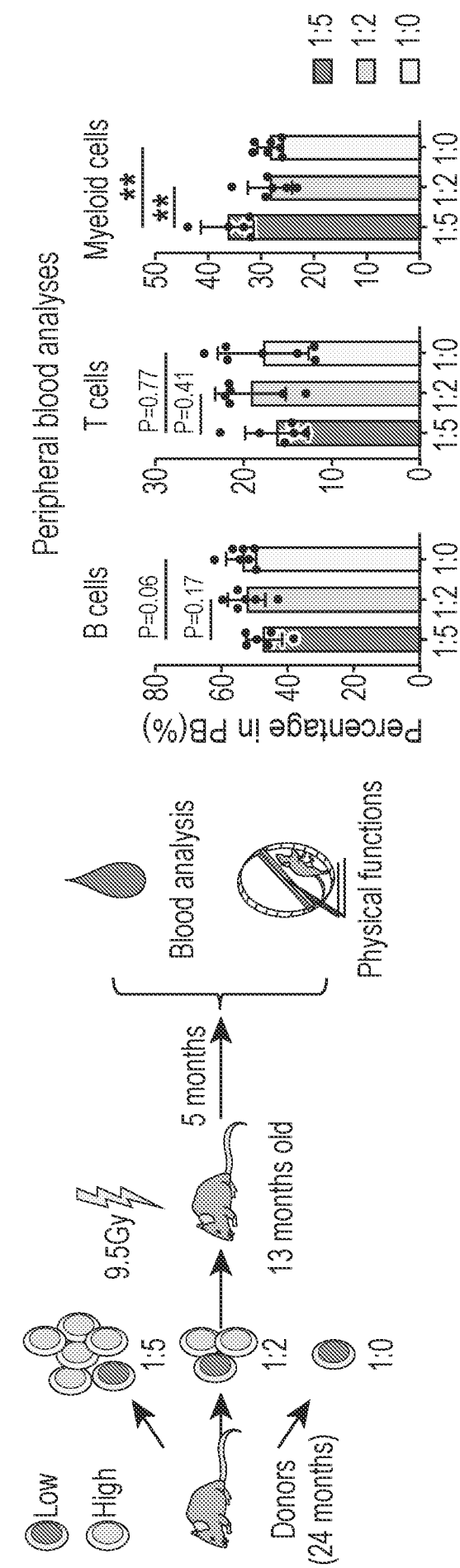
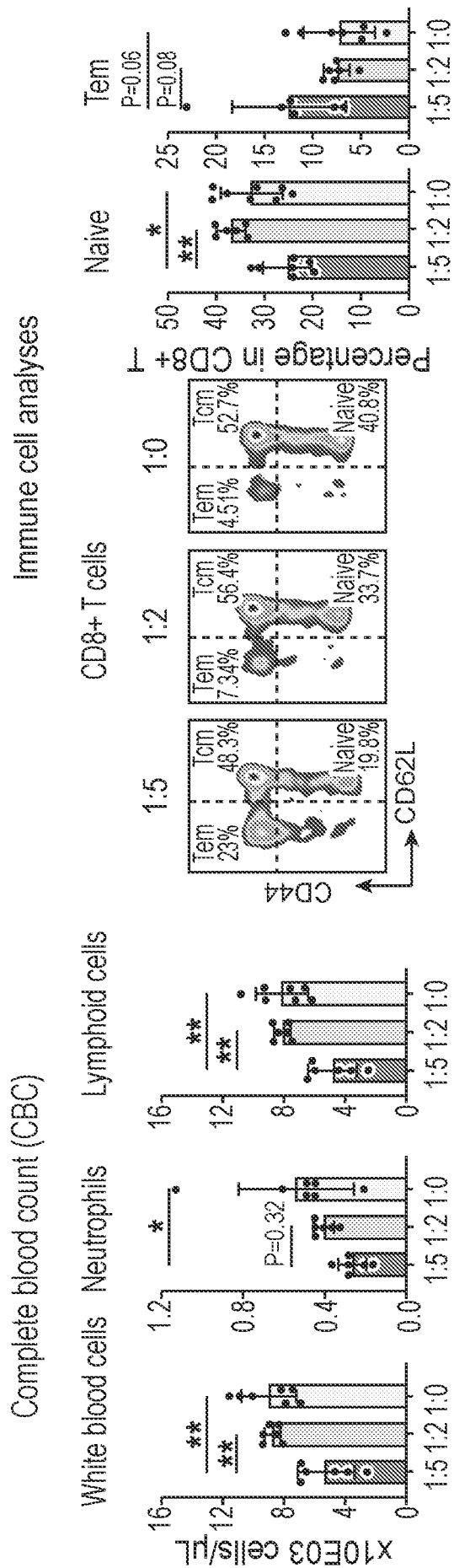
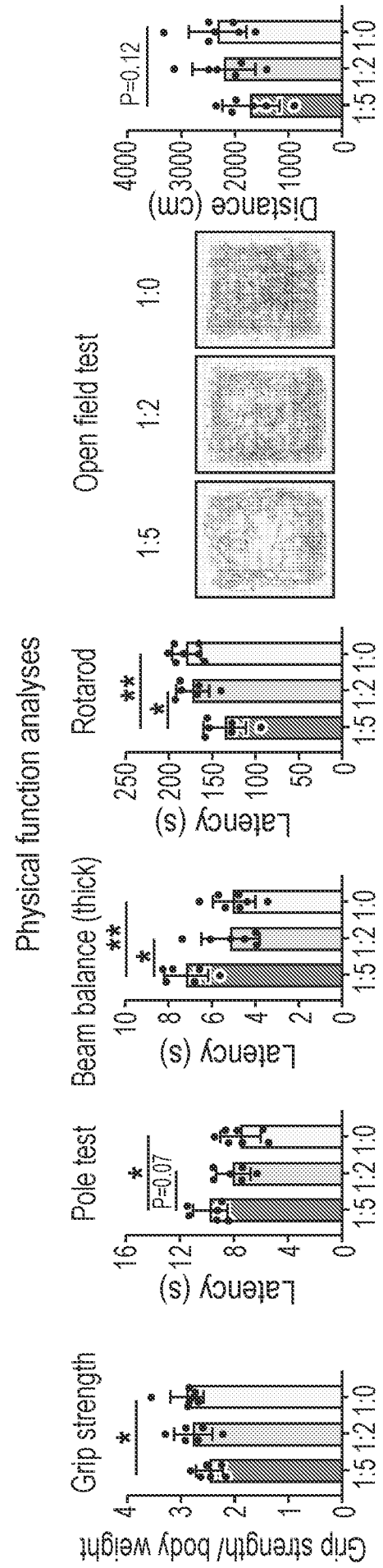


FIG. 6B

FIG. 6A



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W
C
D
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L

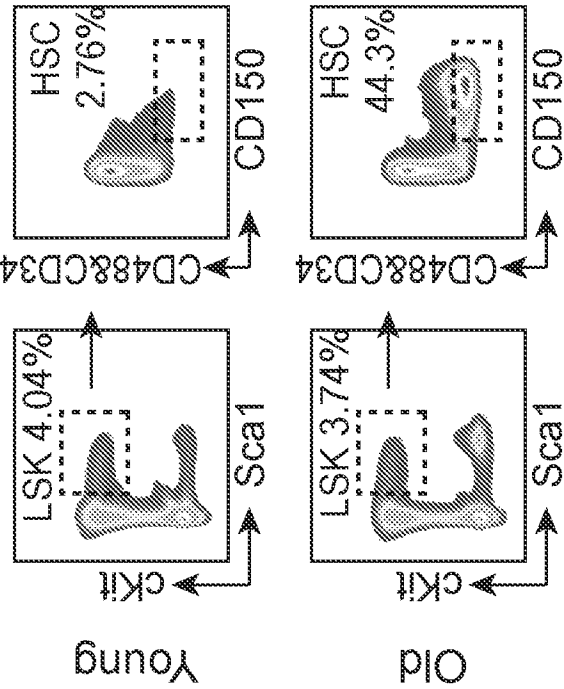


FIG. 7A

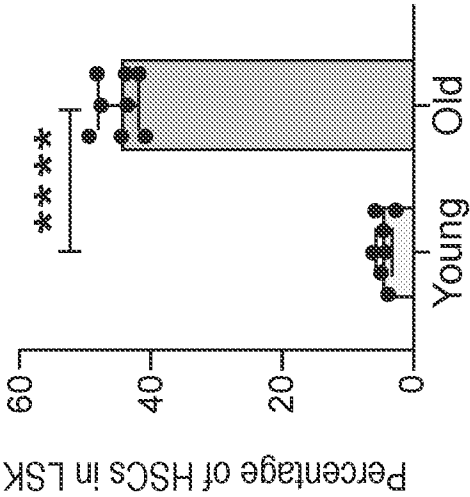


FIG. 7B

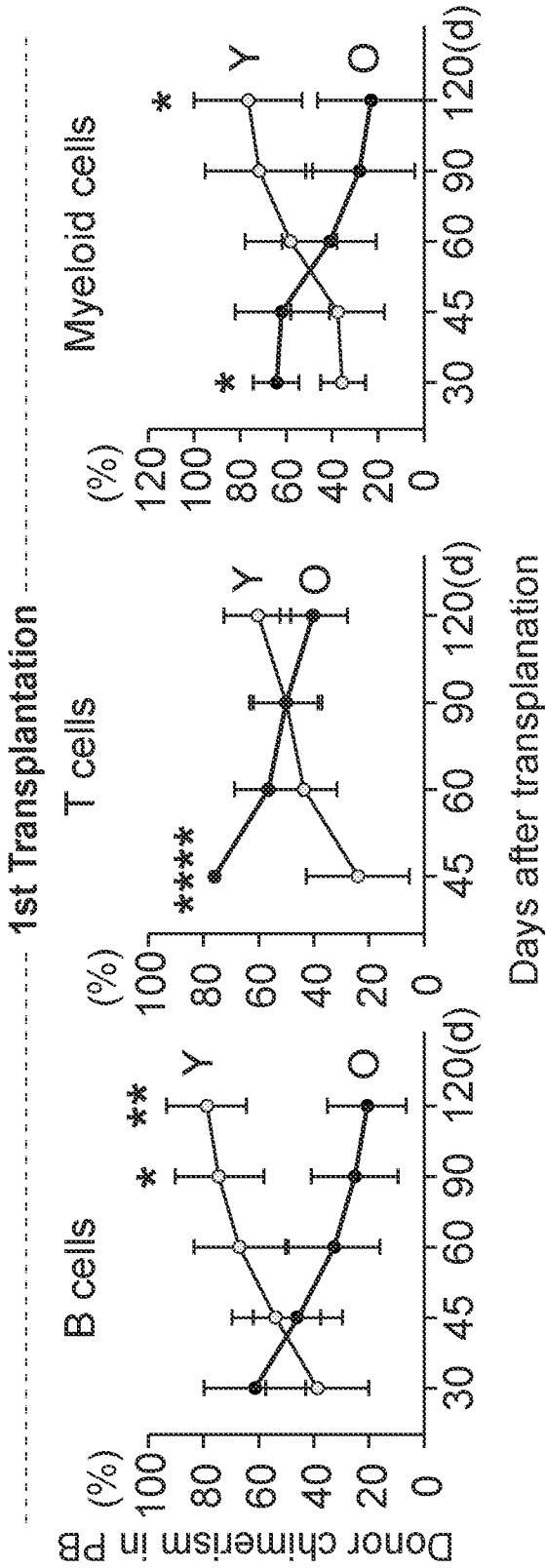


FIG. 7C

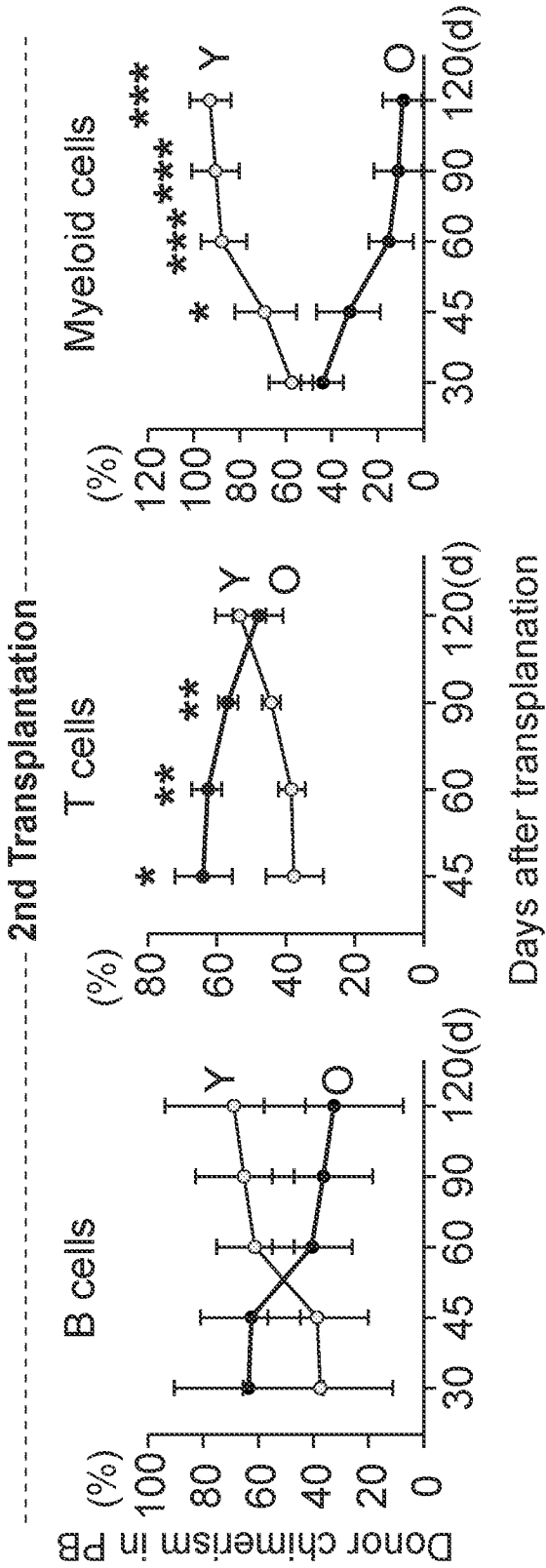


FIG. 7D

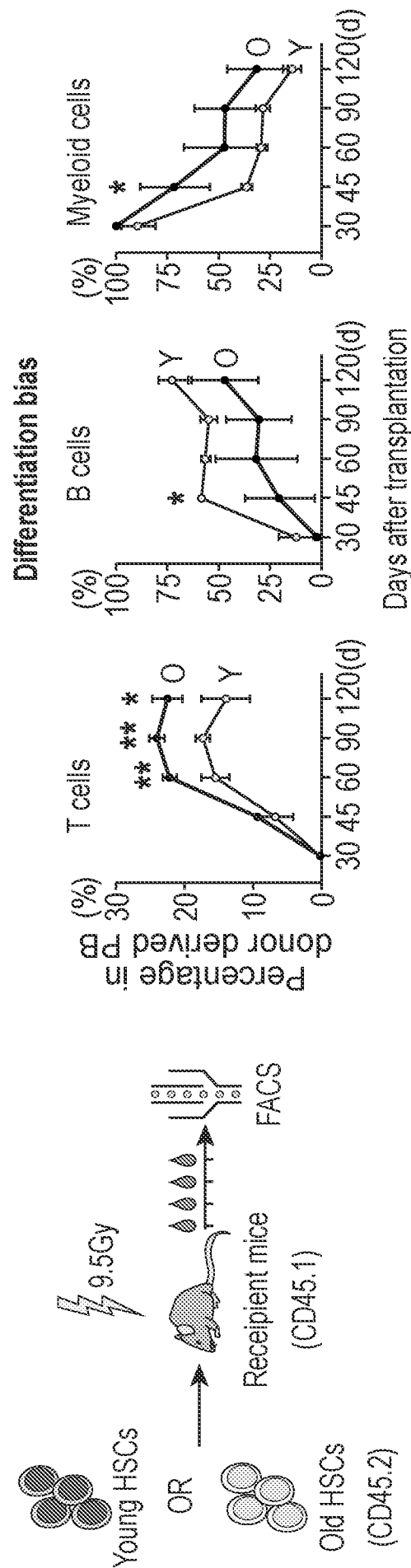


FIG. 7E

FIG. 7F

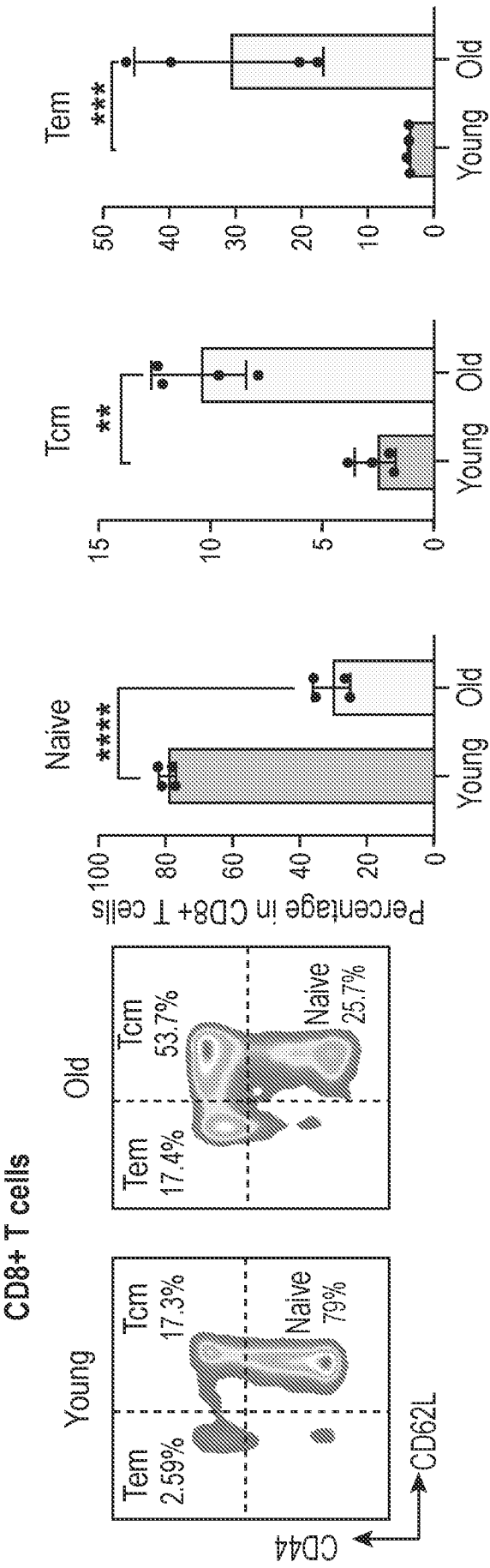


FIG. 8A

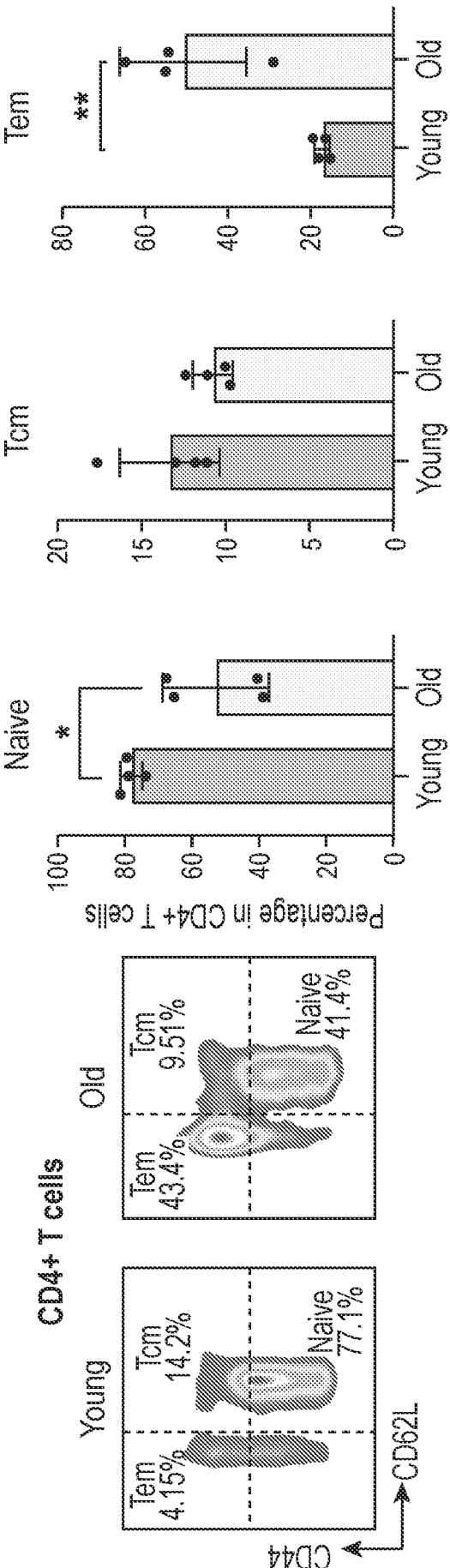


FIG. 8B

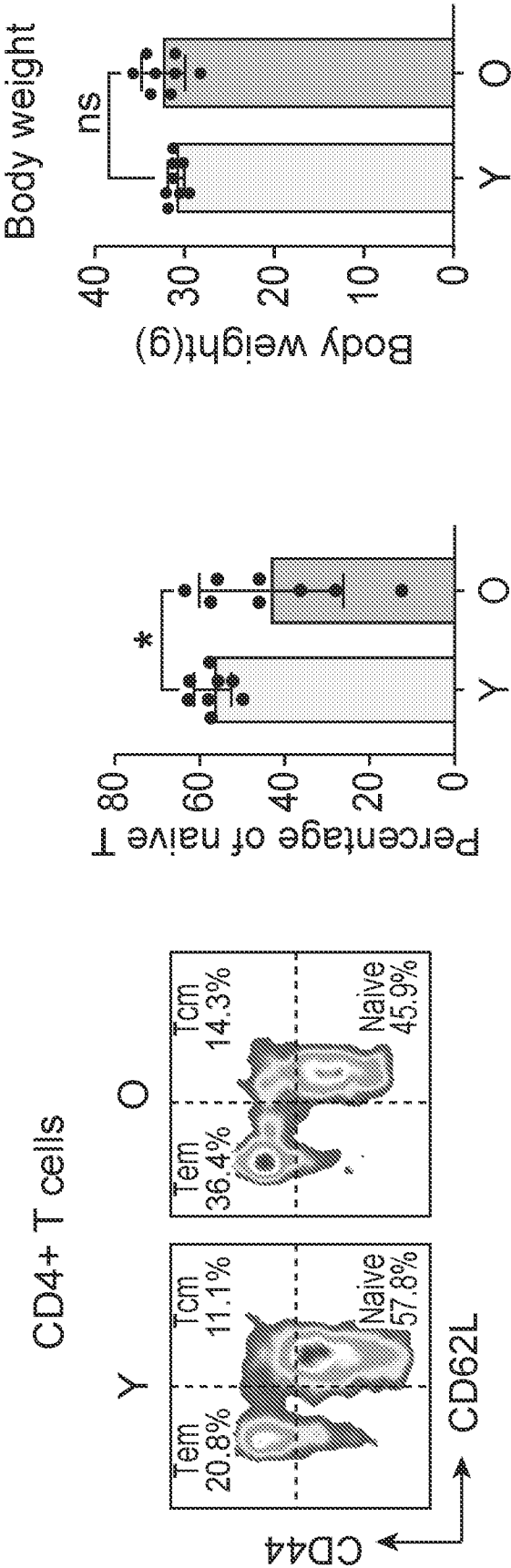


FIG. 9A

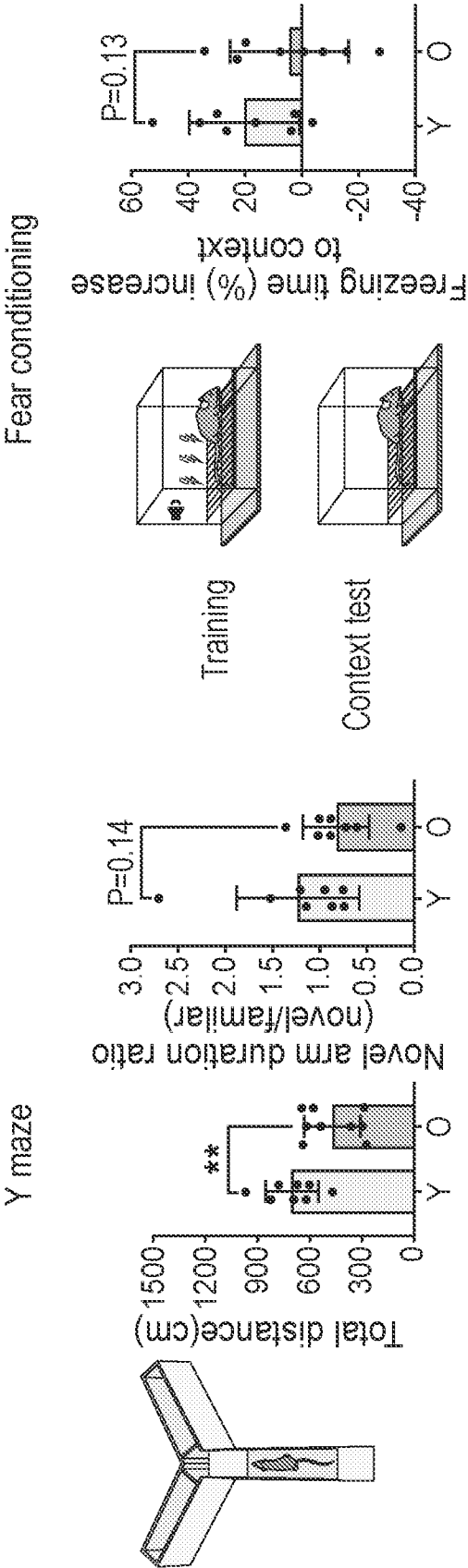


FIG. 9B

FIG. 9C

FIG. 9D

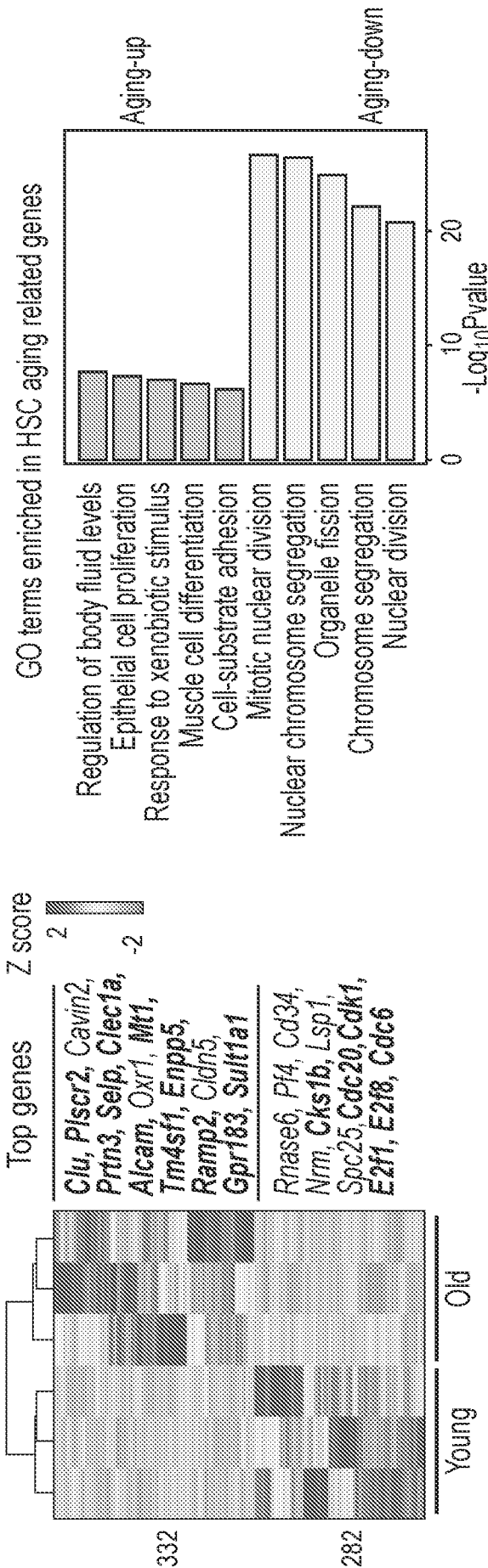


FIG. 10A

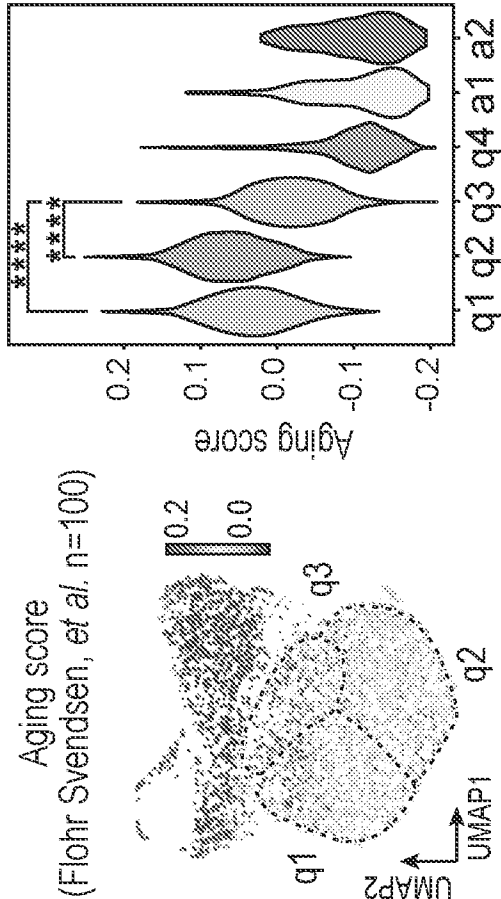
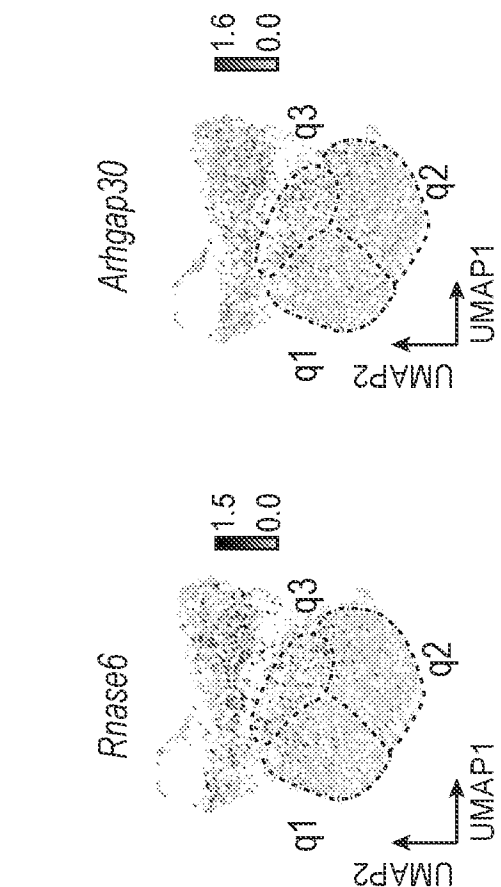


FIG. 10B



(Flohr Svendsen, et al. n=100)

FIG. 10C

FIG. 10D

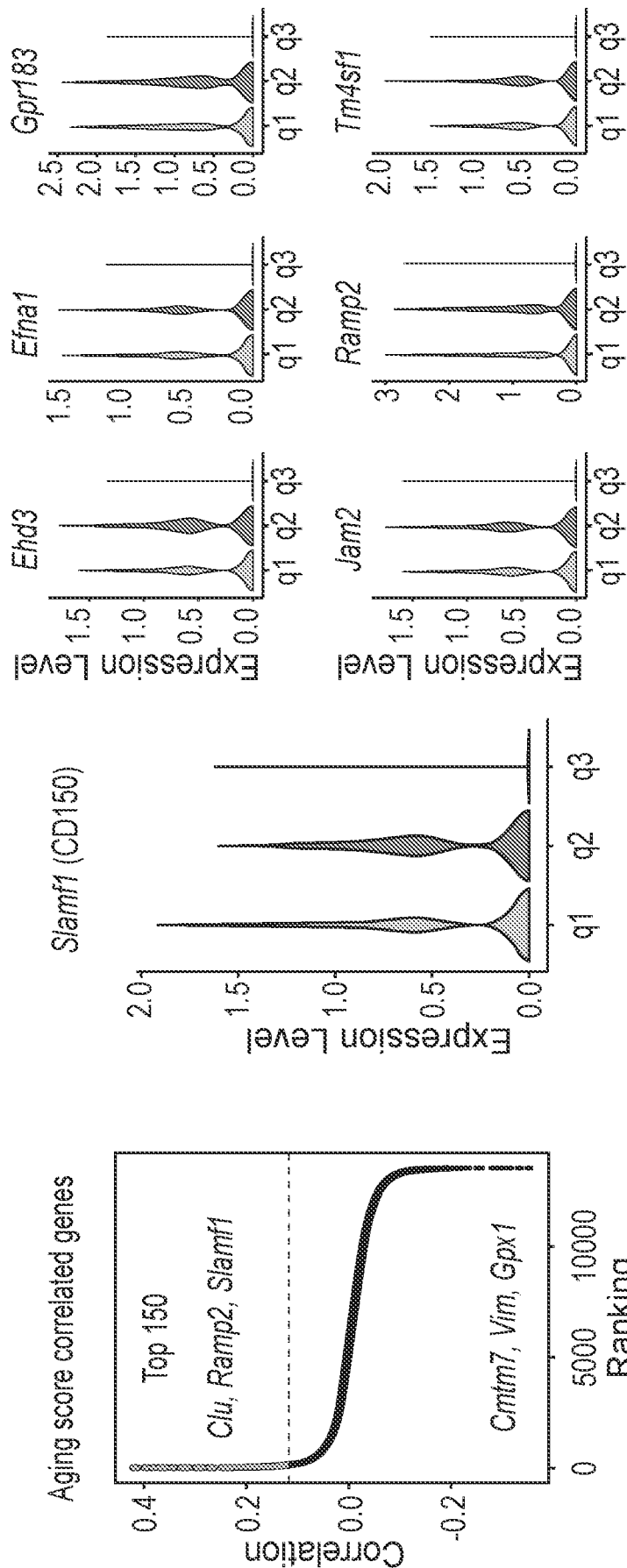


FIG. 11A

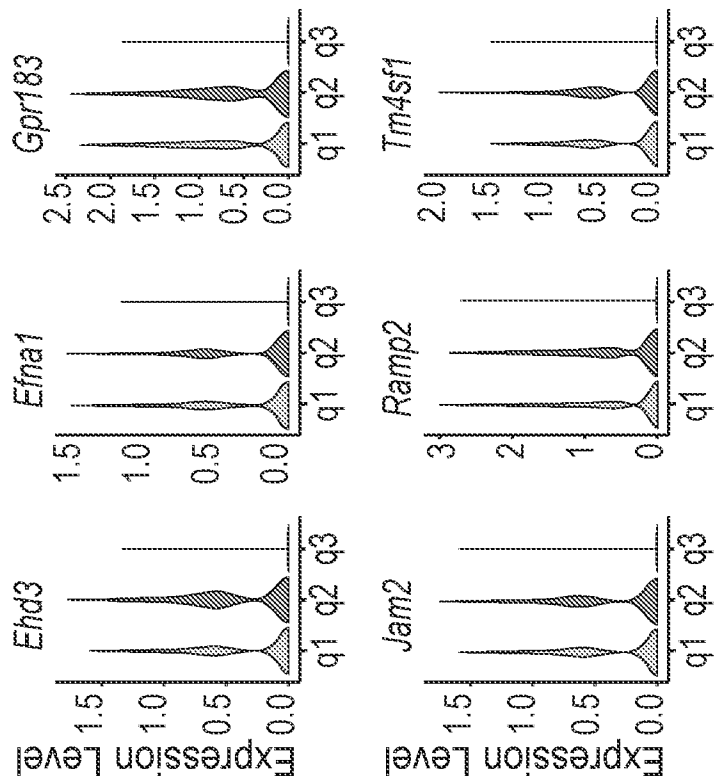
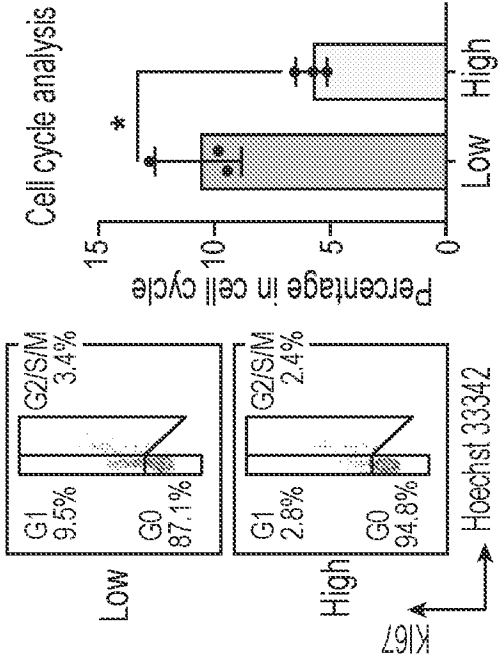
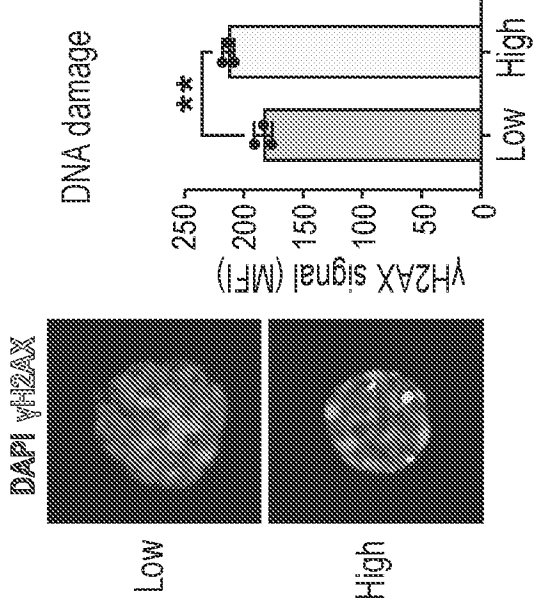
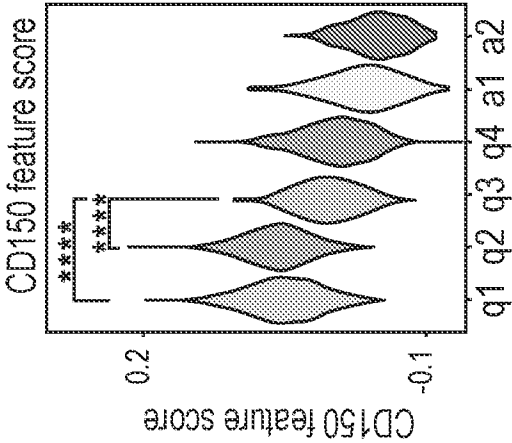
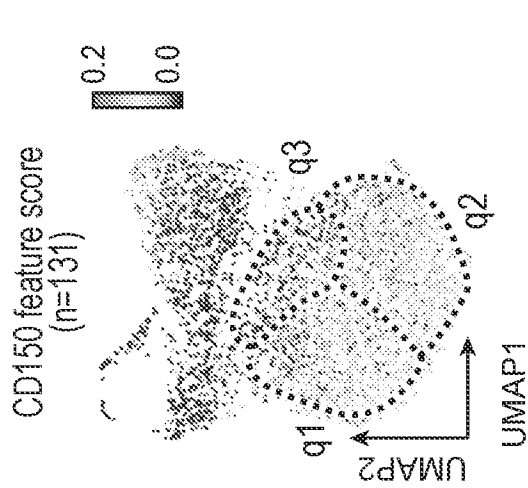
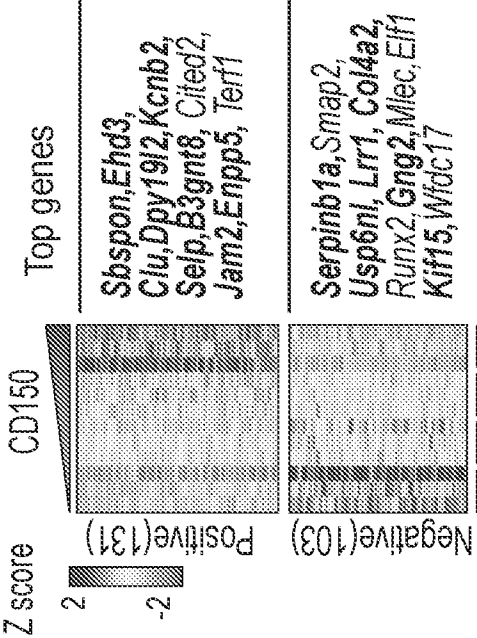
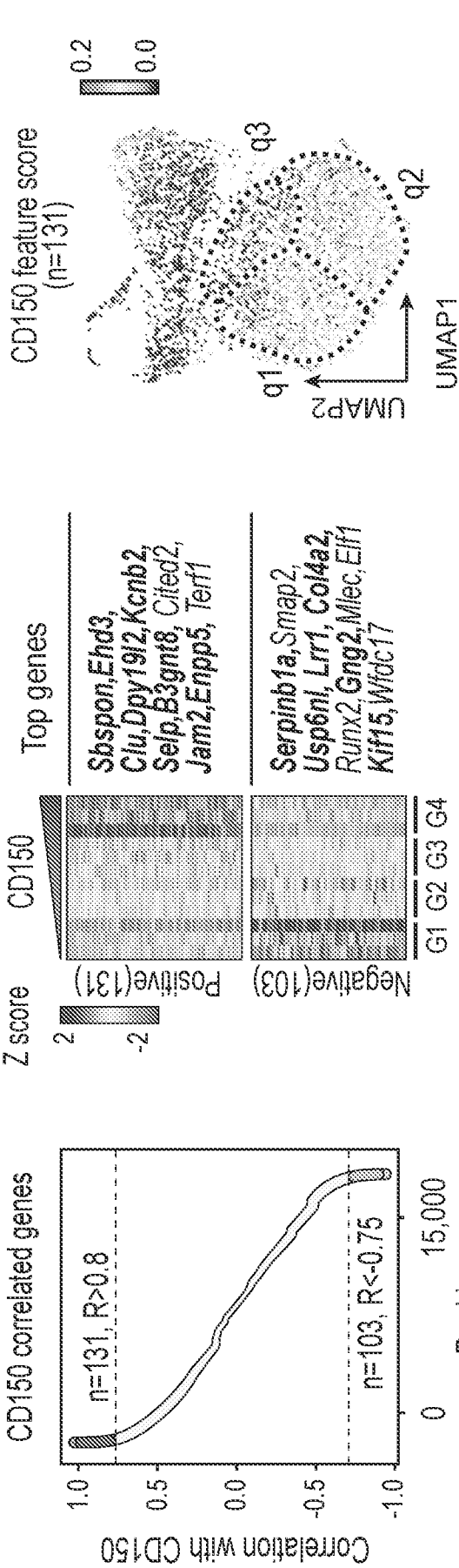


FIG. 11B



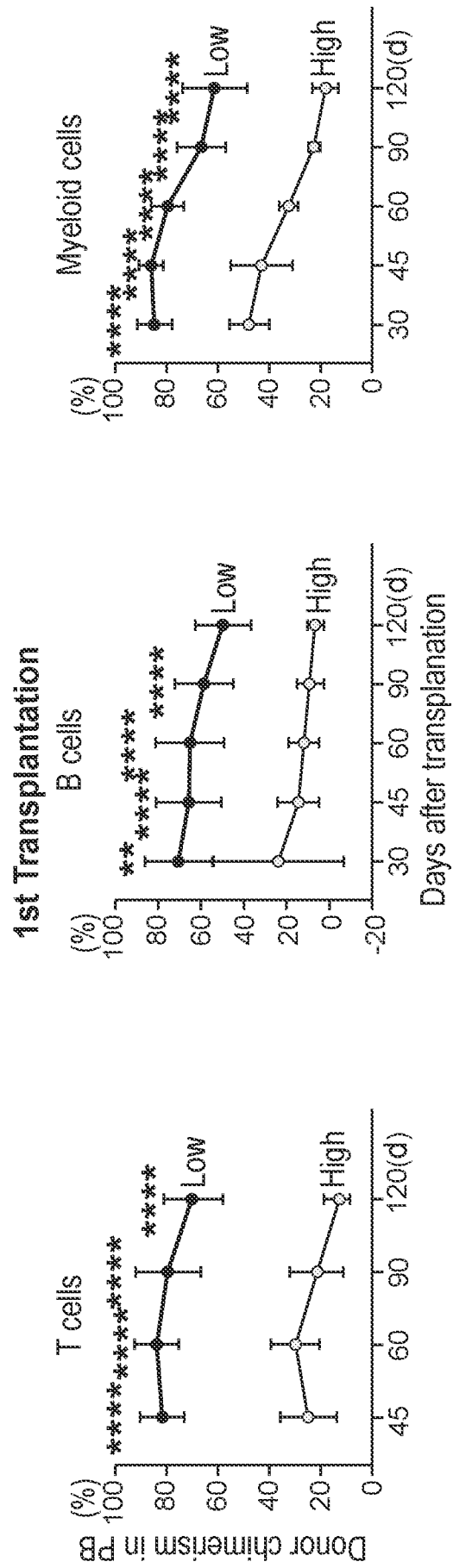


FIG. 12A

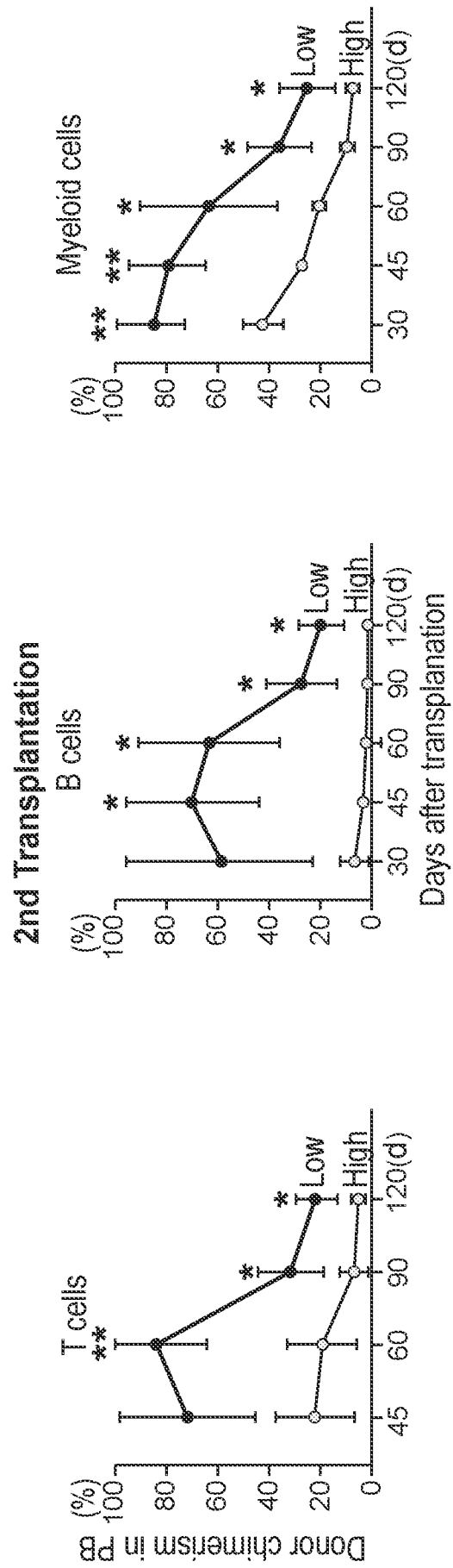


FIG. 12B

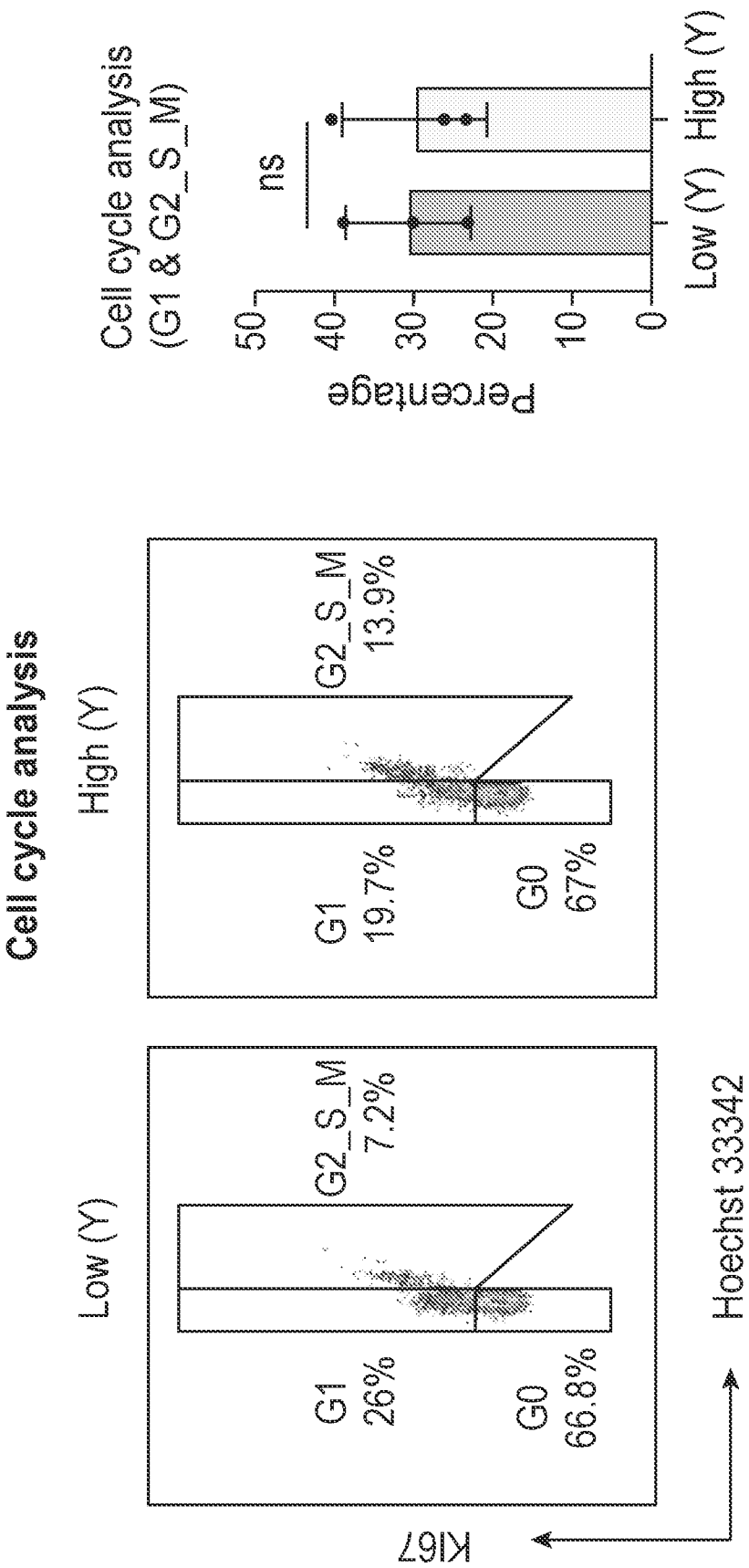


FIG. 13A

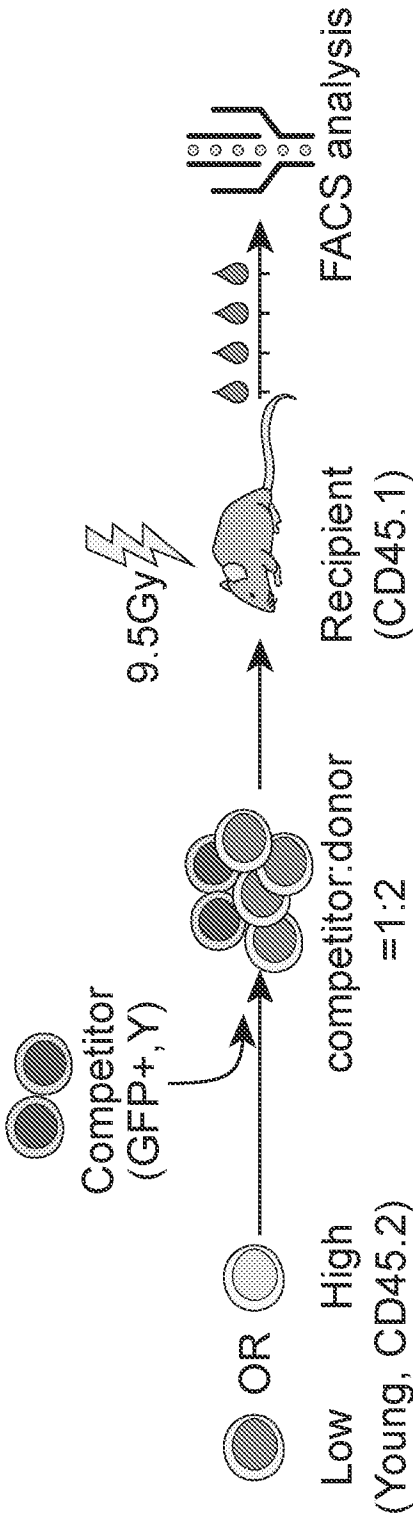


FIG. 13B

Donor chimerism

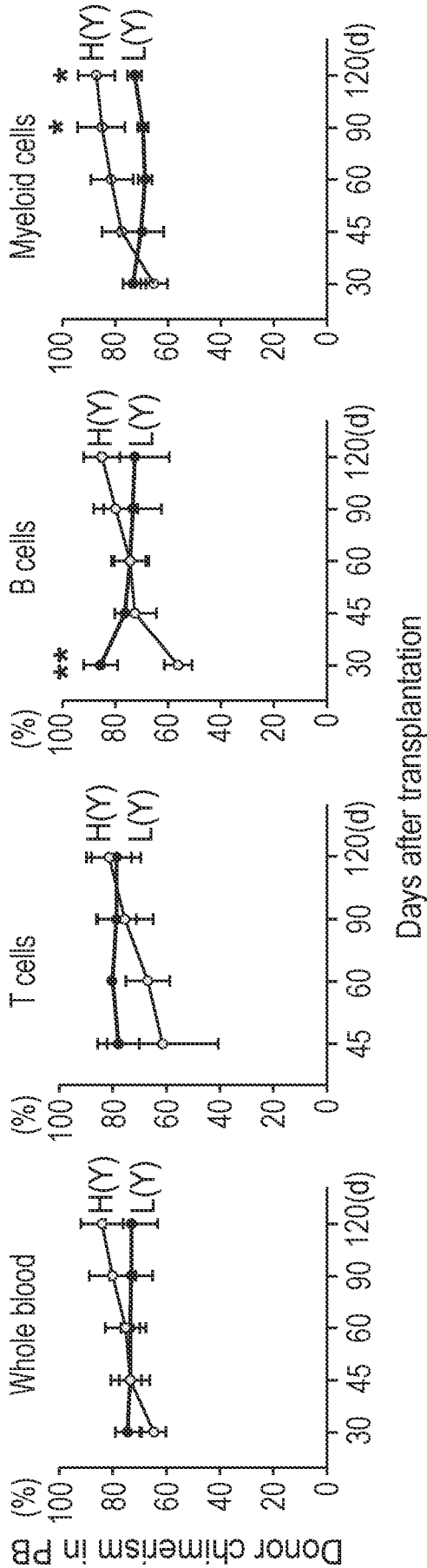


FIG. 13C

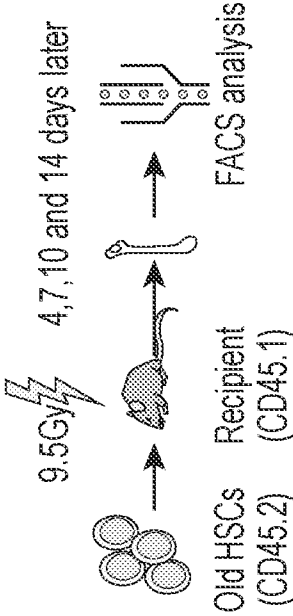
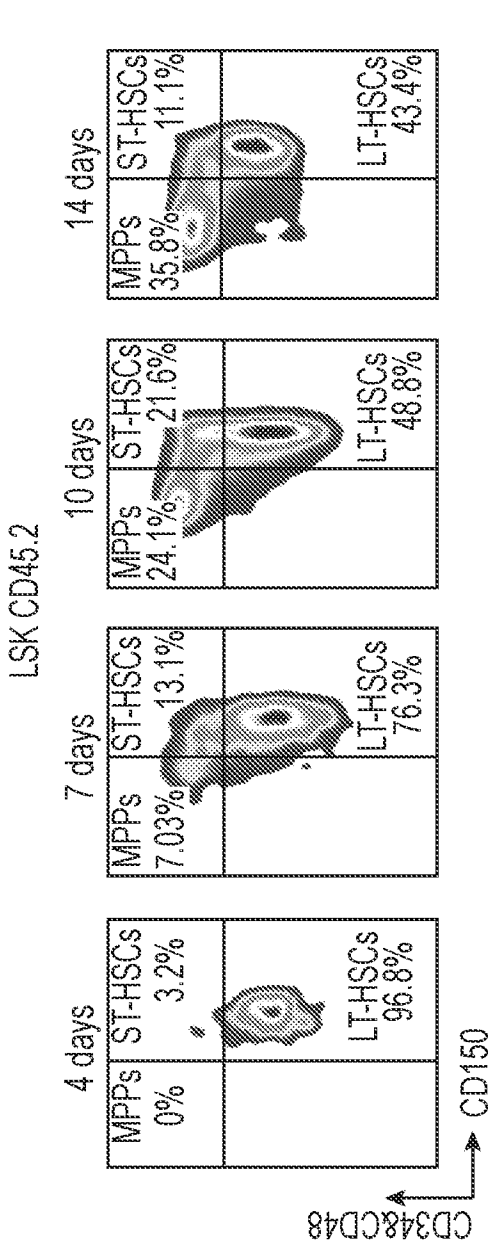
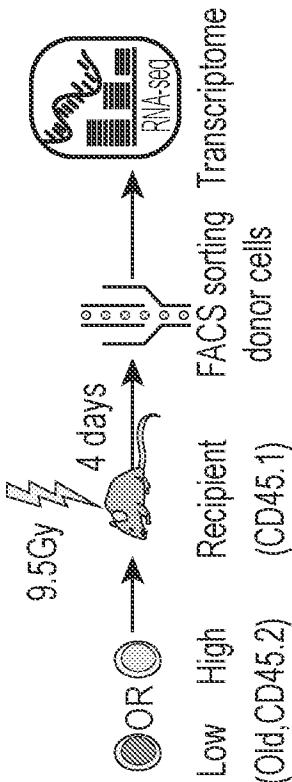
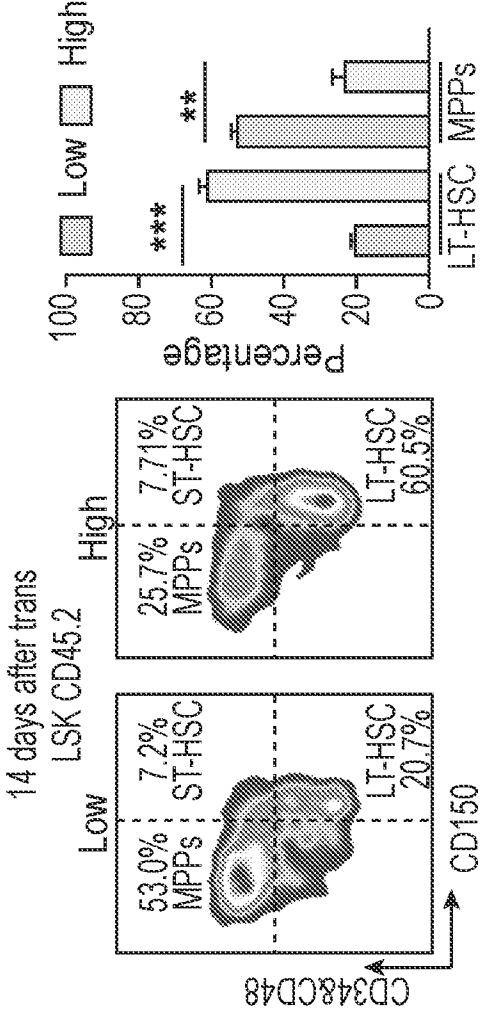


FIG. 14A



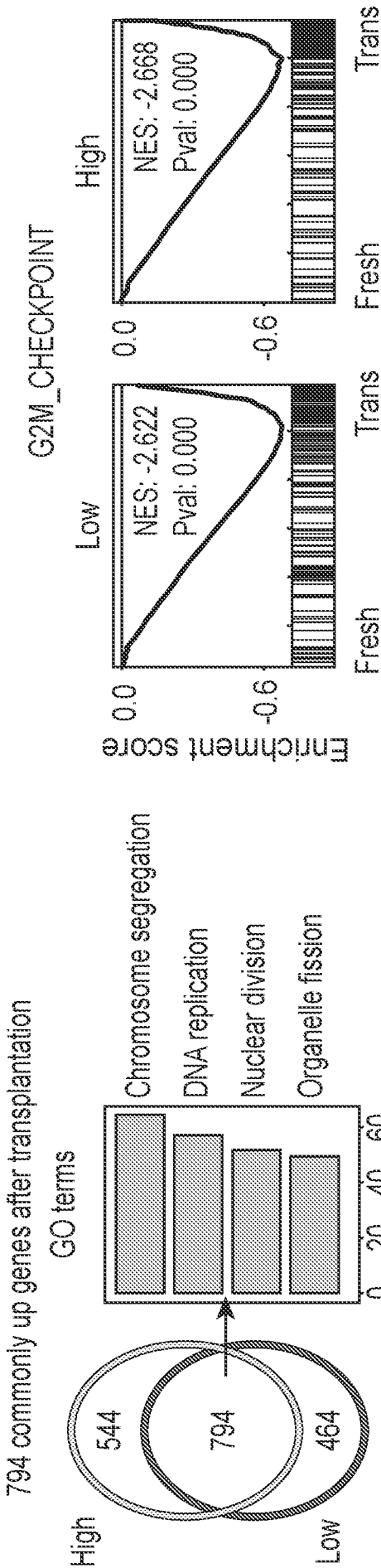


FIG. 14F

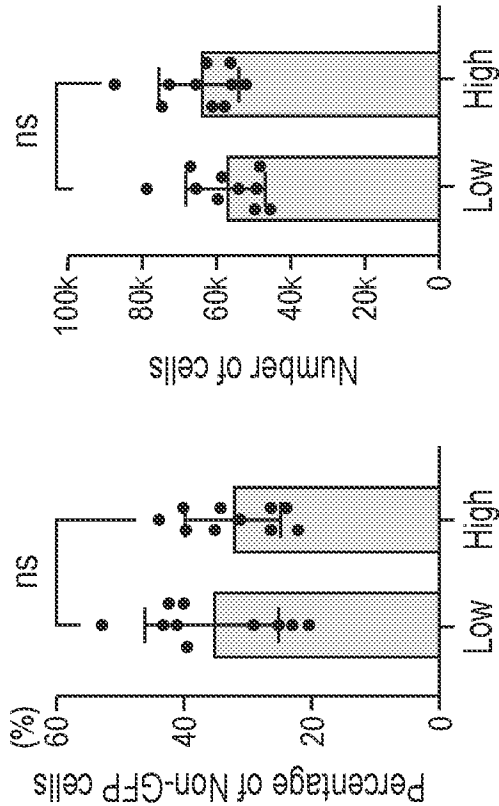
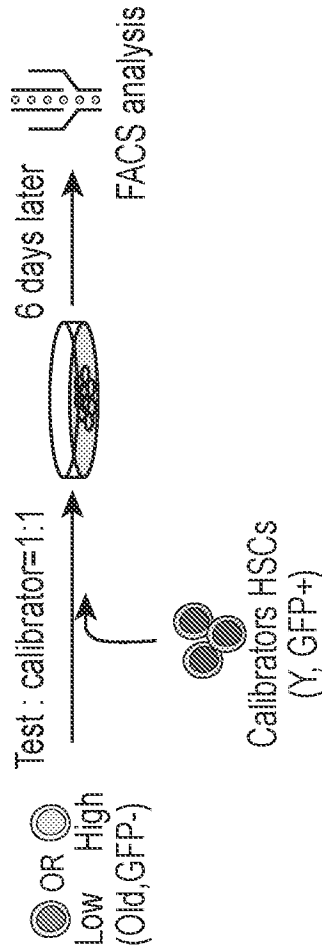


FIG. 14I



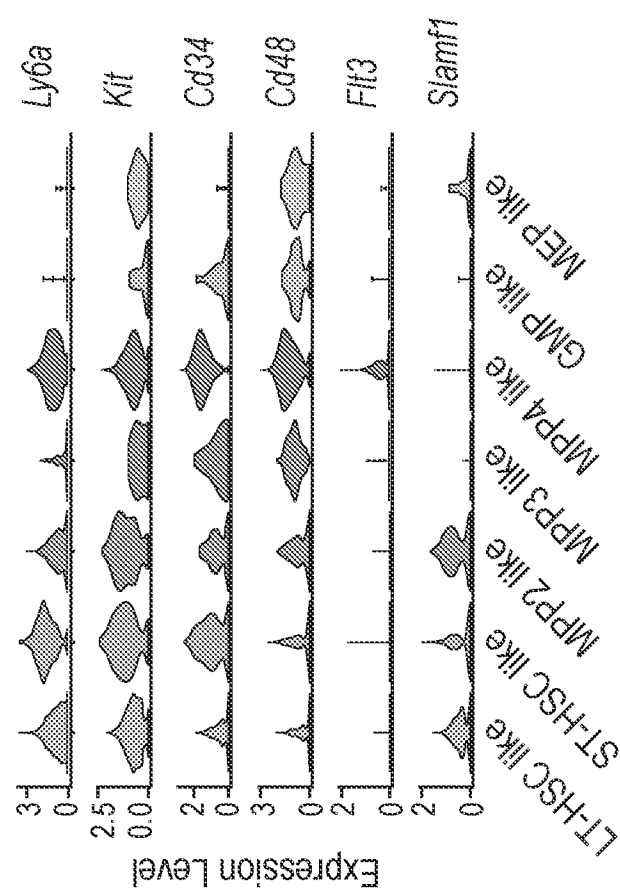


FIG. 14K

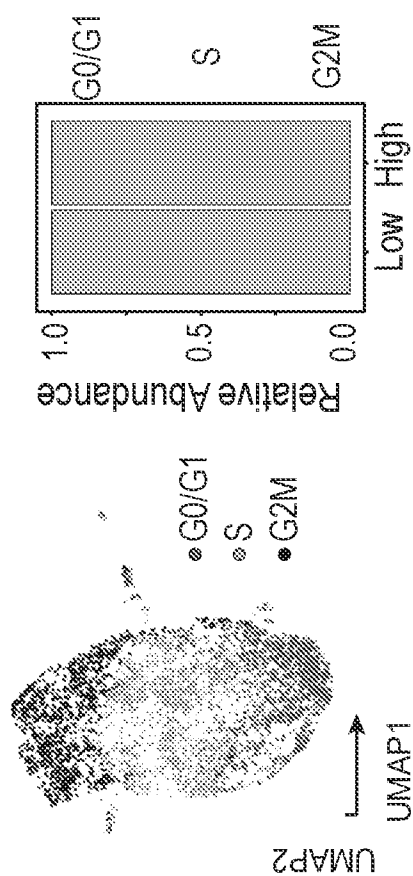


FIG. 14J

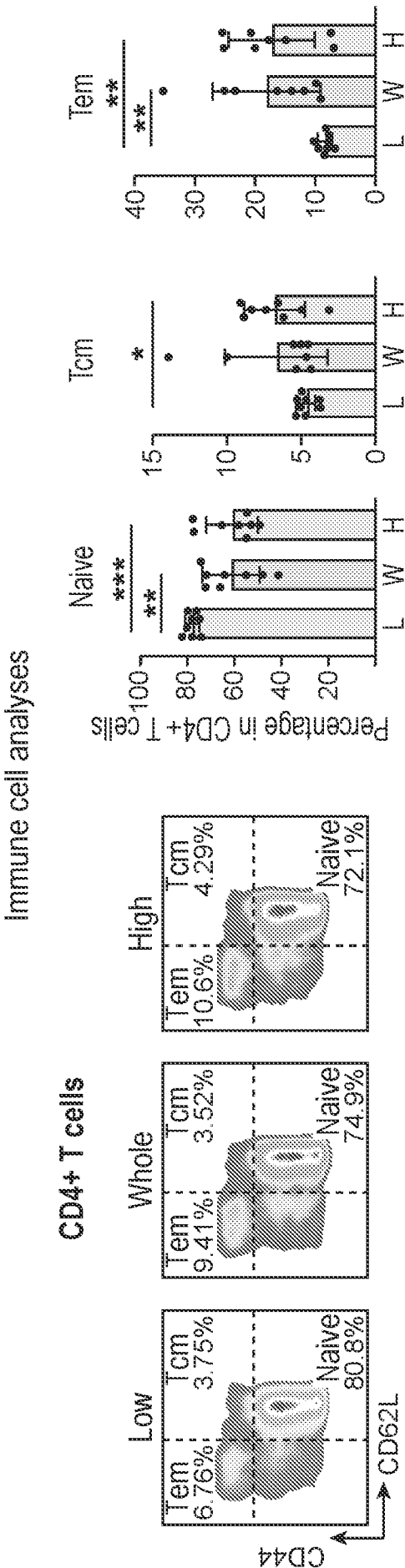


FIG. 15A

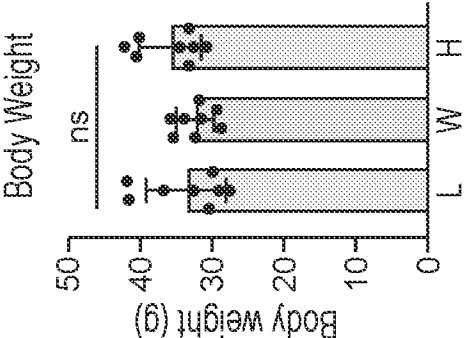


FIG. 15B

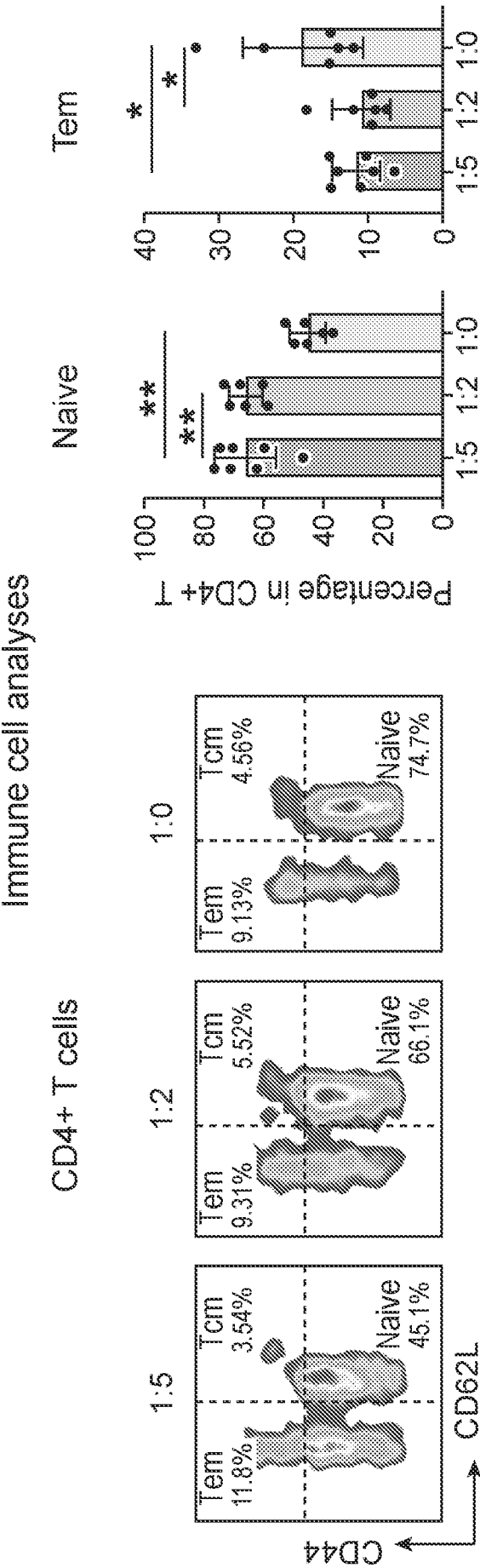


FIG. 16A

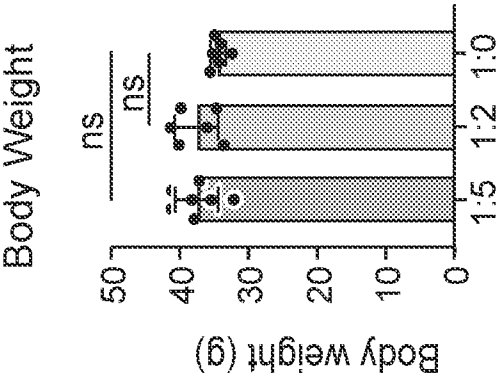


FIG. 16B

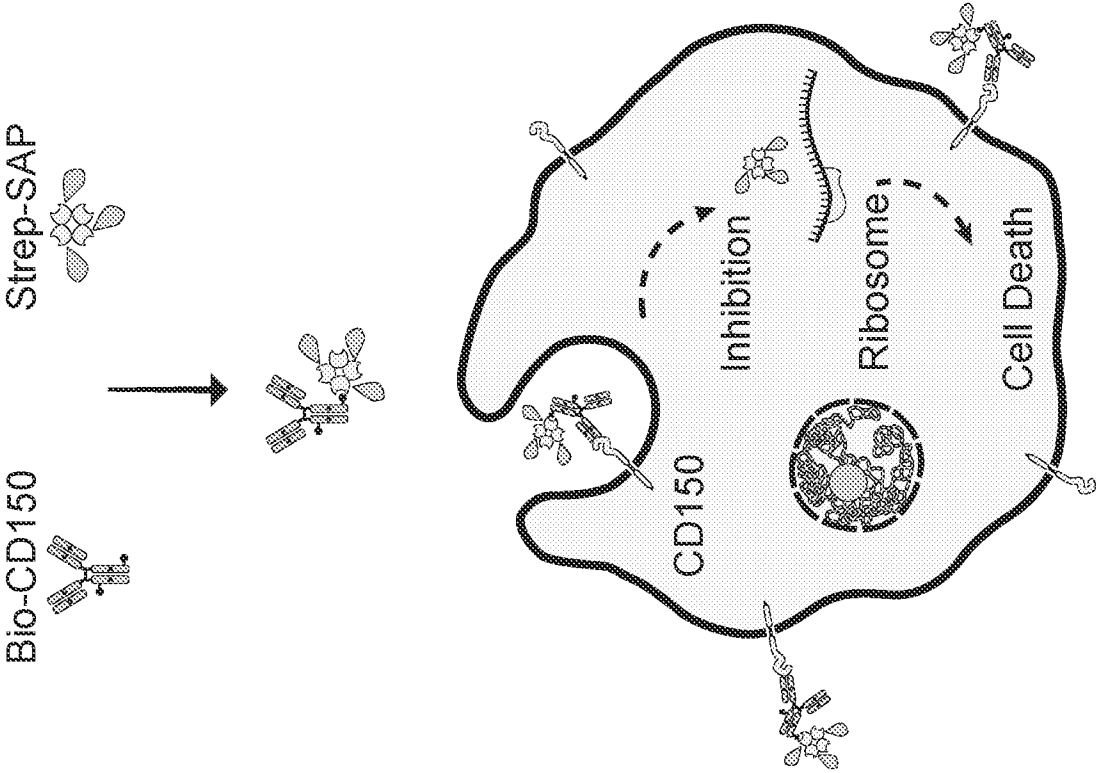


FIG. 17A

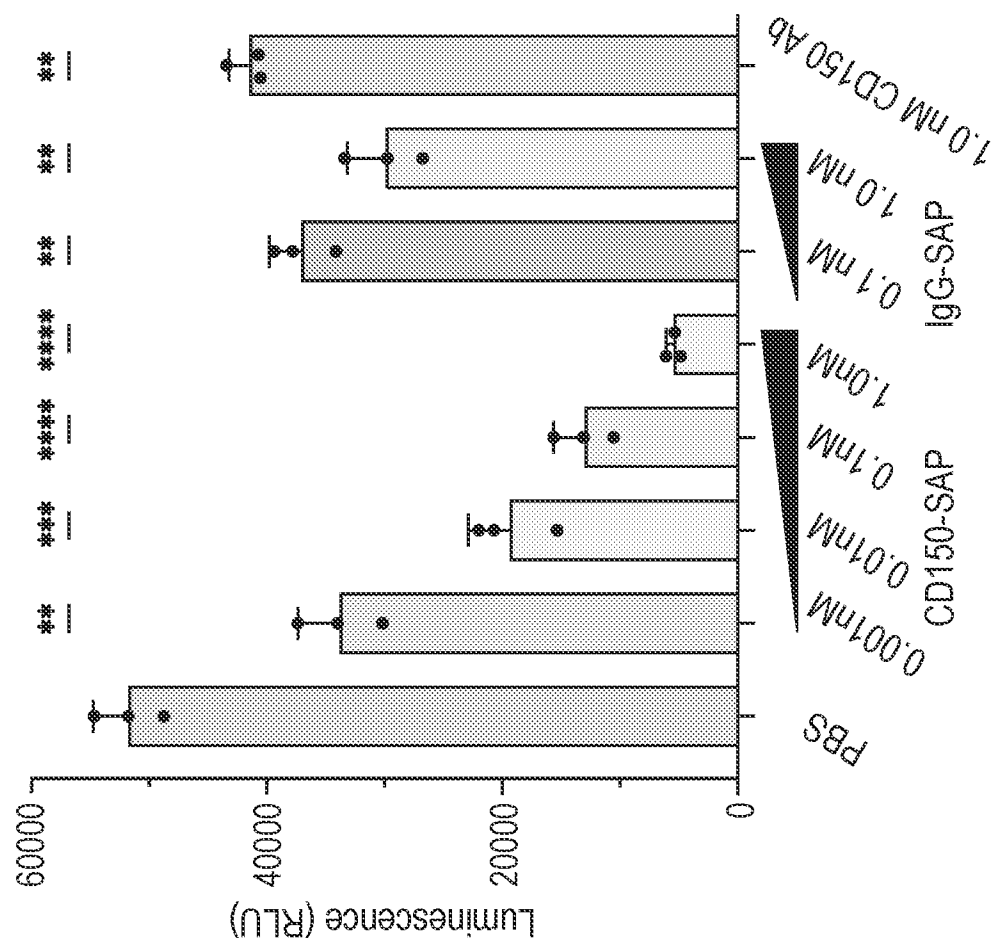


FIG. 17B

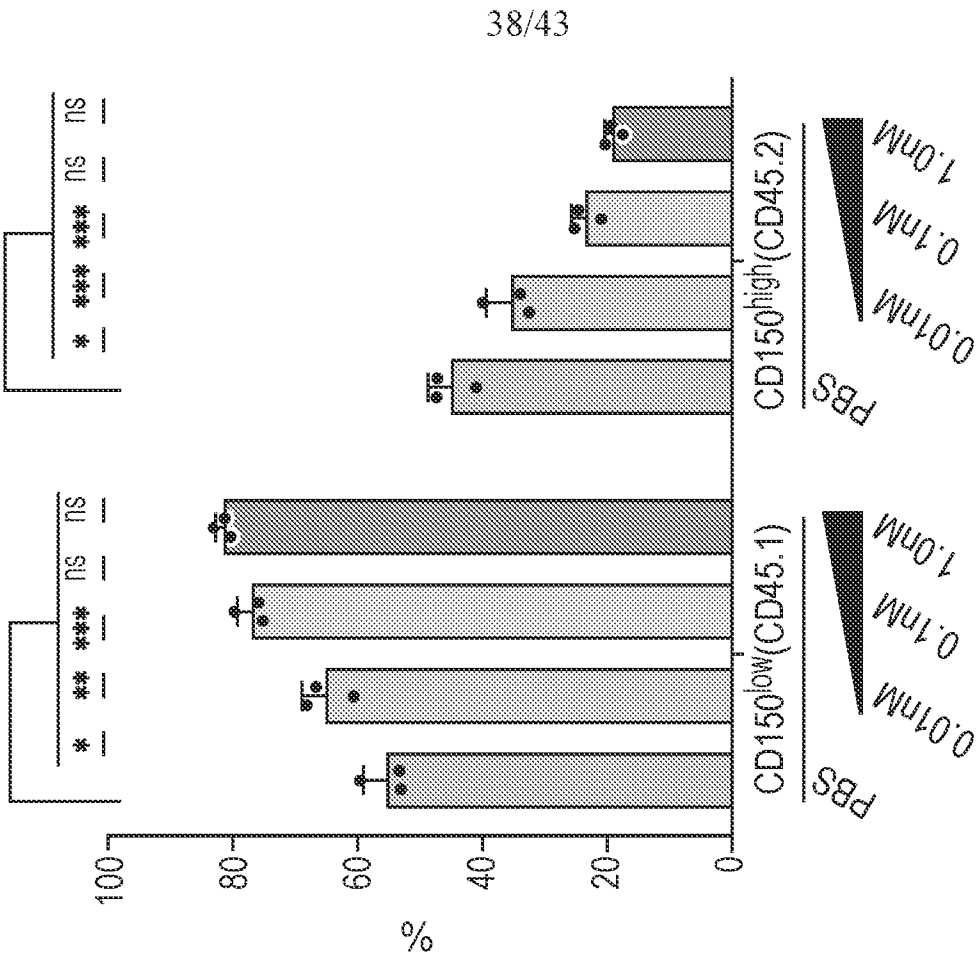


FIG. 17D

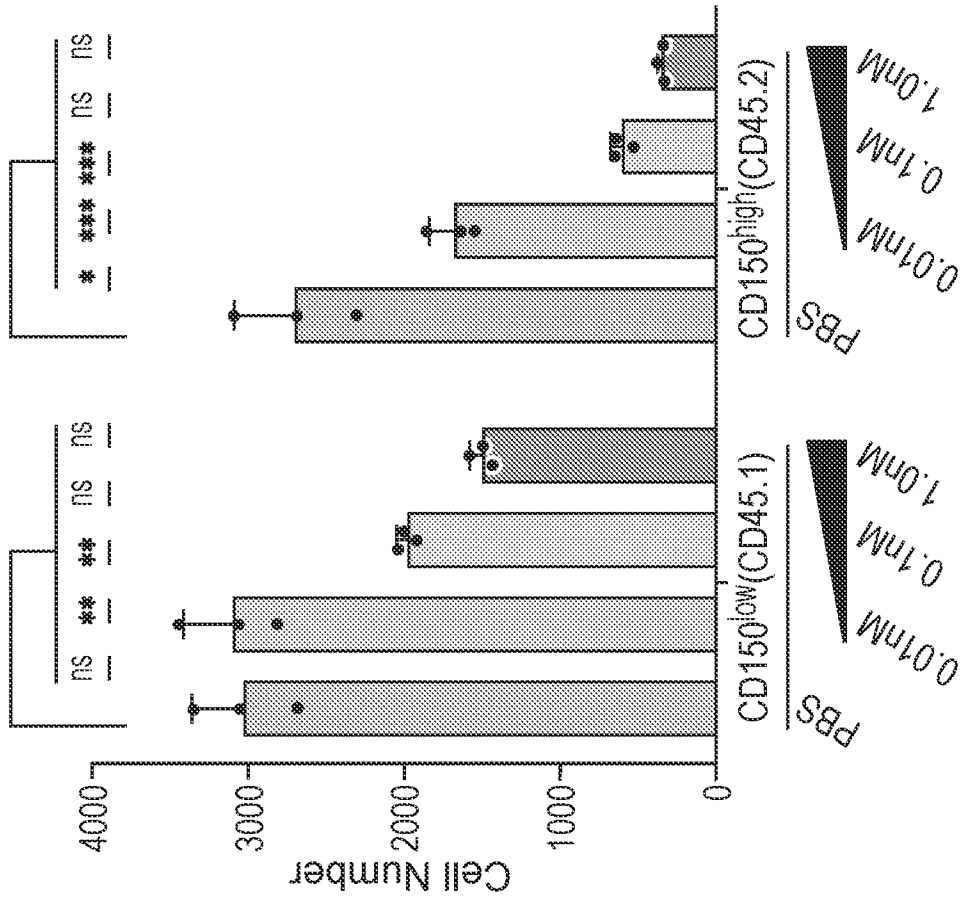


FIG. 17C

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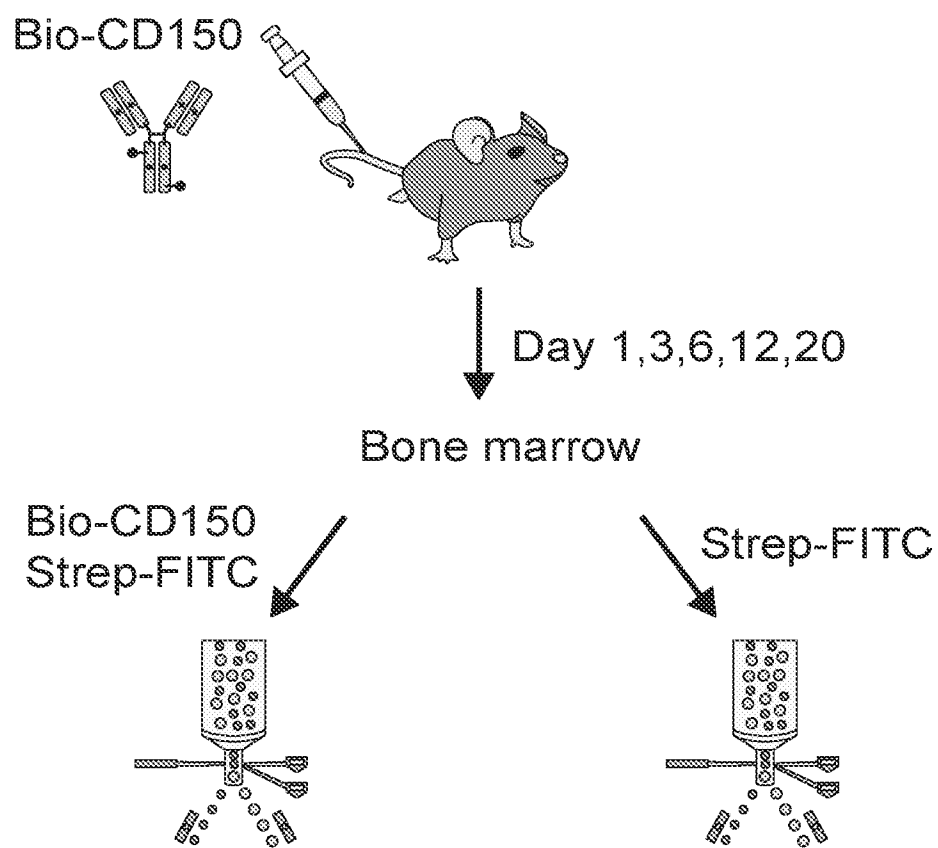


FIG. 18A

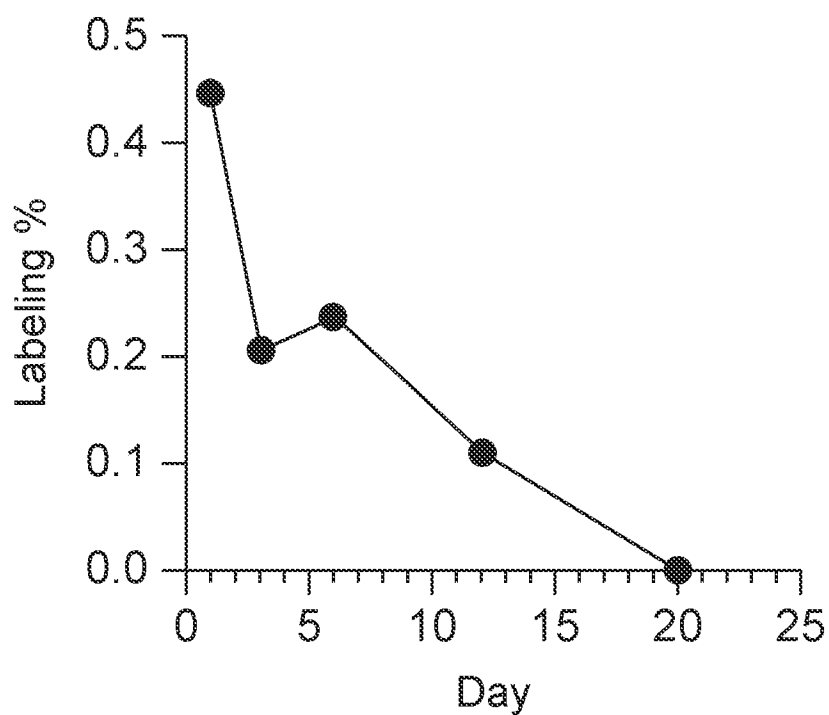


FIG. 18B

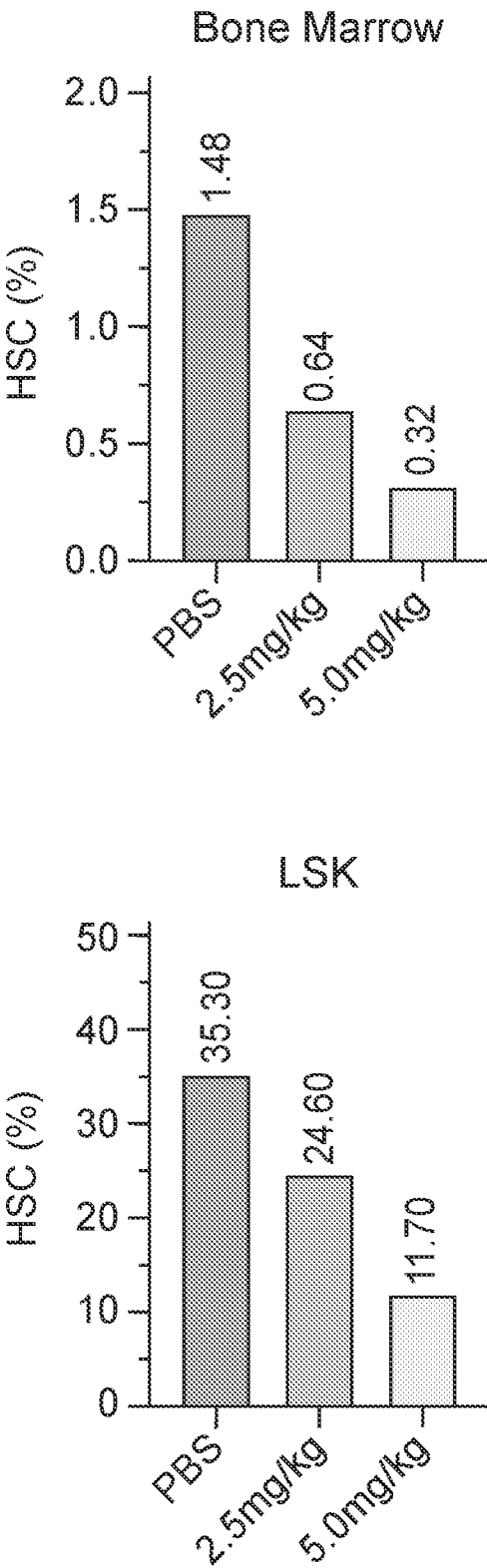


FIG. 18C

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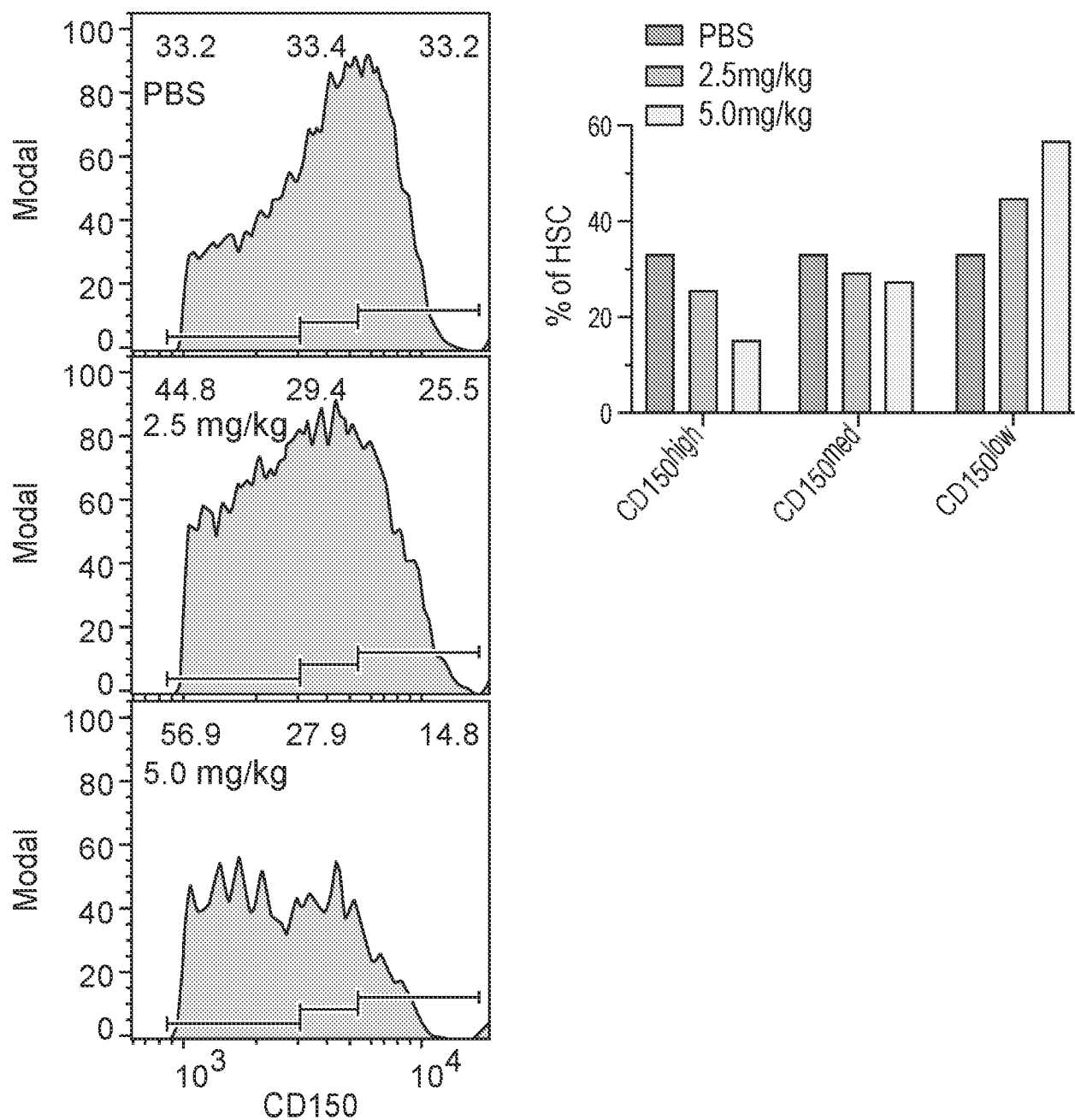


FIG. 18D

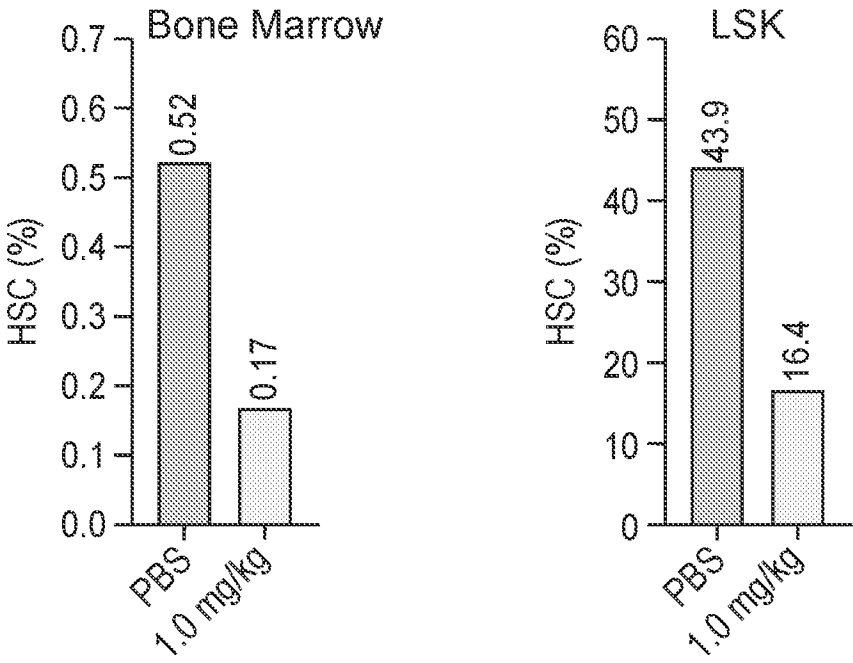


FIG. 19A

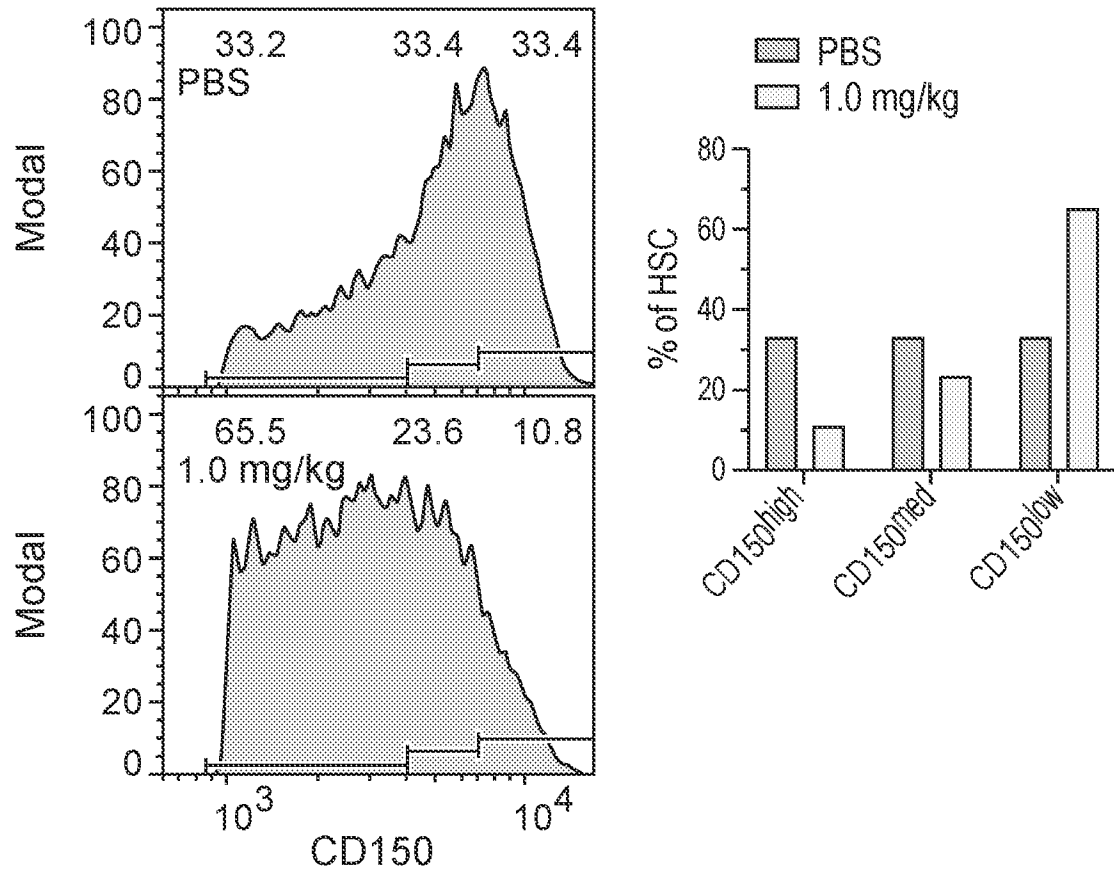


FIG. 19B

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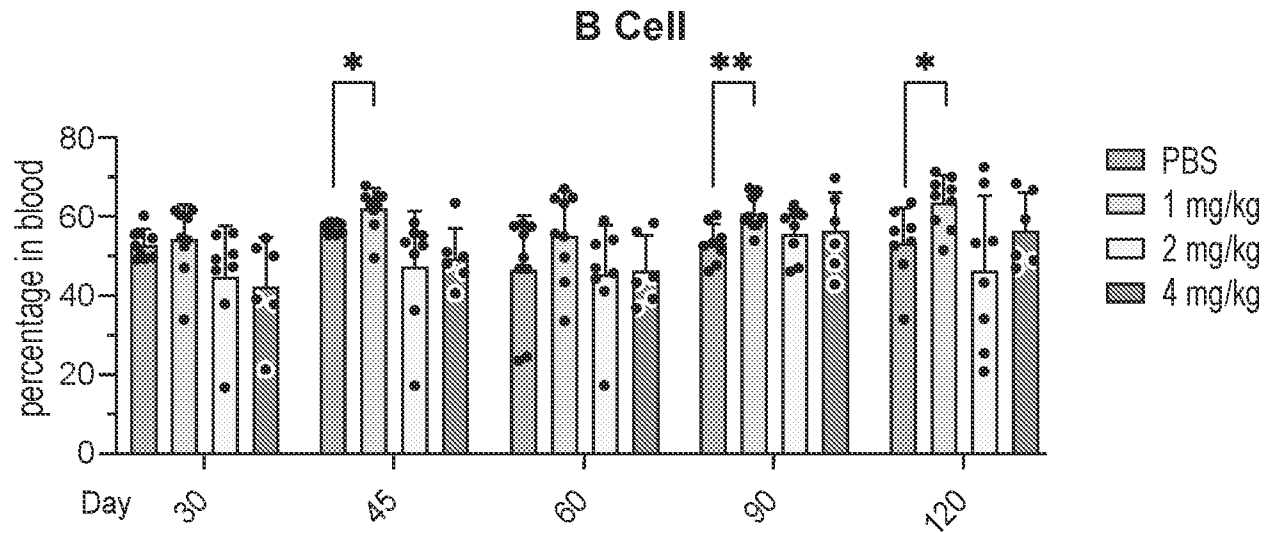


FIG. 19C

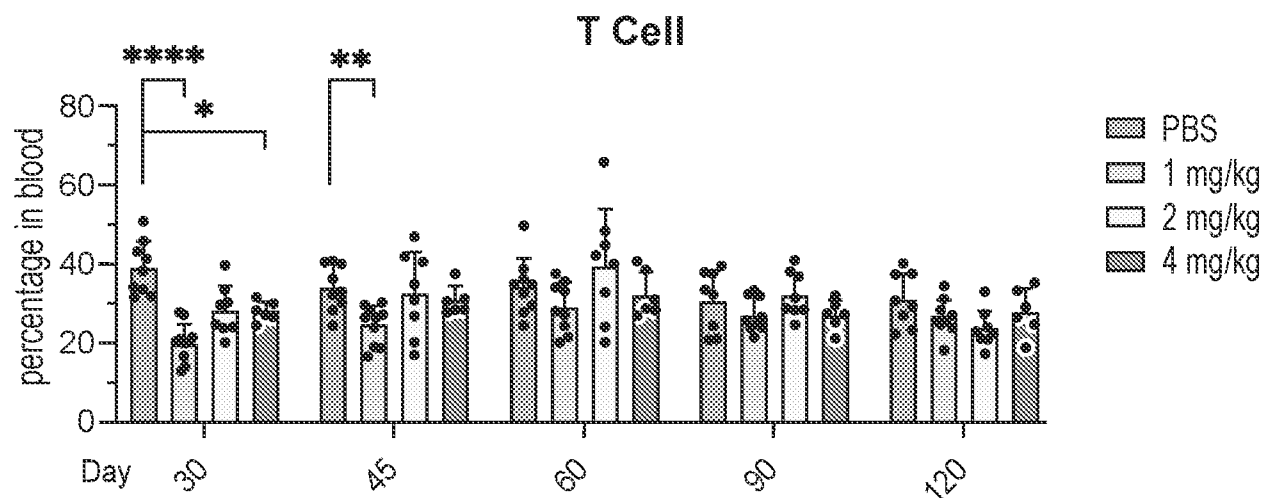


FIG. 19D

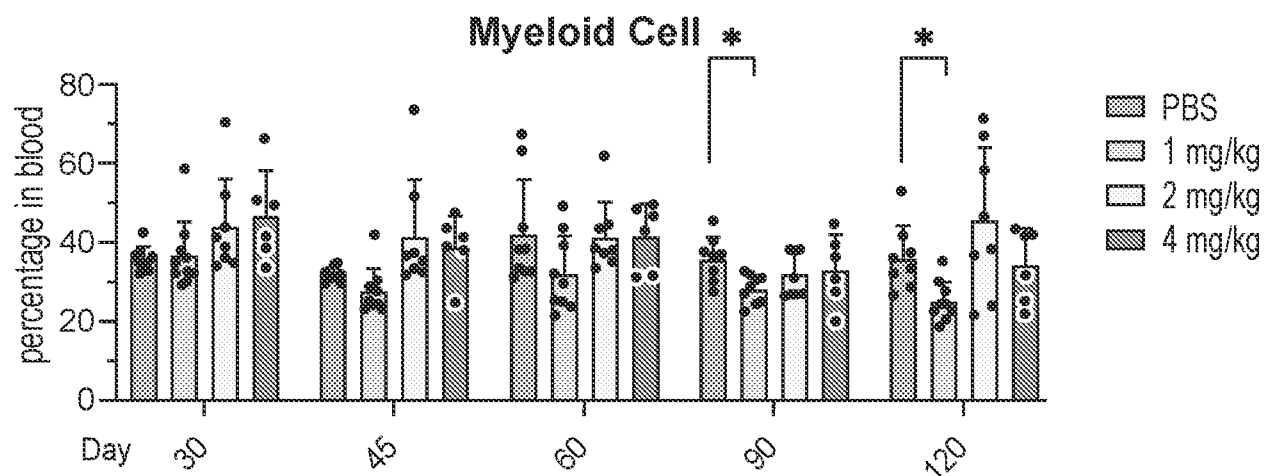


FIG. 19E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033619

A. CLASSIFICATION OF SUBJECT MATTERIPC: **A61K 35/28** (2024.01); **A61P 7/00** (2024.01); **C12N 5/074** (2024.01); **C12N 5/0789** (2024.01)CPC: **A61K 35/28**; **C12N 5/0647**; **A61P 7/00**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/211893 A1 (HACKENSACK MERIDIAN HEALTH CENTER FOR DISCOVERY AND INNOVATION) 21 October 2021 (21.10.2021)	1, 7, 9
Y	entire document	3, 4, 8, 10
A	WO 2021/073610 A1 (SUN YAT-SEN UNIVERSITY) 22 April 2021 (22.04.2021)	1, 3, 4, 7-10
Y	WILSON et al., Combined Single-Cell Functional and Gene Expression Analysis Resolves Heterogeneity within Stem Cell Populations, Cell Stem Cell, Vol. 16, 04 June 2015, Pgs. 712–724	3, 8, 10
Y	JUNG et al., Cellular Mechanisms of Somatic Stem Cell Aging, Curr Top Dev Biol., Vol. 107, 2014, Pgs. 1-26	4

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

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“D” document cited by the applicant in the international application

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

09 August 2024 (09.08.2024)

Date of mailing of the international search report

23 October 2024 (23.10.2024)

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033619

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LUCHSINGER et al., Harnessing Hematopoietic Stem Cell Low Intracellular Calcium Improves Their Maintenance In Vitro, Cell Stem Cell, Vol. 25, 01 August 2019, Pgs. 225–240	1, 3, 4, 7-10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033619

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033619

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **14-23, 27-39, 44, 50-52, 58-73**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Group I: claim 1, 4 and also claims 3 and 7-10 (in-part) are drawn to methods for characterizing hematopoietic stem cell (HSC) functionality.

Group II: claims 2, 5, 6 and also claims 3 and 7-10 (in-part) are drawn to methods for selecting a hematopoietic stem cell (HSC) for engraftment in a subject in need thereof.

Group III: claims 11-13 are drawn to methods of rejuvenating blood components of an aging or aged subject or alleviating aging-related hematopoietic cell functional decline.

Group IV: claims 24-26 are drawn to methods of improving physical health and aging-related hematopoietic cell functional decline of a selected subject.

Group V: claims 40-43, 45 and 46 are drawn to compositions and use of compositions.

Group VI: claims 47-49, 53-57 are drawn to methods of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs).

Group VII: claims 74-76 are drawn to methods kits or articles of manufacture.

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

The special technical features of Group I, methods for characterizing hematopoietic stem cell (HSC) functionality, are not present in Groups II-VII; the special technical features of Group II, methods for selecting a hematopoietic stem cell (HSC) for engraftment in a subject in need thereof, are not present in Groups I and III-VII; the special technical features of Group III, methods of rejuvenating blood components of an aging or aged subject or alleviating aging-related hematopoietic cell functional decline, are not present in Groups I, II, and IV-VII; the special technical features of Group IV, methods of improving physical health and aging-related hematopoietic cell functional decline of a selected subject, are not present in Groups I-III and V-VII; the special technical features of Group V, compositions and use of compositions, are not present in Groups I-IV, VI, and

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

VII; the special technical features of Group VI, methods of reducing or eliminating dysfunctional or defective CD150-expressing hematopoietic stem cells (HSCs), are not present in Groups I-V and VII; and the special technical features of Group VII, kits or articles of manufacture, are not present in Groups I-VI.

Additionally, even if Groups I-VII were considered to share the technical features of a method; detecting the level of CD150 polypeptide or polynucleotide in an HSC; administering to the subject an effective amount of hematopoietic stem cells (HSCs) expressing low levels of CD150 polypeptide (CD150low HSCs) or a composition thereof comprising a physiologically acceptable carrier, excipient or diluent; wherein the CD150 low HSCs differentiate into hematopoietic cell types; composition comprising an effective amount of hematopoietic stem cells (HSCs) expressing a low abundance of the CD150 glycoprotein (CD150low HSCs), wherein the CD150high HSCs express one or more age-related marker genes selected from the group consisting of Sbspon, Ehd3, Clu, Dpyl912, Kcnb2, Selp, B3gnt8, Jam2, Enpp5, Gpr183, and Ramp2; and flow cytometry or fluorescence activated cell sorting (FACS) analysis, these shared technical features do not represent a contribution over the prior art as disclosed by WO 2021/211893 A1 to Hackensack Meridian Health Center for Discovery and Innovation (hereinafter, "Hackensack"), WO 2021/073610 A1 to Sun Yat-Sen University (hereinafter, "Sun") and the publication entitled "Harnessing Hematopoietic Stem Cell Low Intracellular Calcium Improves Their Maintenance In Vitro" by Luchsinger et al. (hereinafter, "Luchsinger").

Hackensack teaches a method (abstract); wherein the CD150high HSCs (HSC gene expression associated with aging in aged HSCs comprise upregulation of SLAMF1; paragraph [0425]) express one or more age-related marker genes selected from the group consisting of Sbspon, Ehd3, Clu, Dpyl912, Kcnb2, Selp, B3gnt8, Jam2, Enpp5, Gpr183, and Ramp2 (HSC gene expression associated with aging in aged HSCs comprise upregulation of one or more of SELP, JAM2, and CLU; paragraph [0425]); a composition thereof comprising a physiologically acceptable carrier, excipient or diluent (paragraph [0469]).

Sun teaches detecting an HSC (detection of cell surface markers using flow cytometry and cell sorting assays; page 10, first paragraph); administering to the subject an effective amount of hematopoietic stem cells (HSCs) (present disclosure relates to a therapeutic composition comprising a cell population with expanded HSCs derived from not more than one or two cord blood units. In some embodiments, the present disclosure relates to a therapeutic composition containing a total amount of cells of at least about 10^5 , at least about 10^6 , at least about 10^7 , at least about 10^8 or at least about 10^9 cells with about 20% to about 100%, for example between about 43% to about 80%, of total cells being CD34+ cells, page 109, fourth paragraph; A dose of the expanded hematopoietic stem cell composition of the disclosure is deemed to have achieved a therapeutic benefit if it alleviates a sign or a symptom of the disease, page 111, third paragraph); and a composition comprising an effective amount of hematopoietic stem cells (HSCs) (present disclosure relates to a therapeutic composition comprising a cell population with expanded HSCs derived from not more than one or two cord blood units. In some embodiments, the present disclosure relates to a therapeutic composition containing a total amount of cells of at least about 10^5 , at least about 10^6 , at least about 10^7 , at least about 10^8 or at least about 10^9 cells with about 20% to about 100%, for example between about 43% to about 80%, of total cells being CD34+ cells, page 109, fourth paragraph; A dose of the expanded hematopoietic stem cell composition of the disclosure is deemed to have achieved a therapeutic benefit if it alleviates a sign or a symptom of the disease, page 111, third paragraph); and flow cytometry or fluorescence activated cell sorting (FACS) analysis (detection of cell surface markers using flow cytometry and cell sorting assays; page 10, first paragraph).

Luchsinger teaches expressing low levels of CD150 polypeptide (CD150low HSCs) (CD150lo HSC; page 228, second column, second paragraph), wherein the CD150 low HSCs differentiate into hematopoietic cell types (CD150lo HSC provided more lymphoid-biased repopulation; page 228, second column, second paragraph).

The inventions listed in Groups I-VII therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

INTERNATIONAL SEARCH REPORT

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1, 3, 4, 7-10**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.