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Canadian Intellectual Property Office

CA 3213384 A1 2022/11/10

(21) **3 213 384**

(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2022/05/02
(87) **Date publication PCT/PCT Publication Date:** 2022/11/10
(85) **Entrée phase nationale/National Entry:** 2023/09/25
(86) **N° demande PCT/PCT Application No.:** EP 2022/061666
(87) **N° publication PCT/PCT Publication No.:** 2022/233767
(30) **Priorité/Priority:** 2021/05/05 (EP21172290.5)

(51) **Cl.Int./Int.Cl.** ***A61K 31/37*** (2006.01), ***A61P 37/04*** (2006.01), ***A61P 7/04*** (2006.01), ***A61P 7/06*** (2006.01)

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(54) **Titre : UROLITHINE POUR AUGMENTER LA FONCTION DE CELLULES SOUCHES**
(54) **Title: UROLITHIN FOR INCREASING STEM CELL FUNCTION**

(57) **Abrégé/Abstract:**
Use of a urolithin for increasing stem cell function in a population of haematopoietic stem and/or progenitor cells (HSPCs), wherein the stem cell function is increased for at least 40 weeks.

Date Submitted: 2023/09/25

CA App. No.: 3213384

Abstract:

Use of a urolithin for increasing stem cell function in a population of haematopoietic stem and/or progenitor cells (HSPCs), wherein the stem cell function is increased for at least 40 weeks.

UROLITHIN FOR INCREASING STEM CELL FUNCTION

FIELD OF THE INVENTION

The invention relates to agents and methods for increasing stem cell function in haematopoietic stem and progenitor cells (HSPCs), for example increasing engraftment by a population of HSPCs, and/or increasing capacity for self-renewal and differentiation. In particular, the invention relates to a long term increase in stem cell function.

BACKGROUND TO THE INVENTION

The haematopoietic system is a complex hierarchy of cells of different mature cell lineages. These include cells of the immune system that offer protection from pathogens, cells that carry oxygen through the body and cells involved in wound healing. All these mature cells are derived from a pool of haematopoietic stem cells (HSCs) that are capable of self-renewal and differentiation into any blood cell lineage.

HSCs differ from their committed progeny by relying primarily on anaerobic glycolysis rather than mitochondrial oxidative phosphorylation for energy production (Simsek, T. et al. (2010) Cell Stem Cell 7: 380-90; Takubo, K. et al. (2013) Cell Stem Cell 12: 49-61; Vannini, N. et al. (2016) Nat Commun 7: 13125; Yu, W.M. et al. (2013) Cell Stem Cell 12: 62-74). This distinct metabolic state is believed to protect the HSCs from cellular damage inflicted by reactive oxygen species (ROS) in active mitochondria, thereby maintaining the cells' long-term in vivo function (Chen, C. et al. (2008) J Exp Med 205: 2397-408; Ito, K. et al. (2004) Nature 431: 997-1002; Ito, K. et al. (2006) Nat Med 12: 446-51; Tothova, Z. et al. (2007) Cell 128: 325-39).

Mitochondrial membrane potential, indicated by tetramethylrhodamine methyl ester (TMRM) fluorescence, has previously been used as a surrogate for the metabolic state of cells, and it has been demonstrated that phenotypically defined HSCs have lower mitochondrial membrane potential compared to progenitors (Vannini, N. et al. (2016) Nat Commun 7: 13125). In the same study it was found that artificial lowering of mitochondrial membrane potential, by chemical uncoupling of the mitochondrial electron transport chain, forces the HSCs to maintain their functionality under culture conditions that normally induce rapid differentiation (Vannini, N. et al. (2016) Nat Commun 7: 13125). Importantly, similar mechanisms were observed in human HSCs where artificial lowering of mitochondrial membrane potential by supplementing the culture media with nicotinamide riboside (an NAD and vitamin B3 precursor) resulted in significantly higher levels of engraftment and were capable of sustaining long-term blood production in both primary and secondary recipient humanised mice.

However, there remains a significant need for additional approaches that increase stem cell function in HSCs in vivo and in vitro in the long term, in particular approaches that increase engraftment by a population of HSPCs (e.g. during a haematopoietic stem cell transplant procedure), and increase capacity for self-renewal and differentiation by HSCs.

5 **SUMMARY OF THE INVENTION**

The inventors observed that urolithin A (UroA) ameliorates haematopoietic stem cell (HSC) function, such as through increasing engraftment and self-renewal.

In addition, the inventors have observed, for example through serial transplantation studies, that UroA treatment of HSPCs may provide for a long term increase in stem cell function. In
10 particular, the inventors have found that increased stem cell function may be sustained for at least 40 weeks.

While not wishing to be bound by theory, the inventors' studies indicate that a long term increase in stem cell function may be achieved using a relatively short exposure of HSPCs to UroA through an effect on the epigenetic signature of the cells.

15 In one aspect the invention provides use of a urolithin for increasing stem cell function in a population of haematopoietic stem and/or progenitor cells (HSPCs), wherein the stem cell function is increased for at least 40 weeks.

In some embodiments, the use is in vitro use. In some embodiments, the use is ex vivo use.

In another aspect the invention provides a method for increasing stem cell function in a
20 population of haematopoietic stem and/or progenitor cells (HSPCs) comprising contacting the population with a urolithin, wherein the stem cell function is increased for at least 40 weeks.

In some embodiments, the stem cell function is increased for at least 41 weeks. In some embodiments, the stem cell function is increased for at least 42 weeks. In some embodiments, the stem cell function is increased for at least 43 weeks.

25 In preferred embodiments, the stem cell function is increased for at least 44 weeks.

In some embodiments, the population is an isolated population of HSPCs.

In some embodiments, the HSPCs have a CD34+ phenotype.

In some embodiments, the HSPCs have a CD34+CD38- phenotype.

In some embodiments, the method comprises the steps:

(a) providing a population of HSPCs;

(b) optionally isolating a sub-population of HSPCs characterised by low mitochondrial membrane potential; and

(c) contacting the population of (a) or the sub-population of (b) with the urolithin.

In another aspect the invention provides a urolithin for use in a method of therapy by increasing stem cell function in haematopoietic stem and/or progenitor cells (HSPCs), wherein the stem cell function is increased for at least 40 weeks.

In some embodiments, the urolithin is for use in increasing haematopoietic stem cell function in a subject.

In some embodiments, the method comprises contacting the HSPCs with the urolithin prior to administration of the HSPCs to a subject.

In some embodiments, the method comprises administering the urolithin to a subject.

In some embodiments, the urolithin is administered to a subject enterally or parenterally, preferably enterally. In preferred embodiments, the urolithin is administered to a subject orally.

In some embodiments, the method of therapy is treatment or prevention of (a) anaemia, leukopenia and/or thrombocytopenia; (b) an infection; and/or (c) cancer.

In some embodiments, the method of therapy is treatment or prevention of anaemia, leukopenia and/or thrombocytopenia. In some embodiments, the method of therapy is treatment or prevention of an infection. In some embodiments, the method of therapy is treatment or prevention of cancer.

In some embodiments, the cancer is a haematological cancer. In some embodiments, the cancer is leukaemia, lymphoma or myeloma.

In some embodiments, the stem cell function comprises one or more of: capacity for engraftment; self-renewal; and differentiation of blood and immune cell production.

In some embodiments, the stem cell function comprises capacity for engraftment. In some embodiments, the stem cell function comprises self-renewal. In some embodiments, the stem cell function comprises differentiation of blood and immune cell production.

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In some embodiments, the stem cell function is capacity for engraftment. In some embodiments, the stem cell function is self-renewal. In some embodiments, the stem cell function is differentiation of blood and immune cell production.

In some embodiments, the increased stem cell function increases blood cell levels in a subject.

5 In preferred embodiments, the urolithin is urolithin A.

In some embodiments, the population or sub-population of HSPCs is contacted with the urolithin for up to and including 7 days.

In some embodiments, the population or sub-population of HSPCs is contacted with the urolithin for 1-3 days. In some embodiments, the population or sub-population of HSPCs is 10 contacted with the urolithin for 1-5 days. In some embodiments, the population or sub-population of HSPCs is contacted with the urolithin for 1-7 days.

In some embodiments, the population or sub-population of HSPCs is contacted with the urolithin for 3-7 days. In some embodiments, the population or sub-population of HSPCs is contacted with the urolithin for 5-7 days.

15 In some embodiments, the population or sub-population of HSPCs is contacted with the urolithin for 3-5 days.

In some embodiments, the urolithin is in the form of a pharmaceutical or nutritional composition.

In some embodiments, the urolithin is in the form of a food product, food supplement, 20 nutraceutical, food for special medical purpose (FSMP), nutritional supplement, dairy-based drink, low-volume liquid supplement or meal replacement beverage.

In some embodiments, a subject has or is at risk of having subnormal amounts of haematopoietic cells, for example erythrocytes, leukocytes and/or platelets.

In some embodiments, a subject has or is at risk of having anaemia, leukopenia and/or 25 thrombocytopenia.

In some embodiments, a subject has undergone an intervention selected from the group consisting of a haematopoietic stem cell transplant; a bone marrow transplant; myeloablative conditioning; chemotherapy; radiotherapy; and surgery.

In some embodiments, a subject is an immune-compromised subject.

In some embodiments, the subject is 3-4 weeks post-intervention.

In some embodiments, a subject is a human or non-human mammal, preferably a human, optionally a human adult, child or infant.

In some embodiments, the urolithin is in a combined preparation for simultaneous, separate or sequential use with an agent selected from the group consisting of nicotinamide riboside, a G-CSF analogue, a TPO receptor analogue, SCF, TPO, Flt3-L, FGF-1, IGF1, IGFBP2, IL-3, IL-6, G-CSF, M-CSF, GM-CSF, EPO and combinations thereof.

In preferred embodiments, the urolithin is in a combined preparation for simultaneous, separate or sequential use with nicotinamide riboside.

In another aspect, the invention provides a method of expanding an isolated population of haematopoietic stem and/or progenitor cells (HSPCs) comprising contacting the population with a urolithin, wherein stem cell function of the HSPCs is increased for at least 40 weeks.

In some embodiments, the contacting comprises culturing the population in the presence of the urolithin.

In some embodiments, the method comprises the steps:

(a) providing a population of HSPCs;

(b) optionally culturing the population of HSPCs, preferably in a HSPC expansion or maintenance culture medium;

(c) optionally isolating a sub-population of HSPCs characterised by low mitochondrial membrane potential; and

(d) contacting the population of (a) or (b), or the sub-population of (c) with a urolithin.

In some embodiments, the population provided in step (a) is obtained from bone marrow, mobilised peripheral blood or umbilical cord blood.

In some embodiments, the product of step (d) is enriched in cells having long-term multi-lineage blood reconstitution capability.

In another aspect, the invention provides a population of haematopoietic stem and/or progenitor cells (HSPCs) obtainable by the method of the invention.

In another aspect, the invention provides a pharmaceutical composition comprising the population of haematopoietic stem and/or progenitor cells (HSPCs) of the invention.

In another aspect, the invention provides a method of engrafting a subject with haematopoietic stem and/or progenitor cells (HSPCs) comprising contacting an isolated population of HSPCs with a urolithin, and administering the population of HSPCs to the subject in need thereof, wherein stem cell function of the HSPCs is increased for at least 40 weeks.

In another aspect, the invention provides a method of increasing haematopoietic stem cell function comprising contacting a population of haematopoietic stem and/or progenitor cells (HSPCs) with a urolithin, wherein the stem cell function is increased for at least 40 weeks.

In another aspect, the invention provides a method of increasing haematopoietic stem cell function in a subject comprising contacting a population of haematopoietic stem and/or progenitor cells (HSPCs) with a urolithin, and administering the population of HSPCs to the subject in need thereof, wherein the stem cell function is increased for at least 40 weeks.

In another aspect the invention provides a method of increasing capacity for engraftment by a population of haematopoietic stem and/or progenitor cells (HSPCs) comprising contacting the population of HSPCs with a urolithin, wherein the capacity for engraftment and blood reconstitution capability are increased for at least 40 weeks. In another aspect the invention provides a method of increasing haematopoietic stem cell self-renewal comprising contacting a population of haematopoietic stem and/or progenitor cells (HSPCs) with a urolithin, wherein the stem cell self-renewal is increased for at least 40 weeks. In another aspect the invention provides a method of increasing haematopoietic stem cell differentiation comprising contacting a population of haematopoietic stem and/or progenitor cells (HSPCs) with a urolithin, wherein the stem cell differentiation is increased for at least 40 weeks. In some embodiments, the engraftment, self-renewal and/or differentiation are increased in a subject and the method further comprises administering the population of HSPCs to the subject in need thereof.

In some embodiments, the method is an ex vivo method. In some embodiments, the method is an in vivo method.

In some embodiments, the population is an isolated population of HSPCs.

In another aspect, the invention provides a method of increasing haematopoietic stem cell function comprising administering a urolithin to a subject in need thereof, wherein the stem cell function is increased for at least 40 weeks.

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In another aspect, the invention provides a method of increasing haematopoietic stem cell engraftment comprising administering a urolithin to a subject in need thereof, wherein the engraftment is increased for at least 40 weeks. In another aspect the invention provides a method of increasing haematopoietic stem cell self-renewal comprising administering a urolithin to a subject in need thereof, wherein the stem cell self-renewal is increased for at least 40 weeks. In another aspect the invention provides a method of increasing haematopoietic stem cell differentiation comprising administering a urolithin to a subject in need thereof, wherein the stem cell differentiation is increased for at least 40 weeks.

DESCRIPTION OF THE DRAWINGS

FIGURE 1

UroA induces lowering of mitochondrial membrane potential. A) Bone marrow derived murine HSCs cultured in basal media (control) supplemented with various concentrations of UroA. The proportion of cells in the TMRM low gate increases and the MFI TMRM decreases in a dose-dependent manner. Mitochondrial mass (measured by Mitotracker) decreases with increasing concentration of UroA in culture. B) Human cord blood derived HSPCs cultured in basal media (control) with various concentrations of UroA for 7 days. FACS analysis shows lowering of TMRM signal at all three time points [Day 3 (top), 5 (middle) and 7 (lower)]. The proportion of cells in the CD34+ TMRM low gate increases while MFI TMRM decreases in a dose dependent manner.

FIGURE 2

In vitro UroA treatment enhances mHSC and hHSPC in vivo function. A) HSCs were isolated from bone marrow of mice, and cultured in basal media with or without 20 μM UroA. At the end of the culture period cells were transferred into lethally irradiated recipient mice via intra venous tail injections. Mice injected with UroA-cultured cells show higher blood reconstitution over a period of 24 weeks. The increase is also reflected in the myeloid and lymphoid lineages. B) Human cord blood derived HSPCs were cultured in basal media with or without 50 μM UroA. Two functional assays were carried out. Five days post culture cells were injected in irradiated NSG-SGM3 new born pups. Seven days post culture cells were plated in methylcellulose plates to estimate their colony forming capacity (CFU assay). C) UroA treated cells produced significantly higher number of colonies as compared to the control (Ctrl) group after 15 days of methylcellulose culture. D) Mice transplanted with UroA-treated cells show a significant increase of human cell chimerism in the peripheral blood. E) UroA treatment increases blood cell counts primarily in the human lymphoid lineage (T and B cells).

FIGURE 3

UroA drives the expression of metabolic genes in mHSCs. A) QPCR analysis carried out on bone marrow derived mHSCs cultured in basal media with or without 20 µM UroA. Higher expression of mito/autophagy, glycolytic and ROS protection genes was found in UroA treated cells.

FIGURE 4

UroA treatment improves survival of recipient mice post transplantation. A) Human cord blood derived HSPCs were cultured in basal media with or without 50uM UroA. Three days post culture cells were counted and limiting dose (40,000 cells) was injected in each irradiated recipient adult NSG mouse and survival was monitored over several months. 12 mice each for Control and UroA condition were transplanted. B) Mice transplanted with UroA treated cells had significantly improved survival over a period of eight months.

FIGURE 5

Serial transplantation analysis demonstrates in vitro UroA treatment enhances HSC in vivo function in the long term. (Fig. 5a) HSCs were isolated from bone marrow of mice, and cultured in basal media with or without 20 µM UroA. At the end of the culture period cells were transferred into a lethally irradiated recipient first mouse via intravenous tail injection. Blood analysis was carried out over a period of 24 weeks (Fig. 5b), followed by analysis of spleen (Fig. 5d) and bone marrow (Fig. 5e) samples. Bone marrow of the first mouse and transferred into a second lethally irradiated recipient mouse via intravenous tail injection. Blood analysis was carried out over a period of 20 weeks (Fig. 5c), followed by analysis of spleen (Fig. 5f) and bone marrow (Fig. 5g) samples. UroA-cultured cells show higher blood reconstitution over a total period of at least 44 weeks. The increase is also reflected in the myeloid and lymphoid lineages.

FIGURE 6

Gene expression analysis of UroA-treated mouse HSCs. (Fig. 6a) RNA sequencing analysis was carried out on HSCs after a short ex vivo UroA treatment. (Fig. 6b) Gel electrophoresis and fragment analyser analysis. (Fig. 6c) Multi-dimensional scaling (MDS) plot of the RNA sequencing data, and differential expression analysis. (Fig. 6d) Analysis of biological pathways altered by UroA treatment. (Fig. 6e) Differential expression analysis of mitochondrial genes.

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DETAILED DESCRIPTION OF THE INVENTION

The terms “comprising”, “comprises” and “comprised of” as used herein are synonymous with “including” or “includes”; or “containing” or “contains”, and are inclusive or open-ended and do
5 not exclude additional, non-recited members, elements or steps. The terms “comprising”, “comprises” and “comprised of” also include the term “consisting of”.

Haematopoietic stem cells

A stem cell is able to differentiate into many cell types. A cell that is able to differentiate into all cell types is known as totipotent. In mammals, only the zygote and early embryonic cells
10 are totipotent. Stem cells are found in most, if not all, multicellular organisms. They are characterised by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialised cell types. The two broad types of mammalian stem cells are embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in adult tissues. In a developing embryo, stem cells can differentiate
15 into all of the specialised embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialised cells, but also maintaining the normal turnover of regenerative organs, such as blood, skin or intestinal tissues.

Haematopoietic stem cells (HSCs) are multipotent stem cells that may be found, for example, in peripheral blood, bone marrow and umbilical cord blood. HSCs are capable of self-renewal
20 and differentiation into any blood cell lineage. They are capable of recolonising the entire immune system, and the erythroid and myeloid lineages in all the haematopoietic tissues (such as bone marrow, spleen and thymus). They provide for life-long production of all lineages of haematopoietic cells.

Haematopoietic progenitor cells have the capacity to differentiate into a specific type of cell.
25 In contrast to stem cells however, they are already far more specific: they are pushed to differentiate into their “target” cell. A difference between stem cells and progenitor cells is that stem cells can replicate indefinitely, whereas progenitor cells can only divide a limited number of times. Haematopoietic progenitor cells can be rigorously distinguished from HSCs only by functional in vivo assay (i.e. transplantation and demonstration of whether they can give rise
30 to all blood lineages over prolonged time periods).

A differentiated cell is a cell which has become more specialised in comparison to a stem cell or progenitor cell. Differentiation occurs during the development of a multicellular organism as the organism changes from a single zygote to a complex system of tissues and cell types.

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Differentiation is also a common process in adults: adult stem cells divide and create fully-differentiated daughter cells during tissue repair and normal cell turnover. Differentiation dramatically changes a cell's size, shape, membrane potential, metabolic activity and responsiveness to signals. These changes are largely due to highly-controlled modifications
5 in gene expression. In other words a differentiated cell is a cell which has specific structures and performs certain functions due to a developmental process which involves the activation and deactivation of specific genes. Here, a differentiated cell includes differentiated cells of the haematopoietic lineage such as monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells, T-cells, B-cells and NK-
10 cells. For example, differentiated cells of the haematopoietic lineage can be distinguished from stem cells and progenitor cells by detection of cell surface molecules which are not expressed or are expressed to a lesser degree on undifferentiated cells. Examples of suitable human lineage markers include CD33, CD13, CD14, CD15 (myeloid), CD19, CD20, CD22, CD79a (B), CD36, CD71, CD235a (erythroid), CD2, CD3, CD4, CD8 (T), CD56 (NK).

15 *HSC source*

In some embodiments, haematopoietic stem cells are obtained from a tissue sample.

For example, HSCs can be obtained from adult and foetal peripheral blood, umbilical cord blood, bone marrow, liver or spleen. They may be obtained after mobilisation of the cells in vivo by means of growth factor treatment.

20 Mobilisation may be carried out using, for example, G-CSF, plerixaphor or combinations thereof. Other agents, such as NSAIDs, CXCR2 ligands (Grobeta) and dipeptidyl peptidase inhibitors may also be useful as mobilising agents.

With the availability of the stem cell growth factors GM-CSF and G-CSF, most haematopoietic stem cell transplantation procedures are now performed using stem cells collected from the
25 peripheral blood, rather than from the bone marrow. Collecting peripheral blood stem cells provides a bigger graft, does not require that the donor be subjected to general anaesthesia to collect the graft, results in a shorter time to engraftment and may provide for a lower long-term relapse rate.

Bone marrow may be collected by standard aspiration methods (either steady-state or after
30 mobilisation), or by using next-generation harvesting tools (e.g. Marrow Miner).

In addition, HSCs may be derived from induced pluripotent stem cells.

HSC characteristics

HSCs are typically of low forward scatter and side scatter profile by flow cytometric procedures. Some are metabolically quiescent, as demonstrated by Rhodamine labelling which allows determination of mitochondrial activity. HSCs may comprise certain cell surface markers such as CD34, CD45, CD133, CD90 and CD49f. They may also be defined as cells lacking the expression of the CD38 and CD45RA cell surface markers. However, expression of some of these markers is dependent upon the developmental stage and tissue-specific context of the HSC. Some HSCs called “side population cells” exclude the Hoechst 33342 dye as detected by flow cytometry. Thus, HSCs have descriptive characteristics that allow for their identification and isolation.

Negative markers

CD38 is the most established and useful single negative marker for human HSCs.

Human HSCs may also be negative for lineage markers such as CD2, CD3, CD14, CD16, CD19, CD20, CD24, CD36, CD56, CD66b, CD271 and CD45RA. However, these markers may need to be used in combination for HSC enrichment.

By “negative marker”, it is to be understood that human HSCs lack the expression of these markers.

Positive markers

CD34 and CD133 are the most useful positive markers for HSCs.

Some HSCs are also positive for lineage markers such as CD90, CD49f and CD93. However, these markers may need to be used in combination for HSC enrichment.

By “positive marker”, it is to be understood that human HSCs express these markers.

In some embodiments, the HSCs have a CD34+ phenotype.

In some embodiments, the HSCs have a CD34+CD38- phenotype.

Further separations may be carried out to obtain, for example, CD34+CD38-CD45RA-CD90+CD49f+ cells.

Stem cell function

The term “stem cell function” as used herein refers to characteristics of a cell that are typically associated with a stem cell, for example the ability to engraft, the ability differentiate into specific cellular lineages and/or the ability to self renew.

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The term "engraftment" as used herein refers to the ability of the haematopoietic stem and/or progenitor cells to populate and survive in a subject following their transplantation, i.e. in the short and/or long term after transplantation. For example, engraftment may refer to the number and/or percentages of haematopoietic cells descended from the transplanted haematopoietic stem and/or progenitor cells (e.g. graft-derived cells) that are detected about 1 day to 24 weeks, 1 day to 10 weeks, or 1-30 days or 10-30 days after transplantation. In some embodiments, engraftment is assessed at about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or 30 days after transplantation. In other embodiments, engraftment is assessed at about 4, 5, 6, 7, 8, 9, 10 ,11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 weeks after transplantation. In other embodiments, engraftment is assessed at about 16-24 weeks, preferably 20 weeks, after transplantation.

Engraftment may be readily analysed by the skilled person. For example, the transplanted haematopoietic stem and/or progenitor cells may be engineered to comprise a marker (e.g. a reporter protein, such as a fluorescent protein), which can be used to quantify the graft-derived cells. Samples for analysis may be extracted from relevant tissues and analysed ex vivo (e.g. using flow cytometry).

The term "self renewal" as used herein refers to the ability of a cell to undergo multiple cycles of cell division while maintaining an undifferentiated state.

Cell numbers and/or percentages in certain states (e.g. live, dead or apoptotic cells) may be quantified using any of a number of methods known in the art, including use of haemocytometers, automated cell counters, flow cytometers and fluorescence activated cell sorting machines. These techniques may enable distinguishing between live, dead and/or apoptotic cells. In addition or in the alternative, apoptotic cells may be detected using readily available apoptosis assays (e.g. assays based on the detection of phosphatidylserine (PS) on the cell membrane surface, such as through use of Annexin V, which binds to exposed PS; apoptotic cells may be quantified through use of fluorescently-labelled Annexin V), which may be used to complement other techniques.

Haematopoietic stem and/or progenitor cells, and cells differentiated therefrom may be identified and/or quantified using the characteristics and/or markers (e.g. CD34 and CD38) disclosed herein.

"Increased stem cell function" may refer to an increase in the stem cell function, for example the capacity for engraftment, self-renewal and/or differentiation, compared to the stem cell function in the absence of the urolithin. Stem cell function may be readily analysed by the

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skilled person, for example using the methods disclosed herein (e.g. disclosed in the Examples).

Stem cell function (e.g. self-renewal and/or differentiation) may be determined using a Colony Forming Unit (CFU) assay, such as disclosed in the Examples herein. For example, experiments may be conducted in which populations of HSPCs are cultured in the presence or absence of the urolithin, but under otherwise substantially identical conditions, before conducting a CFU assay on each of the populations of HSPCs. Levels of stem cell function may be determined by analysing the number of colonies in each CFU assay.

Stem cell function (e.g. capacity for engraftment, self-renewal and/or differentiation) may be determined using an in vivo transplantation assay, such as disclosed in the Examples herein. For example, experiments may be conducted in which populations of human HSPCs are cultured in the presence or absence of the urolithin, but under otherwise substantially identical conditions, before the populations of HSPCs are transplanted into irradiated mice. Engraftment may be determined by analysing the number of human cells in the mice, for example as disclosed herein. Self-renewal and/or differentiation may be determined by analysing blood reconstitution levels, in particular blood reconstitution levels over time, for example as disclosed herein. Blood may be further analysed for levels of particular blood cell lineages.

The increased stem cell function (e.g. capacity for engraftment, self-renewal and/or differentiation) may be an increase of the stem cell function of at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400% or 500% compared to the stem cell function in the absence of the urolithin. The increased stem cell function may be an increase of the stem cell function of at least about 0.5-fold, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold compared to the stem cell function in the absence of the urolithin.

The increased stem cell function for a period of time (e.g. at least 40 weeks, 41 weeks, 42 weeks, 43 weeks, preferably at least 44 weeks) may be determined by analysing the stem cell function through blood chimerism analysis over the period of time. For example, in vivo transplantation assays may be carried out in which the stem cell function as disclosed herein is analysed for a relevant period of time. The in vivo transplantation assay may be a primary transplantation assay, for example in which a population of HSPCs is transplanted into a mouse, which is subsequently analysed as disclosed herein. For carrying out analyses over longer periods of time, the in vivo transplantation assay may be a serial transplantation assay, for example in which a population of HSPCs is transplanted into a first mouse, which is

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subsequently analysed over a first period of time; followed by extraction of a population of HSPCs from the first mouse and transplantation of the extracted population of HSPCs into a second mouse, which is subsequently analysed over a second period of time. The total of the first and second periods of time may lead to a longer period of time over which stem cell function may be analysed than might be achievable using a primary transplantation assay alone, for example.

Isolation and enrichment of populations of cells

Populations of cells, such as haematopoietic stem and/or progenitor cells (HSPCs), are disclosed herein. In some embodiments, the population of cells is an isolated population of cells.

The term "isolated population" as used herein refers to a population of cells that is not comprised within the body. An isolated population of cells may have been previously removed from a subject. An isolated population of cells may be cultured and manipulated ex vivo or in vitro using standard techniques known in the art. An isolated population of cells may later be reintroduced into a subject. Said subject may be the same subject from which the cells were originally isolated or a different subject.

A population of cells may be purified selectively for cells that exhibit a specific phenotype or characteristic, and from other cells which do not exhibit that phenotype or characteristic, or exhibit it to a lesser degree. For example, a population of cells that expresses a specific marker (such as CD34) may be purified from a starting population of cells. Alternatively, or in addition, a population of cells that does not express another marker (such as CD38) may be purified.

The term "enriching" as used herein refers to an increase in the concentration of a type of cells within a population. The concentration of other types of cells may be concomitantly reduced.

Purification or enrichment may result in the population of cells being substantially pure of other types of cell.

Purifying or enriching for a population of cells expressing a specific marker (e.g. CD34 or CD38) may be achieved by using an agent that binds to that marker, preferably substantially specifically to that marker.

An agent that binds to a cellular marker may be an antibody, for example an anti-CD34 or anti-CD38 antibody.

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The term "antibody" as used herein refers to complete antibodies or antibody fragments capable of binding to a selected target, and including Fv, ScFv, F(ab') and F(ab')$_2$, monoclonal and polyclonal antibodies, engineered antibodies including chimeric, CDR-grafted and humanised antibodies, and artificially selected antibodies produced using phage display or
5 alternative techniques.

In addition, alternatives to classical antibodies may also be used in the invention, for example "avibodies", "avimers", "anticalins", "nanobodies" and "DARPins".

The agents that bind to specific markers may be labelled so as to be identifiable using any of a number of techniques known in the art. The agent may be inherently labelled, or may be
10 modified by conjugating a label thereto. By "conjugating" it is to be understood that the agent and label are operably linked. This means that the agent and label are linked together in a manner which enables both to carry out their function (e.g. binding to a marker, allowing fluorescent identification, or allowing separation when placed in a magnetic field) substantially unhindered. Suitable methods of conjugation are well known in the art and would be readily
15 identifiable by the skilled person.

A label may allow, for example, the labelled agent and any cell to which it is bound to be purified from its environment (e.g. the agent may be labelled with a magnetic bead or an affinity tag, such as avidin), detected or both. Detectable markers suitable for use as a label include fluorophores (e.g. green, cherry, cyan and orange fluorescent proteins) and peptide tags (e.g.
20 His tags, Myc tags, FLAG tags and HA tags).

A number of techniques for separating a population of cells expressing a specific marker are known in the art. These include magnetic bead-based separation technologies (e.g. closed-circuit magnetic bead-based separation), flow cytometry, fluorescence-activated cell sorting (FACS), affinity tag purification (e.g. using affinity columns or beads, such as biotin columns
25 to separate avidin-labelled agents) and microscopy-based techniques.

It may also be possible to perform the separation using a combination of different techniques, such as a magnetic bead-based separation step followed by sorting of the resulting population of cells for one or more additional (positive or negative) markers by flow cytometry.

Clinical grade separation may be performed, for example, using the CliniMACS® system
30 (Miltenyi). This is an example of a closed-circuit magnetic bead-based separation technology.

It is also envisaged that dye exclusion properties (e.g. side population or rhodamine labelling) or enzymatic activity (e.g. ALDH activity) may be used to enrich for HSCs.

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Urolithins

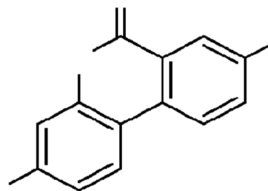
Urolithins are metabolites of dietary ellagic acid derivatives, such as ellagitannins, and are produced in the human gut by gut bacteria.

Ellagitannins are a class of antioxidant polyphenols found in several fruits, particularly
5 pomegranate, strawberries, raspberries and walnuts. Although the absorption of ellagitannins is extremely low, they are rapidly metabolised by the gut microbiota of the large intestine into urolithins.

Due to their superior absorption, urolithins are believed to be the bioactive molecules mediating the effects of ellagitannins. To that end, for example, urolithins were previously
10 shown to have antioxidant and anti-inflammatory properties.

Example urolithins include urolithin A (3,8-dihydroxyurolithin), urolithin B (3-hydroxyurolithin), and urolithin D (3,4,8,9-tetrahydroxyurolithin), urolithin A glucuronide and urolithin B glucuronide.

Urolithin A (UroA) has the structure:



15

In some embodiments, the HSPCs are contacted with the urolithin at a urolithin concentration of 5-250 µM, 5-200 µM, 5-150 µM, 5-100 µM or 5-50 µM. In other embodiments, the HSPCs are contacted with the urolithin at a urolithin concentration of 10-250 µM, 10-200 µM, 10-150 µM, 10-100 µM or 10-50 µM. In other embodiments, the HSPCs are contacted with the urolithin
20 at a urolithin concentration of 20-250 µM, 20-200 µM, 20-150 µM, 20-100 µM or 20-50 µM. In other embodiments, the HSPCs are contacted with the urolithin at a urolithin concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100, 125, 150, 175, 200, 225 or 250 µM.

In preferred embodiments, the HSPCs are contacted with the urolithin at a urolithin
25 concentration of 20-50 µM.

The urolithin of the invention can be present as a salt or ester, in particular a pharmaceutically-acceptable salt or ester.

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Pharmaceutically-acceptable salts of the agents of the invention include suitable acid addition or base salts thereof. A review of suitable pharmaceutical salts may be found in Berge et al. (1977) J Pharm Sci 66: 1-19.

The invention also includes where appropriate all enantiomers and tautomers of the agents.
5 The skilled person will recognise compounds that possess optical properties (e.g. one or more chiral carbon atoms) or tautomeric characteristics. The corresponding enantiomers and/or tautomers may be isolated/prepared by methods known in the art.

Pharmaceutical and nutritional compositions

In some embodiments, the urolithin is in the form of a pharmaceutical composition.

10 The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, diluent or excipient.

In some embodiments, the haematopoietic stem and/or progenitor cells (HSPCs) are in the form of a pharmaceutical composition.

The cells of the invention may be formulated for administration to subjects with a
15 pharmaceutically acceptable carrier, diluent or excipient. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline, and potentially contain human serum albumin.

Handling of the cell therapy product is preferably performed in compliance with FACT-JACIE International Standards for cellular therapy.

20 In some embodiments, the urolithin is in the form of a nutritional composition.

In some embodiments, the urolithin is in the form of a food product, food supplement, nutraceutical, food for special medical purpose (FSMP), nutritional supplement, dairy-based drink, low-volume liquid supplement or meal replacement beverage. In some embodiments, the composition is an infant formula.

25 In some embodiments, the urolithin is in the form of a food additive or a medicament.

A food additive or a medicament may be in the form of tablets, capsules, pastilles or a liquid for example. Food additives or medicaments are preferably provided as sustained release formulations, allowing a constant supply of the urolithin or precursor thereof for prolonged times.

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The composition may be selected from the group consisting of milk-powder based products; instant drinks; ready-to-drink formulations; nutritional powders; nutritional liquids; milk-based products, in particular yoghurts or ice cream; cereal products; beverages; water; coffee; cappuccino; malt drinks; chocolate flavoured drinks; culinary products; soups; tablets; and/or syrups.

The composition may further contain protective hydrocolloids (such as gums, proteins, modified starches), binders, film forming agents, encapsulating agents/materials, wall/shell materials, matrix compounds, coatings, emulsifiers, surface active agents, solubilising agents (oils, fats, waxes, lecithins etc.), adsorbents, carriers, fillers, co-compounds, dispersing agents, wetting agents, processing aids (solvents), flowing agents, taste masking agents, weighting agents, jellifying agents, gel forming agents, antioxidants and antimicrobials.

Further, the composition may contain an organic or inorganic carrier material suitable for oral or enteral administration as well as vitamins, minerals trace elements and other micronutrients in accordance with the recommendations of government bodies such as the USRDA.

The composition of the invention may contain a protein source, a carbohydrate source and/or a lipid source.

Any suitable dietary protein may be used, for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein and pea protein); mixtures of free amino acids; or combinations thereof. Milk proteins such as casein and whey, and soy proteins are particularly preferred.

If the composition includes a fat source, the fat source preferably provides 5% to 40% of the energy of the formula; for example 20% to 30% of the energy. DHA may be added. A suitable fat profile may be obtained using a blend of canola oil, corn oil and high-oleic acid sunflower oil.

A source of carbohydrates may more preferably provide between 40% to 80% of the energy of the composition. Any suitable carbohydrate may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, maltodextrins and mixtures thereof.

Hematopoietic stem cell transplantation

The invention provides a population of haematopoietic stem and/or progenitor cells prepared according to a method of the invention for use in a method of therapy.

The use may be as part of a haematopoietic stem cell transplantation procedure.

Hematopoietic stem cell transplantation (HSCT) is the transplantation of blood stem cells derived from the bone marrow (in this case known as bone marrow transplantation) or blood. Stem cell transplantation is a medical procedure in the fields of haematology and oncology, most often performed for people with diseases of the blood or bone marrow, or certain types of cancer.

Many recipients of HSCTs are multiple myeloma or leukaemia patients who would not benefit from prolonged treatment with, or are already resistant to, chemotherapy. Candidates for HSCTs include paediatric cases where the patient has an inborn defect such as severe combined immunodeficiency or congenital neutropenia with defective stem cells, and also children or adults with aplastic anaemia who have lost their stem cells after birth. Other conditions treated with stem cell transplants include sickle-cell disease, myelodysplastic syndrome, neuroblastoma, lymphoma, Ewing's Sarcoma, Desmoplastic small round cell tumour and Hodgkin's disease. More recently non-myeloablative, or so-called "mini transplant", procedures have been developed that require smaller doses of preparative chemotherapy and radiation. This has allowed HSCT to be conducted in the elderly and other patients who would otherwise be considered too weak to withstand a conventional treatment regimen.

In some embodiments, the haematopoietic stem and/or progenitor cells are administered as part of an autologous stem cell transplant procedure.

In other embodiments, the haematopoietic stem and/or progenitor cells are administered as part of an allogeneic stem cell transplant procedure.

By "autologous stem cell transplant procedure" it is to be understood that the starting population of cells (i.e. before contact with an agent of the invention) is obtained from the same subject as that to which the final cell population is administered. Autologous transplant procedures are advantageous as they avoid problems associated with immunological incompatibility and are available to subjects irrespective of the availability of a genetically matched donor.

By "allogeneic stem cell transplant procedure" it is to be understood that the starting population of cells (i.e. before contact with an agent of the invention) is obtained from a different subject as that to which the final cell population is administered. Preferably, the donor will be genetically matched to the subject to which the cells are administered to minimise the risk of immunological incompatibility.

Method of treatment

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment. The treatment of mammals, particularly humans, is preferred. Both human and veterinary treatments are within the scope of the invention.

Administration

5 Although the agents for use in the invention can be administered alone, they will generally be administered in admixture with a pharmaceutical carrier, excipient or diluent, particularly for human therapy.

In some embodiments, the urolithin is in a combined preparation for simultaneous, separate or sequential use with an agent selected from the group consisting of nicotinamide riboside, a 10 G-CSF analogue, a TPO receptor analogue, and combinations thereof.

The term “combination”, or terms “in combination”, “used in combination with” or “combined preparation” as used herein may refer to the combined administration of two or more agents simultaneously, sequentially or separately.

The term “simultaneous” as used herein means that the agents are administered concurrently, 15 i.e. at the same time.

The term “sequential” as used herein means that the agents are administered one after the other.

The term “separate” as used herein means that the agents are administered independently of each other but within a time interval that allows the agents to show a combined, preferably 20 synergistic, effect. Thus, administration “separately” may permit one agent to be administered, for example, within 1 minute, 5 minutes or 10 minutes after the other.

Dosage

The skilled person can readily determine an appropriate dose of one of the agents of the invention to administer to a subject without undue experimentation. Typically, a physician will 25 determine the actual dosage which will be most suitable for an individual patient and it will depend on a variety of factors including the activity of the specific agent employed, the metabolic stability and length of action of that agent, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. There can of course be 30 individual instances where higher or lower dosage ranges are merited, and such are within the scope of the invention.

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Subject

In some embodiments, a subject is a human or non-human animal.

Examples of non-human animals include vertebrates, for example mammals, such as non-human primates (particularly higher primates), dogs, rodents (e.g. mice, rats or guinea pigs),
5 pigs and cats. The non-human animal may be a companion animal.

Preferably, the subject is a human.

The invention may be, for example, useful for increasing blood cell production in a subject.

The invention may be, for example, useful for increasing blood cell levels in a subject.

In some embodiments, the subject has or is at risk of having subnormal amounts of
10 haematopoietic cells, for example erythrocytes, leukocytes and/or platelets.

A normal range for leukocytes in humans is 4500-10000 cells/µl. A normal range for erythrocytes in male humans is 5-6 million cells/µl, and in female humans is 4-5 million cells/µl. A normal range for platelets is 140000-450000 per µl. Blood cell levels, which may also be referred to as blood cell counts, may be readily measured by the skilled person using any of
15 a number of techniques known in the art, for example the use of haemocytometers and automated blood analysers.

In some embodiments, a subject has or is at risk of having anaemia, leukopenia and/or thrombocytopenia.

In some embodiments, the subnormal amounts of haematopoietic cells is secondary to a
20 primary or autoimmune disorder of the hematopoietic system, for example congenital bone marrow failure syndromes, idiopathic thrombocytopenia, aplastic anaemia and myelodysplastic syndromes.

Subjects at risk of developing a decrease in blood cell levels include patients suffering from anaemia or myelodysplastic syndromes, those undergoing chemotherapy, bone marrow
25 transplant or radiation therapy, and those suffering from autoimmune cytopenias including but not limited to immune thrombocytopenic purpura, pure red cell aplasia and autoimmune neutropenia.

Subjects at risk of developing post-transplantation complications include haematopoietic cell depleted subjects having received an autologous or allogeneic hematopoietic stem or
30 progenitor cell graft from primary or in vitro manipulated HSPCs.

In some embodiments, the subject may have undergone myeloablative conditioning; chemotherapy; radiotherapy; and/or surgery. The myeloablative conditioning; chemotherapy; radiotherapy; and/or surgery may have resulted in subnormal amounts of haematopoietic cells.

5 Subjects having or at risk of developing subnormal amounts of haematopoietic cells include subject suffering from blood cancers (e.g. leukaemia, lymphoma and myeloma), blood disorders (e.g. inherited anaemia, inborn errors of metabolism, aplastic anaemia, beta-thalassaemia, Blackfan-Diamond syndrome, globoid cell leukodystrophy, sickle cell anaemia, severe combined immunodeficiency, X-linked lymphoproliferative syndrome, Wiskott-Aldrich 10 syndrome, Hunter's syndrome, Hurler's syndrome, Lesch Nyhan syndrome, osteopetrosis), subjects undergoing chemotherapy rescue of the immune system, and other diseases (e.g. autoimmune diseases, diabetes, rheumatoid arthritis, systemic lupus erythromatosis). Furthermore, subjects having or at risk of developing subnormal amounts of haematopoietic cells include subjects presenting a severe neutropenia and/or severe thrombocytopenia 15 and/or severe anaemia, such as post-transplanted subjects or subjects undergoing ablative chemotherapy for solid tumours, patients suffering toxic, drug-induced or infectious haematopoietic failure (i.e. benzene-derivatives, chloramphenicol, B19 parvovirus, etc.) as well as patients suffering from myelodysplastic syndromes, from severe immunological disorders, or from congenital haematological disorders whether of central (i.e. Fanconi 20 anaemia) or peripheral origin (i.e. G6PDH deficiency).

The invention may be, for example, useful for the treatment or prevention of anaemia, leukopenia and/or thrombocytopenia; an infection (e.g. a non-viral or viral infection); and/or cancer, such as a haematological cancer (e.g. leukaemia, lymphoma or myeloma).

The agents, compositions and cell populations of the invention may be useful in the treatment 25 of the disorders listed in WO 1998/005635. For ease of reference, part of that list is now provided: cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia, anorexia, acute infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; 30 tumour growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischaemia, ischaemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis; psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; 35 corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis,

eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endosclerosis.

In addition, or in the alternative, the agents, compositions and cell populations of the invention may be useful in the treatment of the disorders listed in WO 1998/007859. For ease of 5 reference, part of that list is now provided: cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g. for treating immune deficiency, including infection with human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumour immunity); regulation of haematopoiesis, e.g. treatment of myeloid or lymphoid 10 diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g. for healing wounds, treatment of burns, ulcers and periodontal disease and neurodegeneration; inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g. for mobilising specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g. for treating haemophilia and stroke); anti-15 inflammatory activity (for treating e.g. septic shock or Crohn's disease); as antimicrobials; modulators of e.g. metabolism or behaviour; as analgesics; treating specific deficiency disorders; in treatment of e.g. psoriasis, in human or veterinary medicine.

In addition, or in the alternative, the agents, compositions and cell populations of the invention may be useful in the treatment of the disorders listed in WO 1998/009985. For ease of 20 reference, part of that list is now provided: macrophage inhibitory and/or T cell inhibitory activity and thus, anti-inflammatory activity; anti-immune activity, i.e. inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation; inhibit the ability of macrophages and T cells to adhere to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells; inhibit 25 unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome 30 or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididimo-orchitis, infertility, orchidal 35 trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or

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inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fondus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillaim-Barre syndrome, Sydenham chora, myasthenia gravis, pseudo-tumour cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g. leukaemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

Methods of expansion and culture media

In another aspect, the invention provides a method of expanding an isolated population of haematopoietic stem and/or progenitor cells (HSPCs) comprising contacting the population with a urolithin, wherein stem cell function of the HSPCs is increased for at least 40 weeks.

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In some embodiments, the contacting comprises culturing the population in the presence of the urolithin.

In some embodiments, the method comprises the steps:

(a) providing a population of HSPCs;

5 (b) optionally culturing the population of HSPCs, preferably in a HSPC expansion or maintenance culture medium;

(c) optionally isolating a sub-population of HSPCs characterised by low mitochondrial membrane potential; and

(d) contacting the population of (a) or (b), or the sub-population of (c) with a
10 urolithin.

In some embodiments, the population provided in step (a) is obtained from bone marrow, mobilised peripheral blood or umbilical cord blood.

In some embodiments, the product of step (d) is enriched in cells having long-term multi-lineage blood reconstitution capability.

15 The terms “expansion culture medium” and “maintenance culture medium” as used herein refer to any standard stem cell culture medium suitable for stem cell expansion and maintenance, respectively, such as for example culture media described herein in the examples or described in Boitano et al. (2010) Science 329: 1345-1348.

In another aspect, the invention provides a cell culture medium comprising a urolithin.

20 In some embodiments, the culture medium comprises cytokines and growth factors. The cytokines and growth factors can be used with or without supporting stromal feeder or mesenchymal cells, and can comprise, but are not restricted to: SCF, TPO, Flt3-L, FGF-1, IGF1, IGFBP2, IL-3, IL-6, G-CSF, M-CSF, GM-CSF, EPO, oncostatin-M, EGF, PDGF-AB, angiopoietin and angiopoietin-like family including Angl5, prostaglandins and eicosanoids
25 including PGE2, Aryl hydrocarbon (AhR) receptor inhibitors such as StemRegeninl (SRI) and LGC006 (Boitano et al. (2010) Science 329: 1345-1348).

Membrane potential in HSC compartments, in particular mitochondrial membrane potential, can be assayed by methods known to the skilled person, such as described herein in the examples, in particular flow cytometry of cells stained with tetramethylrhodamine methyl ester
30 (TMRM).

Kit

In another aspect, the present invention provides a kit comprising the agents and/or cell populations of the invention.

The cell populations may be provided in suitable containers.

5 The kit may also include instructions for use.

The skilled person will understand that they can combine all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

Preferred features and embodiments of the invention will now be described by way of non-10 limiting examples.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, Sambrook, J., Fritsch, E.F. and Maniatis, T. 15 (1989) Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press; Ausubel, F.M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, Ch. 9, 13 and 16, John Wiley & Sons; Roe, B., Crabtree, J. and Kahn, A. (1996) DNA Isolation and Sequencing: Essential Techniques, John Wiley & Sons; Polak, J.M. and McGee, J.O'D. (1990) In Situ Hybridization: Principles and Practice, Oxford University Press; Gait, M.J. 20 (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; and Lilley, D.M. and Dahlberg, J.E. (1992) Methods in Enzymology: DNA Structures Part A: Synthesis and Physical Analysis of DNA, Academic Press. Each of these general texts is herein incorporated by reference.

EXAMPLES

25 **EXAMPLE 1**

RESULTS AND DISCUSSION

UroA induces lowering of mitochondrial membrane potential

We first tested the effect of UroA on bone marrow derived mouse HSCs (mHSCs) (Figure 1A). Freshly isolated mHSCs (LKS CD150+CD48-) were cultured in basal media (Stemline + SCF+ 30 FLT3L + Penicillin/Streptomycin) supplemented with different concentrations of UroA. Cells

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were harvested at day 3 and stained with tetramethylrhodamine methyl ester (TMRM; to measure mitochondria membrane potential) and Mitotracker (to measure mitochondrial mass), and analysed by flow cytometry.

We found a step-wise increase in the proportion of cells in the $TMRM^{low}$ gate with increasing doses of UroA resulting in significant lowering of TMRM fluorescent intensity (Mean Fluorescence Intensity, MFI) (Figure 1A, Top panel). Mitotracker staining showed a decrease in the mitochondrial mass at all concentrations of UroA, with 20 μM resulting in significant reduction (Figure 1A, Bottom panel). We then looked at the effect of UroA on human cord blood derived hematopoietic stem and progenitor cells (hHSPCs) (Figure 1B). Cryopreserved hHSPCs (CD34+) were thawed and cultured in basal media (Stemspan + SCF + FLT3L + TPO + LDLP + Penicillin/Streptomycin) supplemented with different concentrations of UroA. Aliquots of cells were harvested at days 3, 5 and 7, followed by staining for CD34 and TMRM, and analysed by flow cytometry. At all three timepoints we found increasing proportions of cells in the $TMRM^{low}$ gate accompanied by a concomitant decrease of TMRM signal (Median Fluorescence Intensity, MFI) with increasing doses of UroA (Figure 1B).

In vitro UroA treatment enhances mHSC and hHSPC in vivo function

Having previously shown that lowering mitochondrial membrane potential enhances HSC function (Vannini, N. et al. (2016) Nat Commun 7: 13125), we asked if UroA treatment improves in vivo reconstitution potential of HSCs. To that end, we cultured freshly isolated mHSCs in basal media without or with UroA (20 μM). At the end of the culture period (3 days) cells were counted and injected into lethally irradiated recipient mice (Figure 2A). Blood analysis of recipients showed higher reconstitution levels in animals injected with UroA-treated cells (Figure 2A). This trend was reflected in both the myeloid and lymphoid lineages of the blood (Figure 2A).

Next, we cultured cord blood derived human HSPCs without or with UroA (50 μM) and performed two functional assays: Colony Forming Unit (CFU) assay - 7 days post culture and in vivo transplantation assay in new born NSG-SGM3 pups - 5 days post culture (Figure 2B). UroA-treated cells formed significantly higher numbers of colonies in methylcellulose CFU assay plates (Figure 2C), indicating an increased stem and progenitor function of hHSCs exposed to UroA. In the second assay, blood analysis of NSG-SGM3 mice transplanted with cultured cells showed increased human engraftment (both as a proportion and absolute numbers) in the UroA treated condition (Figure 2D). Furthermore, we analysed different human blood cell lineages and found higher human cell numbers in the UroA condition,

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primarily in the lymphoid lineage (T and B cells) (Figure 2E). These data demonstrate that UroA treatment enhances HSC function.

UroA drives the expression of metabolic genes in mHSCs

To have analyse the molecular mechanisms by which UroA enhances HSC function, we
5 performed gene expression analysis on mHSCs cultured in basal media with or without UroA (20 μM). Fold change (delta delta CT) analysis showed increased expression of autophagy (ATG5, PARK2), glycolysis (HK2, Glut1) and ROS protection (Foxo1, SOD2) genes in the UroA treated condition (Figure 3). This is in agreement with our previous work and the literature where autophagy and ROS protection have been shown to be key drivers of HSC
10 self-renewal (Takubo, K. et al. (2013) Cell Stem Cell 12: 49-61; Vannini, N. et al. (2016) Nat Commun 7: 13125; Ito, K. et al. (2006) Nat Med 12: 446-51; Warr, M.R. et al. (2013) Nature 494: 323-327; Ito, K. et al. (2016) Science 354: 1156-1160) and upregulated glycolysis, a key metabolic pathway maintaining HSC stemness (Takubo, K. et al. (2013) Cell Stem Cell 12: 49-61; Yu, W.M. et al. (2013) Cell Stem Cell 12: 62-74).

15 In summary, our findings demonstrate the ability of UroA to ameliorate HSC function via modulation of mitochondrial membrane potential through mitophagy induction, leading to applications of UroA in the context of HSC transplantation for the treatment of blood malignancies.

MATERIALS AND METHODS

20 **Flow cytometry**

Flow cytometry analysis was performed on freshly isolated bone marrow (BM) from C57Bl6 mice. BM was extracted from crushed femora and tibia. Cell suspension was filtered through a 70 μm cell strainer and erythroid cells were eliminated by incubation with red blood cell lysis buffer (eBioscences). Isolation and stains were performed in ice-cold PBS 1 mM EDTA.
25 Lineage positive cells were then removed with a magnetic lineage depletion kit (BD biosciences). Cell suspensions were then stained with specific antibodies for the stem cell compartment and sorted by FACS (BD FACS Aria III) into 1.5 ml Eppendorf tubes.

Antibodies

The following antibodies were used in this study: rat mAbs against cKit (2B8), Sca1 (D7),
30 CD150 (TC-15-12F12.2), CD48 (HM48-1), CD45.2 (104), CD45.1 (A20), Gr1 (RB6-8C5), F4/80 (BM8), CD19 (6D5), CD3 (17A2), CD16/CD32 (2.4G2). The antibodies were purchased from Biolegend, eBiosciences and BD. A mixture of biotinylated mAbs against CD3, CD11b,

CD45R/B220, Ly-6G, Ly-6C and TER-119 was used as lineage marker ("lineage cocktail") and was purchased from BD. Human specific antibodies were: hCD56 (NCAM16.2), hCD16 (3G8), hCD45 (HI30), hCD19 (HIB19), hCD4 (RPA-T4), hCD3 (SK7), hCD14 (M5E2), hCD8b (SIDI8BEE), hCD34 (8G12), hCD38 (HB-7) and were either from eBioscience or BD. DAPI or
5 propidium iodine (PI) staining was used for live/dead cell discrimination.

mHSC and hHSPC culture

Murine HSCs were sorted into 1.5 ml Eppendorf tubes and were cultured in Stemline II (SIGMA) supplemented with 100 ng/ml SCF (R&D) and 2 ng/ml Flt3 (R&D). Different concentrations as indicated of UroA (dissolved in DMSO) were added; an equal amount of
10 DMSO was added to the control well.

Cryopreserved CD34+ cells isolated from foetal liver/cord blood were thawed and cultured in vitro in StemSpan (Stem cell tech) media supplemented with hSCF (100 ng/ml), hFLT3L (100 ng/ml), hTPO (50 ng/ml), hLDLP (10 μg/ml) and different concentrations of UroA (dissolved in DMSO) were added; an equal amount of DMSO was added to the control well. For longer
15 culture periods half of the media was replenished every 2nd or 3rd day.

Analysis of mitochondrial activity

Mouse HSCs already in culture were incubated at 37°C for 1 hour with 200 nM tetramethylrhodamine methyl ester (TMRM; Invitrogen) and 100 nM Mitotracker green. Cells were then washed with FACS buffer and analysed by flow cytometry on a BD LSR II.

20 Human HSCs already in culture were incubated at 37°C for 1 hour with 200 nM TMRM (Invitrogen). Cells were then washed with FACS buffer, followed by staining with CD34 antibody for 1 hour at 4°C. Cells were washed with FACS buffer and analysed by flow cytometry on a BD LSR II.

Mouse and humanised transplantation

25 C57Bl/6 Ly5.2 mice were lethally irradiated with a total 8 Gy dose in a gamma radiator 24 h before transplant. Mice were injected with 200 donor cells post culture derived from C57Bl/6 Ly5.1 mice and 200,000 competitor cells derived from C57Bl/6 Ly5.1/5.2 mice, via tail-vein injection. Peripheral blood was collected every few weeks to determine the percentage of chimerism by FACS analysis.

30 NSG mice were purchased from Jackson Laboratory, bred and maintained under pathogen-free conditions in-house. For transplantation, one day old NSG pups were irradiated with 1 Gy

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(RS-2000, RAD SOURCE) and a few hours later injected intrahepatically with in vitro expanded HSCs. Each pup was injected with a cell mass derived from an initial 50,000 CD34+ cells post in vitro culture. Mice were bled at 12 weeks to estimate human reconstitution levels (% human CD45+ cells) in the peripheral blood. Antibody combinations were used to further
5 estimate human B cells, T cells, monocytes, neutrophils and NK cells.

CFU assay

CFU assays were carried out using H4434 (Stem cell tech) as per the manufacturer's instructions. 1000 cells from each well were plated in duplicate. Colonies were counted 15 days post plating using Stem Vision (Stem cell tech).

10 **QPCR**

RNA was extracted from HSCs post culture using ZR RNA MicroPrep (Zymo Research) and RNA extraction was performed accordingly to the manufacturer's instructions. RNA was retrotranscribed to cDNA with 1st strand cDNA kit (TAKARA) as per the manufacturer's instructions.

15 For qPCR, 0.5 µl of cDNA, 5 µl of Power Syber Green mastermix (Applied Biosystem) and 500 nM of primers were used to a final volume of 10 µl for each reaction. The reactions were performed on the 7900HT system (Applied Biosystem).

Murine primer sequences are as follows:

		SEQ ID NO:
Atg5 F	AAGTCTGTCCTTCCGCAGTC	1
Atg5 R	TGAAGAAAGTTATCTGGGTAGCTCA	2
Park2 F	GAGCTTCCGAATCACCTGAC	3
Park2 R	CATGACTTCTCCTCCGTGGT	4
Hspa9 F	AATGAGAGCGCTCCTTGCTG	5
Hspa9 R	CTGTTCCCCAGTGCCAGAAC	6
Hsp10 F	GGCCCGAGTTCAGAGTCC	7
Hsp10 R	TGTCAAAGAGCGGAAGAAACTT	8
Hsp60 F	TCTTCAGGTTGTGGCAGTCA	9
Hsp60 R	CCCCTCTTCTCCAAACACTG	10
HK2 F	CAAGCTACAGATCAAAGAGAAG	11
HK2 R	CATGAGACCAAGAAACTCTC	12
Glut1 F	TCAACACGGCCTTCACTG	13
Glut1 R	CACGATGCTCAGATAGGACATC	14
Aco1 F	AATTTCTAAAGTGGGGTTCC	15
Aco1 R	TGATCAAACACTACTCTTGC	16
Suclg1 F	AAGAAGGGAAGAATAGGTATCG	17
Suclg1 R	CCAATCAGTATGATGCCTTC	18
Mfn2 F	GTCATACCACCAATTGCTTC	19
Mfn2 R	TCACAGTCTTGACACTCTTC	20

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Foxo1 F	TCACACATCTGCCATGAACC	21
Foxo1 R	TGGACTCCATGTCACAGTCC	22
SOD2 F	CCATTTTCTGGACAAACCTG	23
SOD2 R	GACCTTGCTCCTTATTGAAG	24
Arbp F	AGATTCGGGATATGCTGTTGG	25
Arbp R	AAAGCCTGGAAGAAGGAGGTC	26

EXAMPLE 2

In order to check if a short in vitro treatment with UroA could improve the survival of irradiated recipients post transplantation we designed a limiting transplantation experiment. Human
5 Cord-blood derived HSPCs were cultured for three days in the absence or presence of UroA. Post culture cells were counted and 40,000 cells were injected in each recipient mouse (irradiated NSG adult mice). We followed these mice for several months to check for post transplantation survival. We found the group of mice transplanted with UroA treated cells had a significant improvement in survival especially in the early phases of the post-transplant
10 recovery.

EXAMPLE 3

Serial transplantation analysis demonstrates in vitro UroA treatment enhances HSC in vivo function in the long term.

HSCs were isolated from bone marrow of mice and cultured in the presence or absence of
15 UroA (Figure 5A). At the end of the culture period cells were transferred into a lethally irradiated recipient first mouse via intravenous tail injection.

Blood chimerism analysis was then carried out for the first mouse over a period of 24 weeks (Figure 5B), followed by analysis of spleen (Figure 5D) and bone marrow (Figure 5E) samples from the first mouse.

20 Bone marrow cells were then extracted from bone marrow of the first mouse and transferred into a second lethally irradiated recipient mouse via intravenous tail injection.

Blood chimerism analysis was then carried out for the second mouse over a period of 20 weeks (Figure 5C), followed by analysis of spleen (Figure 5F) and bone marrow (Figure 5G) samples from the second mouse.

25 UroA-cultured cells show higher blood reconstitution over a total period of at least 44 weeks. The increase is also reflected in the myeloid and lymphoid lineages.

EXAMPLE 4

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Gene expression analysis of UroA-treated mouse HSCs.

In order to study the mechanism by which UroA mediates its effects we performed RNA sequencing analysis on HSCs after a short ex vivo UroA treatment (Figure 6A). Post culture we first isolated the RNA from 6 control (D1-6) and 6 UroA treated (U1-U6) samples. Since the number of cells were limiting, the quantity of isolated RNA was found to be quite low. However, gel electrophoresis and fragment analyser analysis confirmed the quality of RNA to be optimal for RNA sequencing (Figure 6B). One of the control samples (D3) had a large peak at the end of the chromatogram but that was concluded to be an artefact of the fragment analyser. Furthermore, a multi-dimensional scaling (MDS) plot of the RNA sequencing data revealed that the UroA samples (U1-6) clustered together while the control samples appeared to be more scattered (Figure 6C). Differential expression analysis revealed several gene candidates that were differentially expressed upon UroA treatment (Figure 6C, volcano plot).

Next, we looked at various biological pathways altered by UroA treatment. We found that the response to topologically incorrect proteins and unfolded proteins was significantly upregulated in the UroA condition (Figure 6D, top left). Moreover, the ~~endoplasmic reticulum~~ unfolded protein response was also significantly upregulated (Figure 6D, top left). Interestingly, we have previously shown that unfolded protein response is one of the key pathways regulating HSC function. Reactome analysis revealed that the activation of mitochondrial biogenesis is downregulated upon UroA treatment (Figure 6D, bottom right). This was in line with results where reduction in mitochondrial mass was observed upon UroA treatment.

Molecular functions and cellular component analysis revealed several candidates, such as histone methyltransferases, histone acetyltransferase complexes and histone deacetylase complexes, involved in epigenetic modifications were significantly downregulated (Figure 6D). This suggests major epigenetic changes occurring in HSCs upon UroA exposure.

Differential expression analysis of mitochondrial genes revealed several candidates with altered expression (Figure 6E).

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the disclosed compositions, uses and methods of the invention will be apparent to the skilled person without departing from the scope and spirit of the invention. Although the invention has been disclosed in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly

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limited to such specific embodiments. Indeed, various modifications of the disclosed modes for carrying out the invention, which are obvious to the skilled person are intended to be within the scope of the following claims.

CLAIMS

1. Use of a urolithin for increasing stem cell function in a population of haematopoietic stem and/or progenitor cells (HSPCs), wherein the stem cell function is increased for at least 40 weeks.

5 2. A method for increasing stem cell function in a population of haematopoietic stem and/or progenitor cells (HSPCs) comprising contacting the population with a urolithin, wherein the stem cell function is increased for at least 40 weeks.

3. The method of claim 2, wherein the method comprises the steps:

   (a) providing a population of HSPCs;

10 (b) optionally isolating a sub-population of HSPCs characterised by low mitochondrial membrane potential; and

   (c) contacting the population of (a) or the sub-population of (b) with the urolithin.

4. A urolithin for use in a method of therapy by increasing stem cell function in
15 haematopoietic stem and/or progenitor cells (HSPCs), wherein the stem cell function is increased for at least 40 weeks.

5. The urolithin for use according to claim 4, wherein the method comprises contacting the HSPCs with the urolithin prior to administration of the HSPCs to a subject.

6. The urolithin for use according to claim 4, wherein the method comprises administering
20 the urolithin to a subject.

7. The urolithin for use according to any one of claims 4-6, wherein the method of therapy is treatment or prevention of (a) anaemia, leukopenia and/or thrombocytopenia; (b) an infection; and/or (c) cancer.

8. The use, method or urolithin for use according to any preceding claim, wherein the
25 stem cell function comprises one or more of: capacity for engraftment; self-renewal; and, blood or immune cell differentiation.

9. The use, method or urolithin for use according to any preceding claim, wherein the increased stem cell function increases blood cell levels in a subject.

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10. The use, method or urolithin for use according to any preceding claim, wherein the urolithin is urolithin A.

11. The use, method or urolithin for use according to any preceding claim, wherein the population or sub-population of HSPCs is contacted with the urolithin for up to and including 7 days.

12. The use, method or urolithin for use according to any preceding claim, wherein the urolithin is in the form of a pharmaceutical or nutritional composition, optionally in the form of a food product, food supplement, nutraceutical, food for special medical purpose (FSMP), nutritional supplement, dairy-based drink, low-volume liquid supplement or meal replacement beverage.

13. The use, method or urolithin for use according to any preceding claim, wherein a subject has or is at risk of having subnormal amounts of haematopoietic cells, optionally wherein the haematopoietic cells are erythrocytes, leukocytes and/or platelets.

14. The use, method or urolithin for use according to any preceding claim, wherein a subject has or is at risk of having anaemia, leukopenia and/or thrombocytopenia.

15. The use, method or urolithin for use according to any preceding claim, wherein a subject has undergone an intervention selected from the group consisting of a haematopoietic stem cell transplant; a bone marrow transplant; myeloablative conditioning; chemotherapy; radiotherapy; and surgery.

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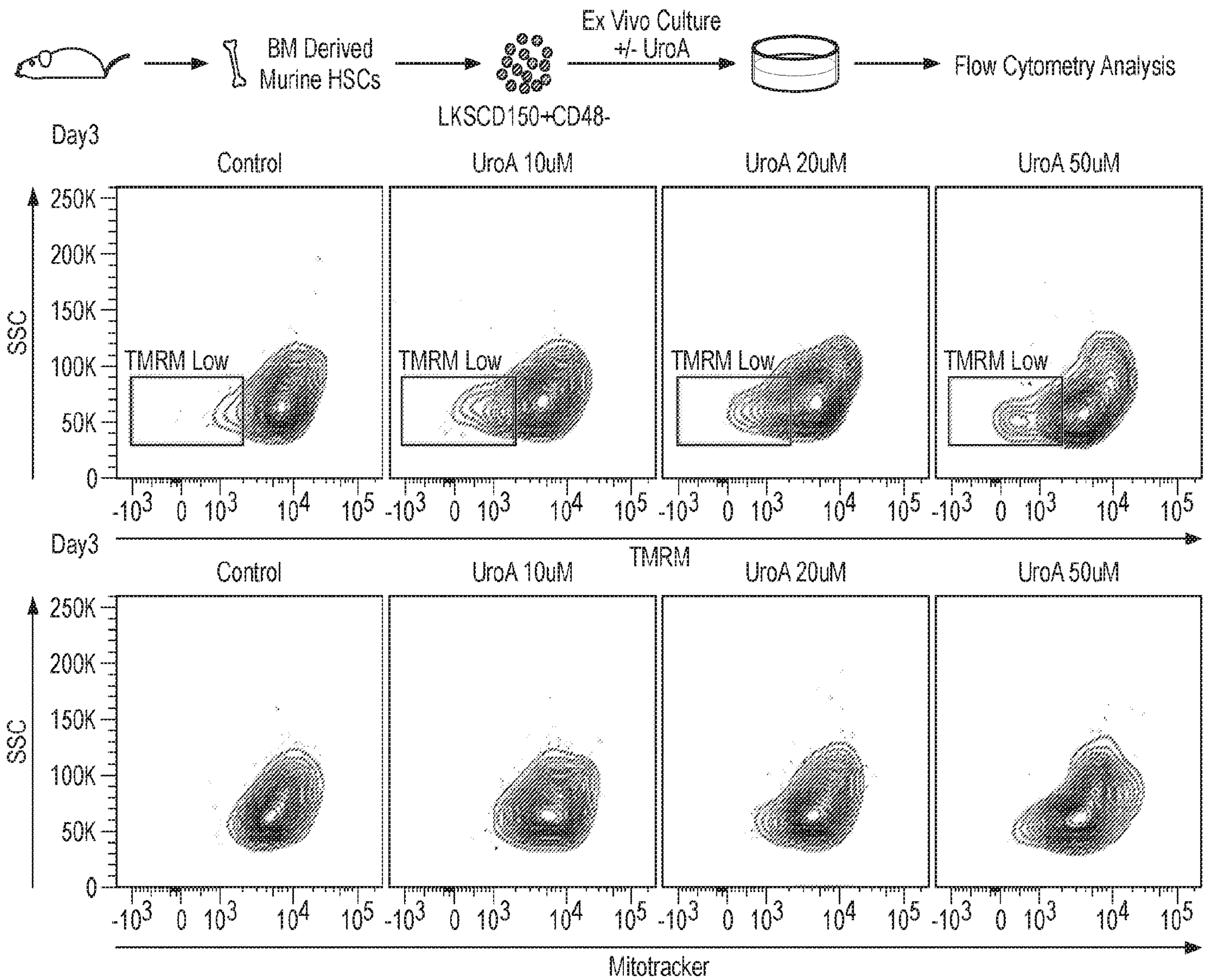
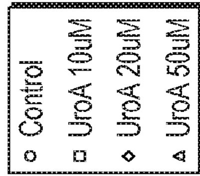


FIG. 1A



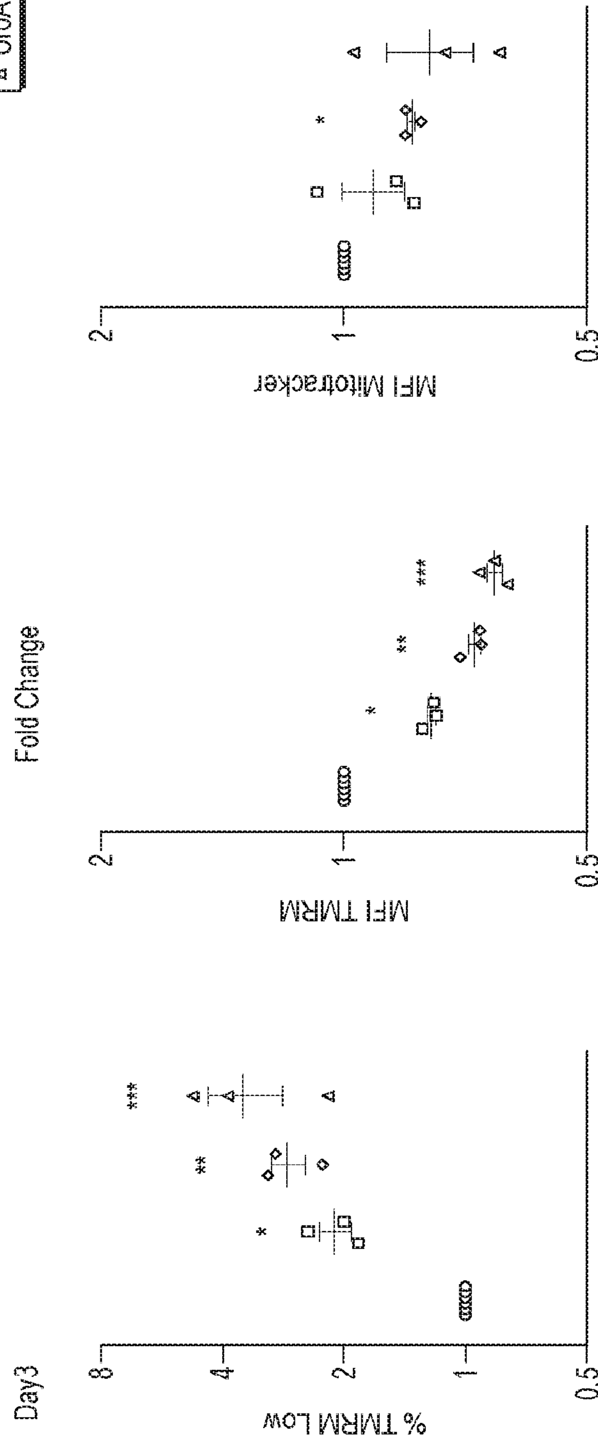
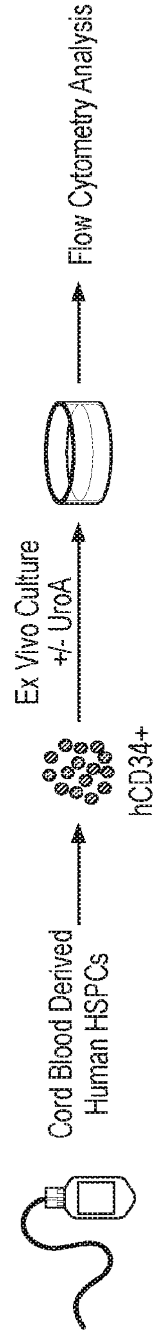


FIG. 1A (Cont.)



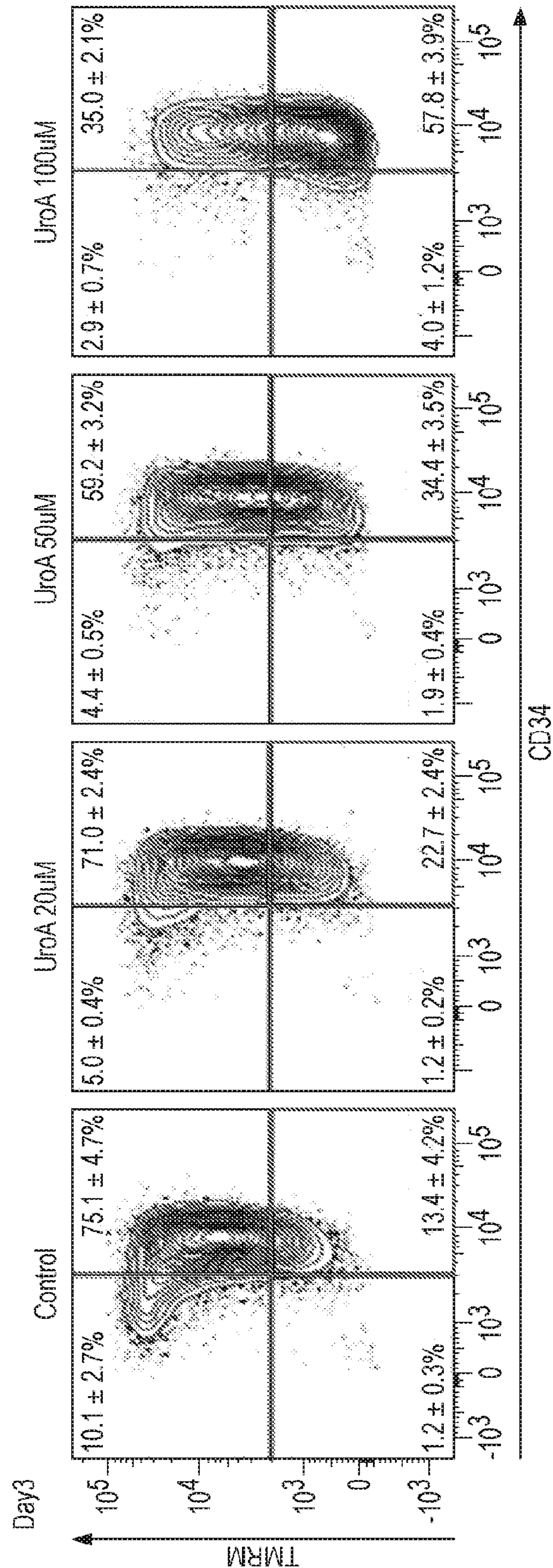


FIG. 1B

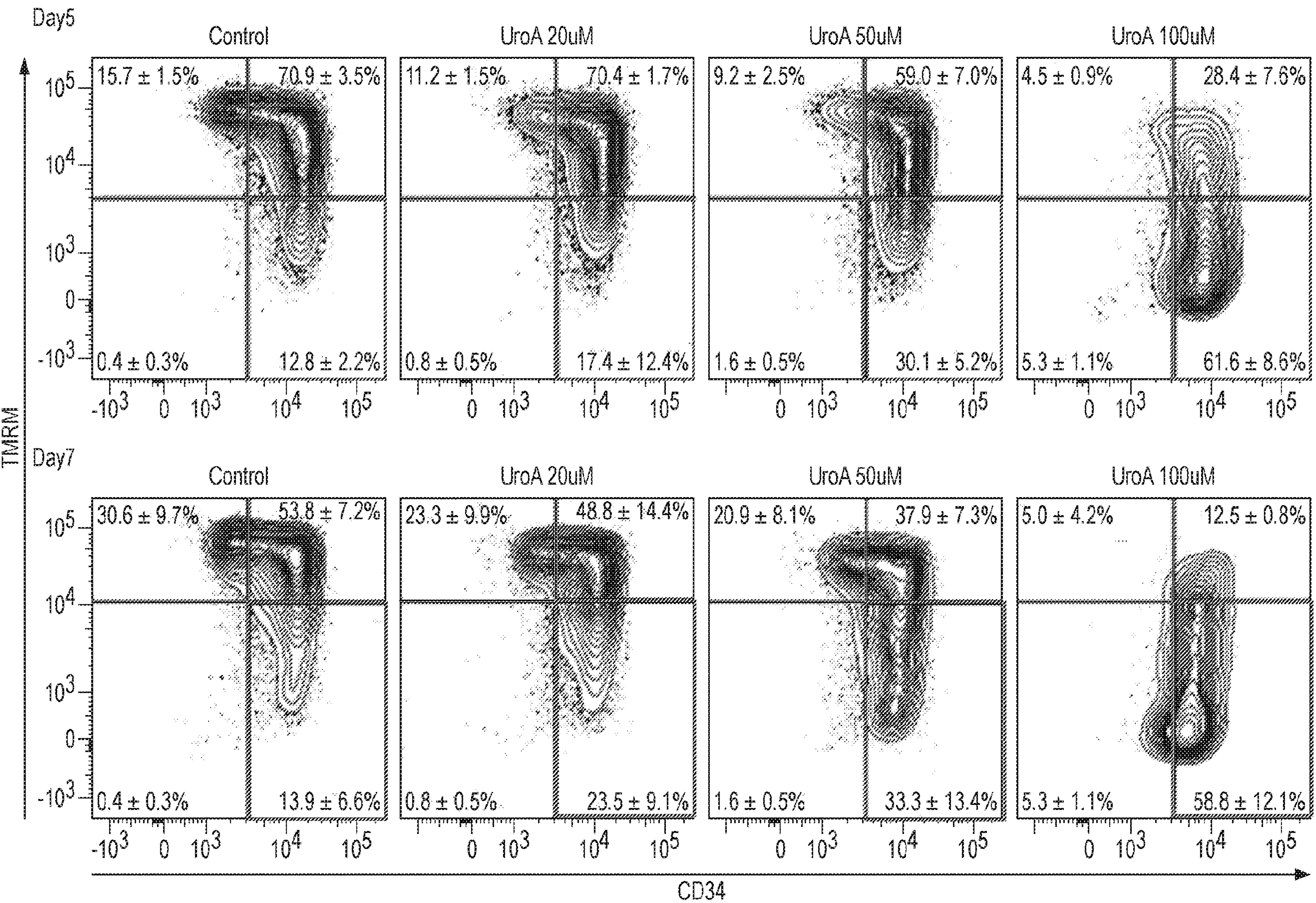
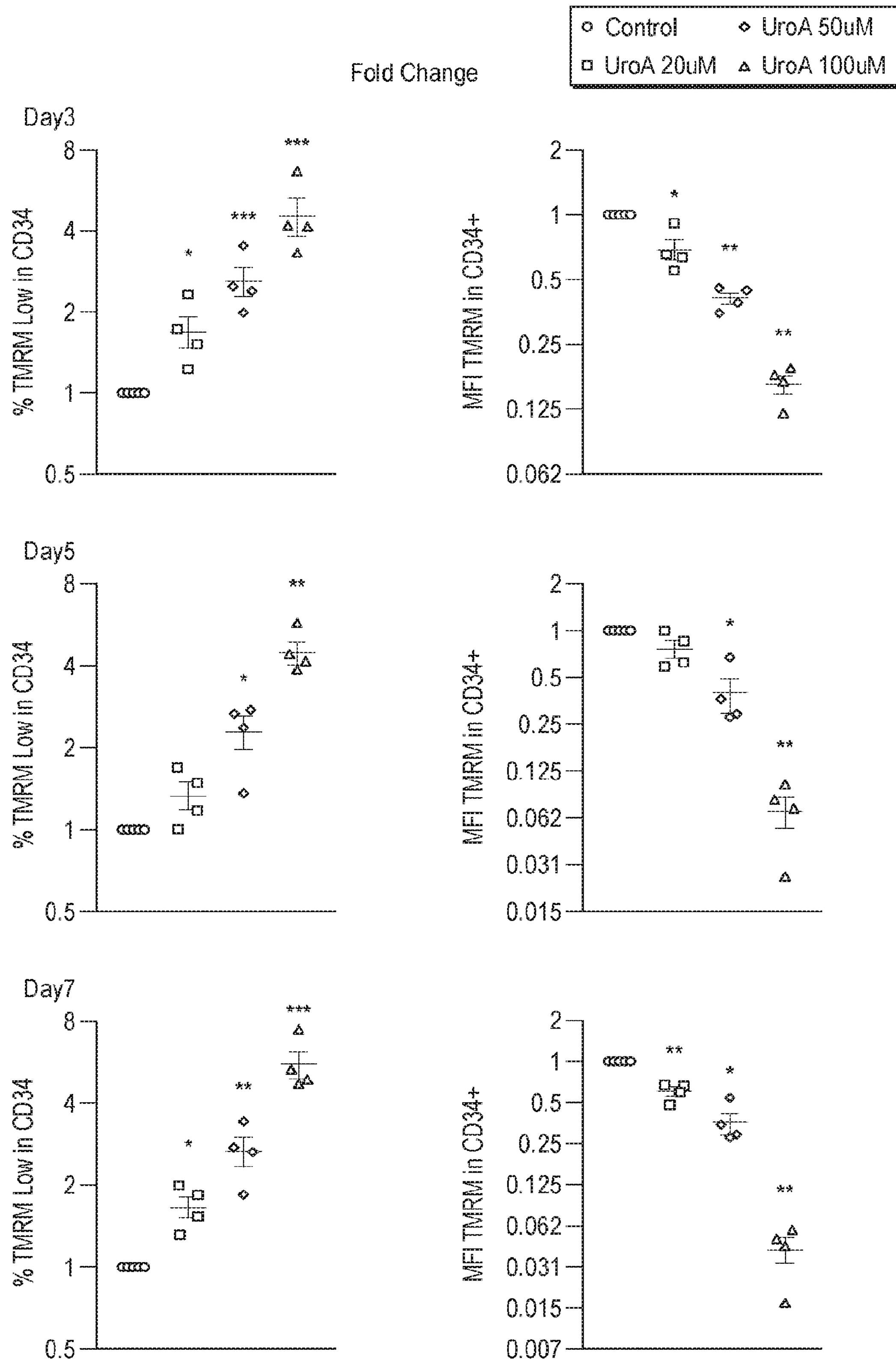


FIG. 1B (Cont.)



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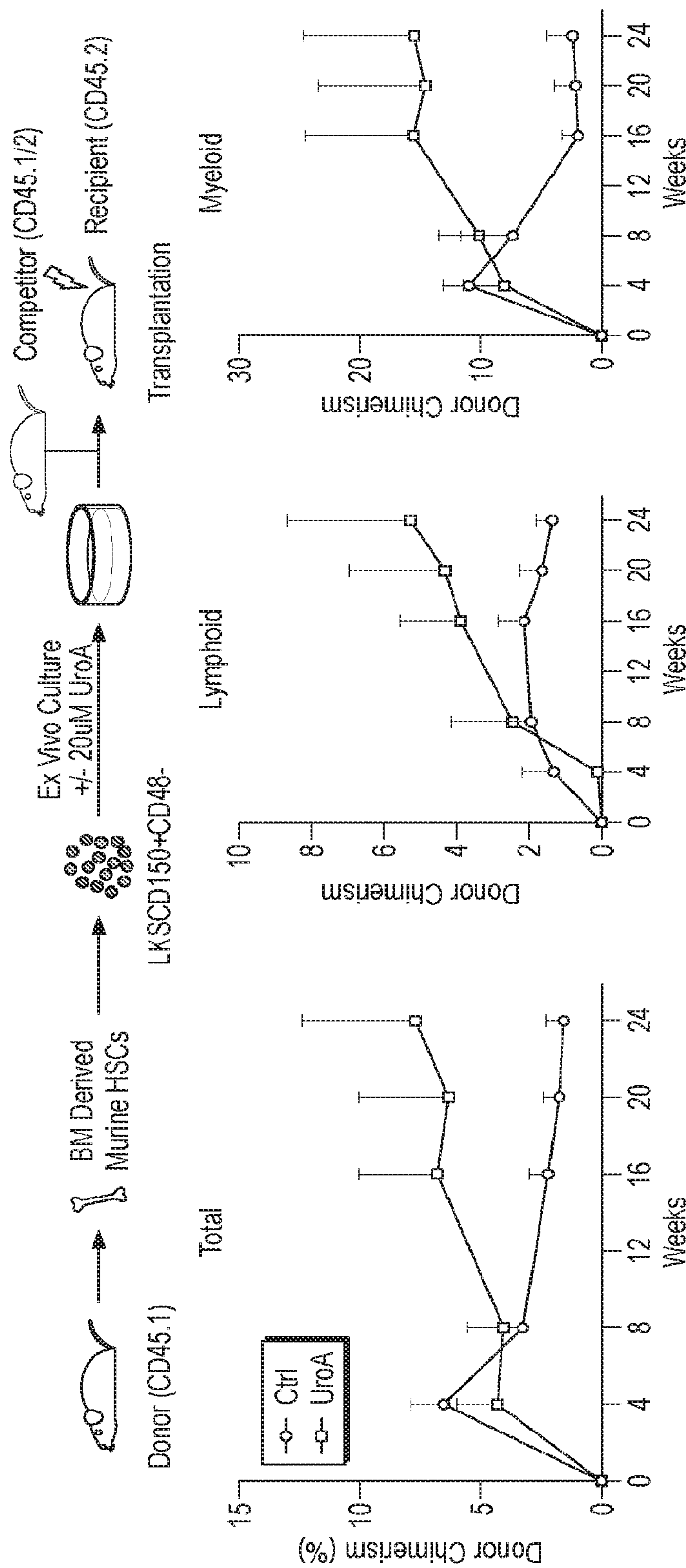


FIG. 2A

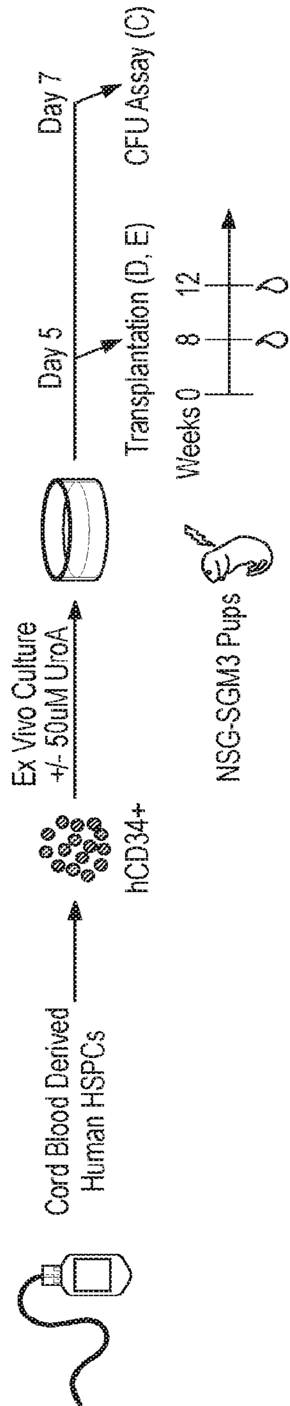


FIG. 2B

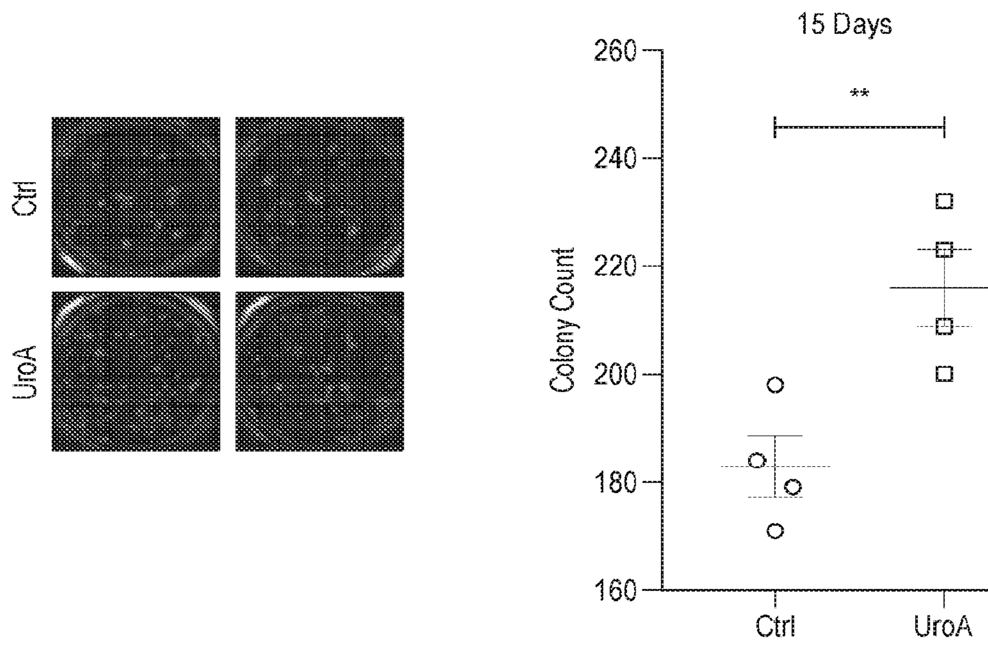


FIG. 2C

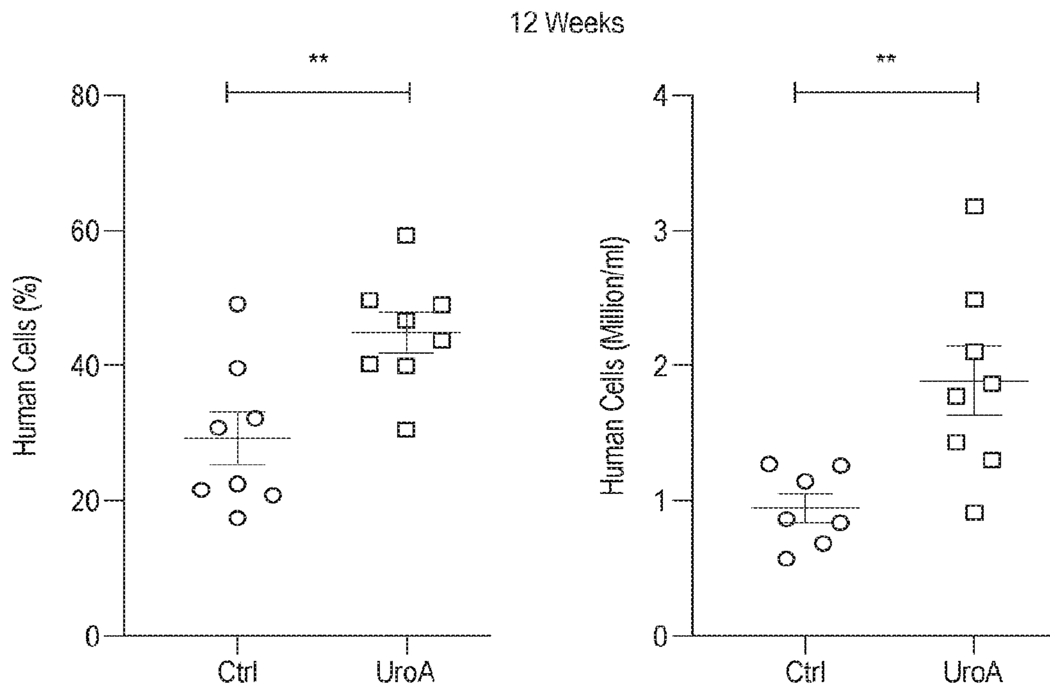


FIG. 2D

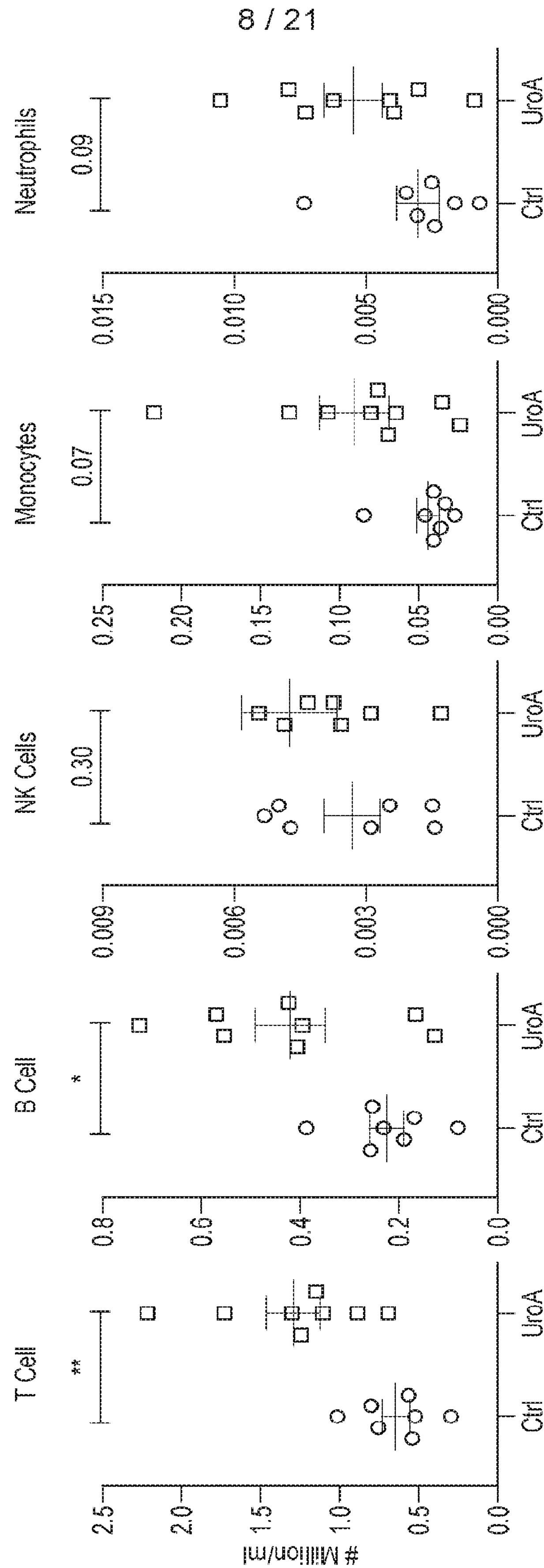
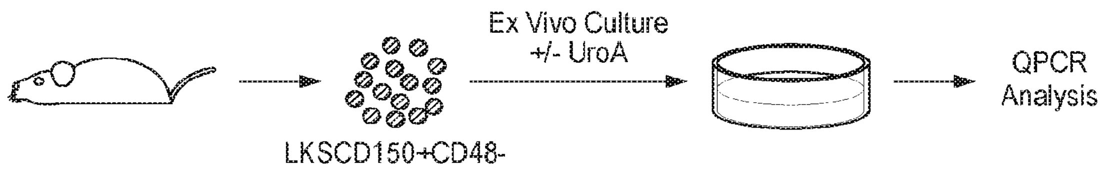


FIG. 2E



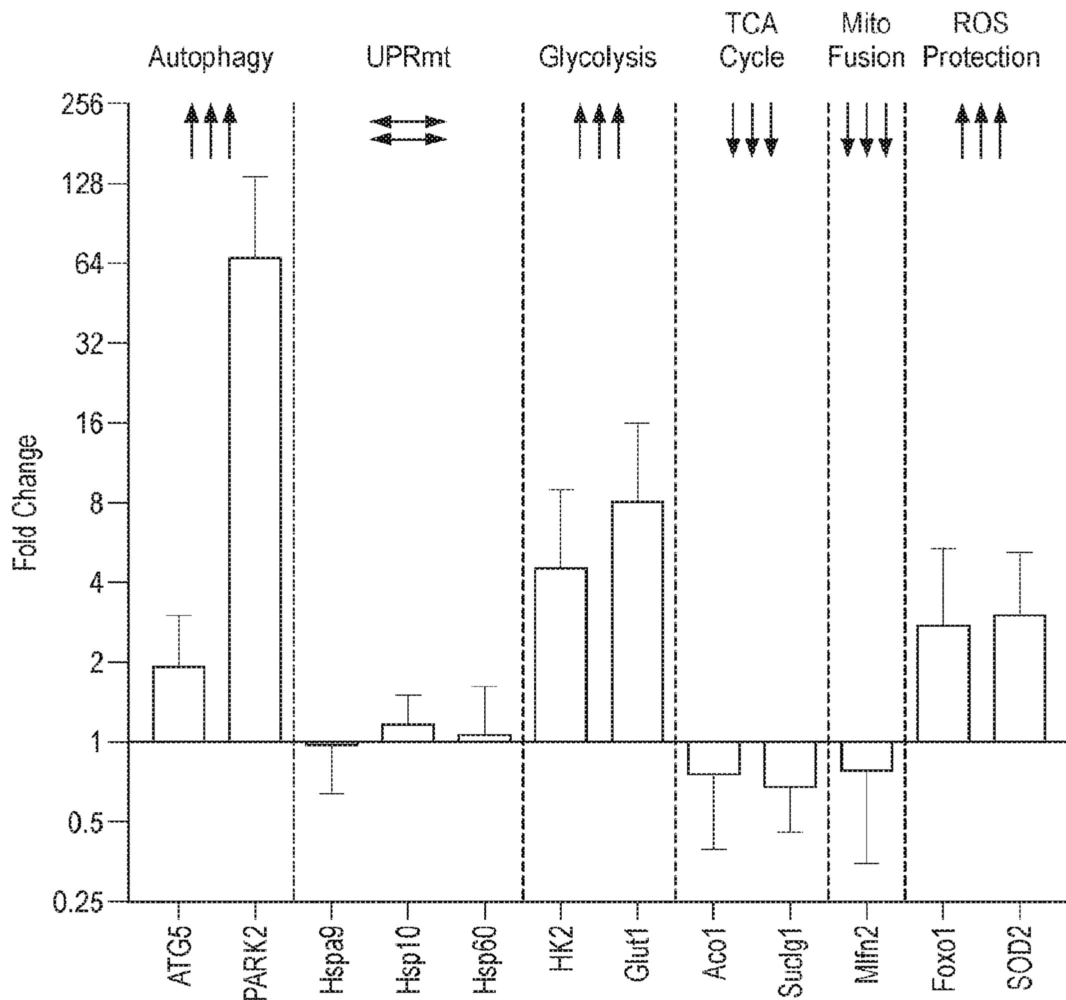


FIG. 3

FIG. 4A

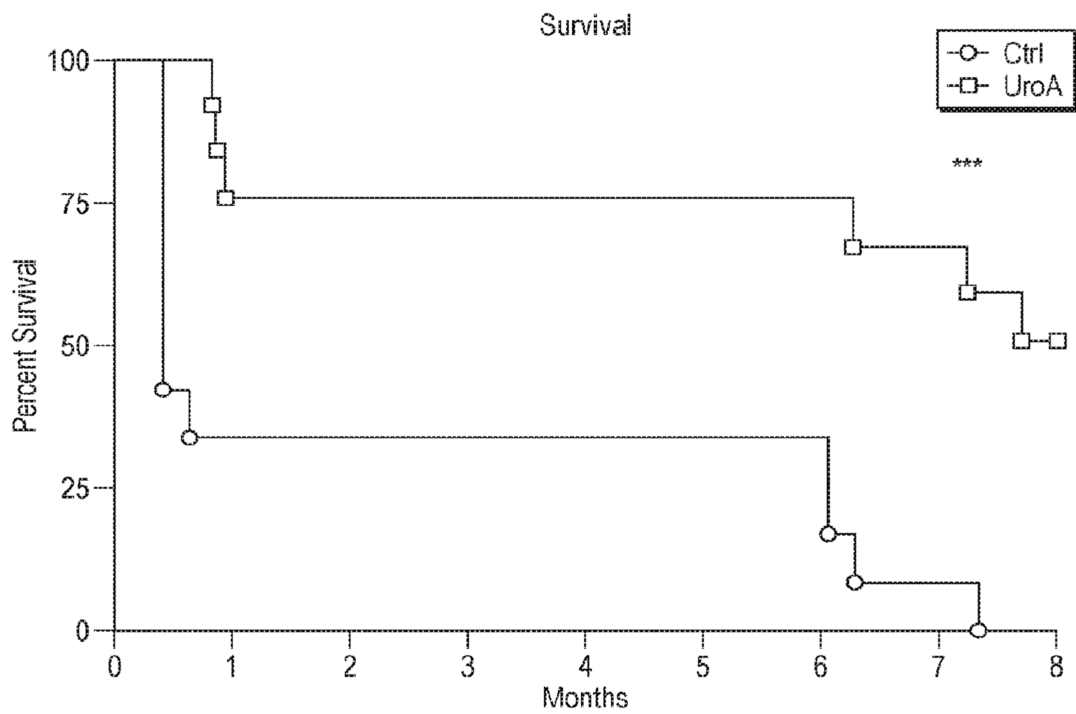


FIG. 4B

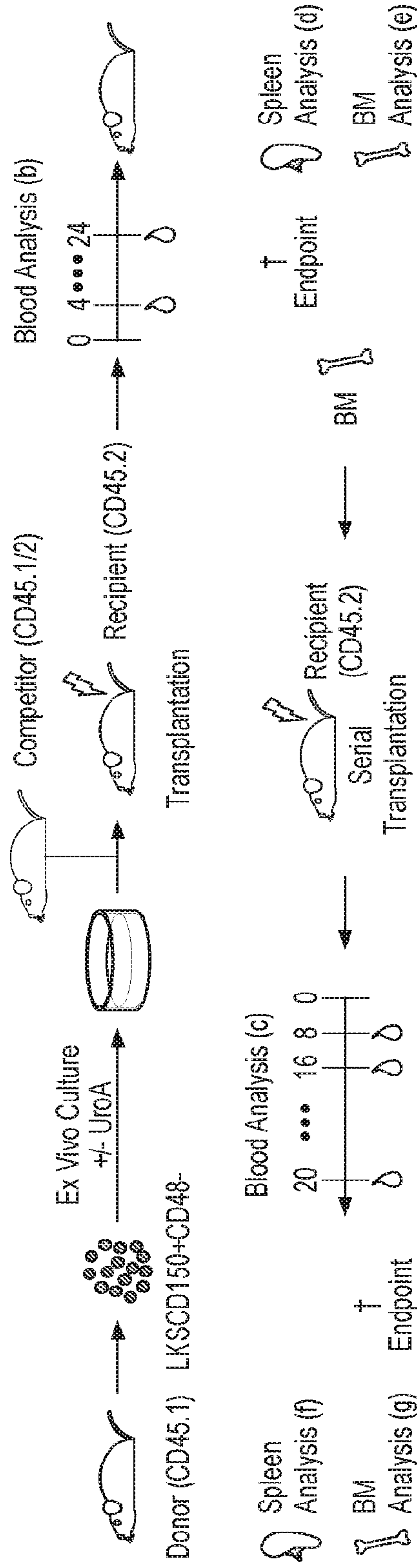


FIG. 5A

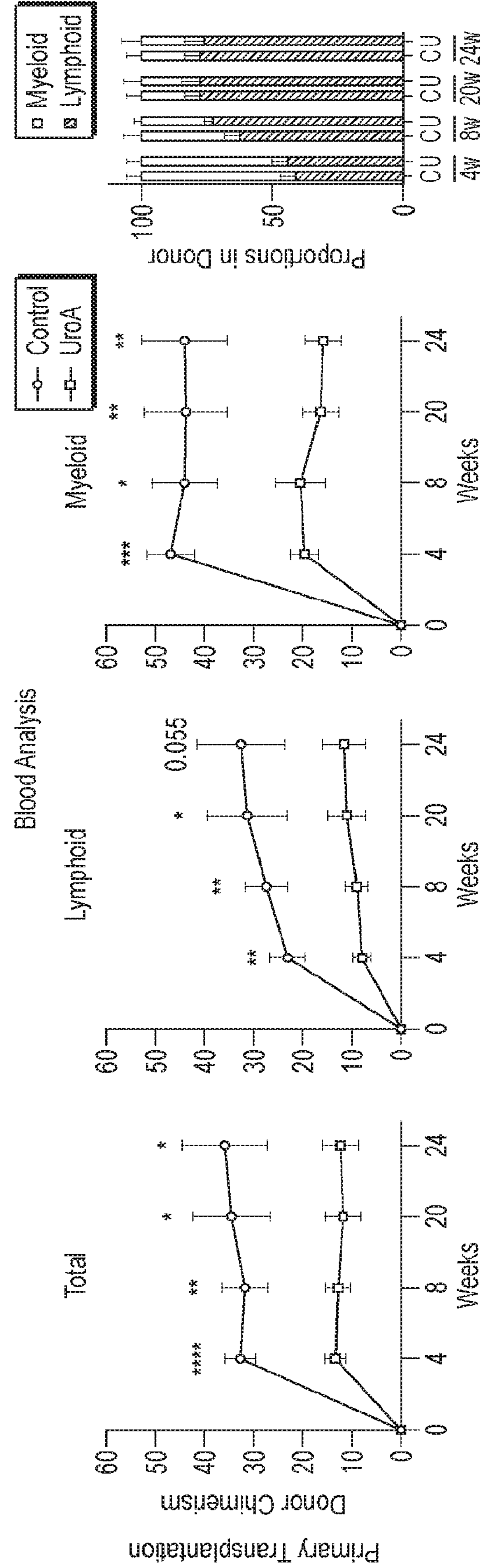


FIG. 5B

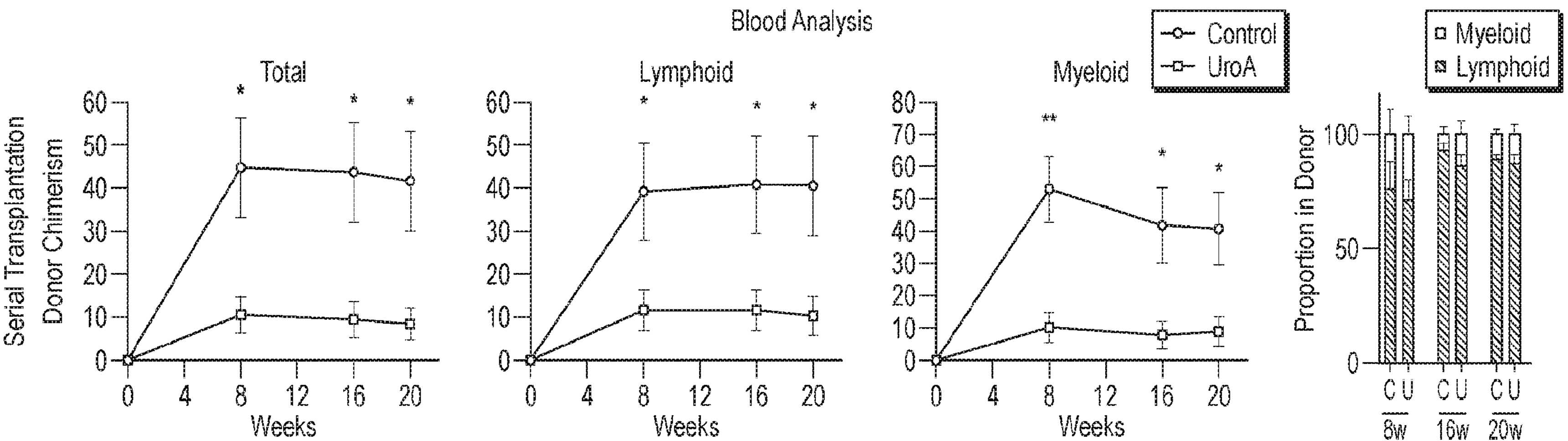


FIG. 5C

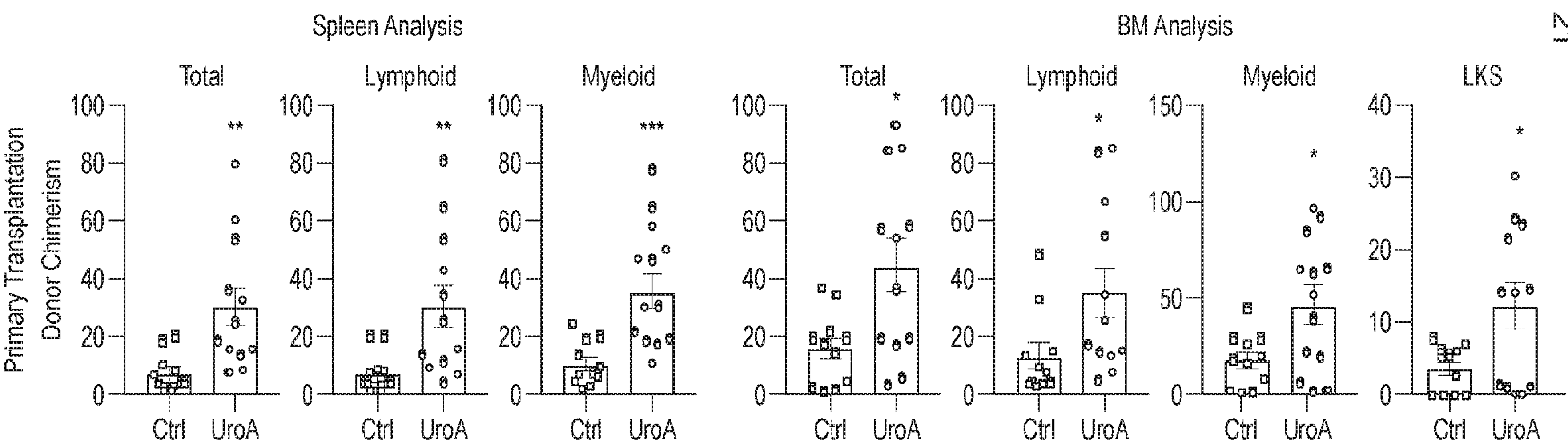


FIG. 5D

FIG. 5E

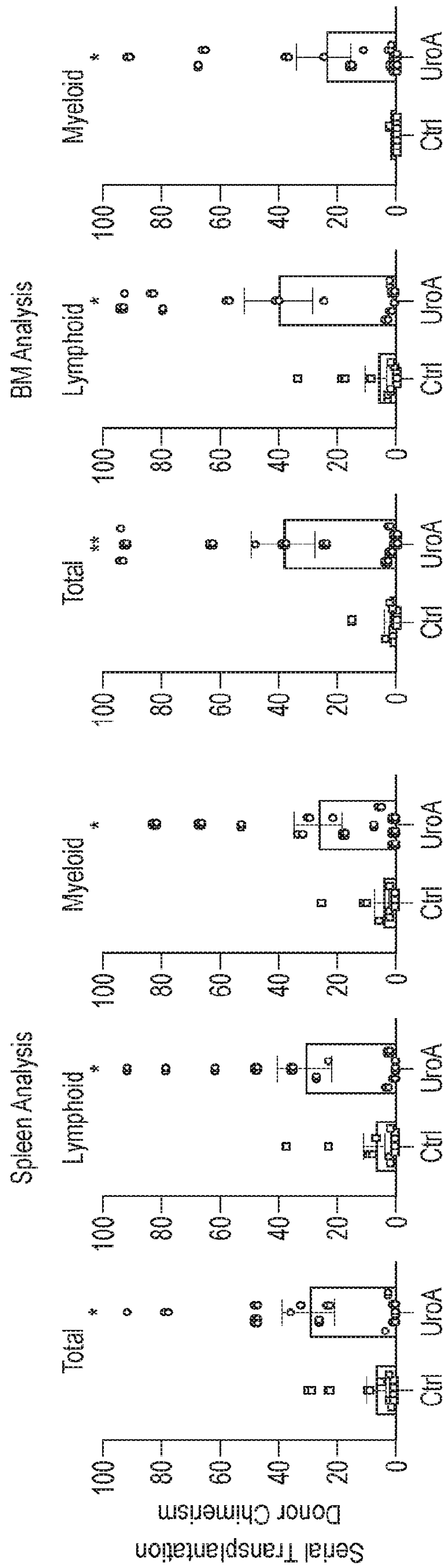


FIG. 5F

FIG. 5G

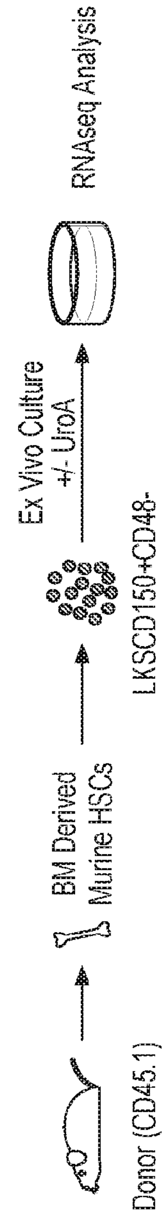


FIG. 6A

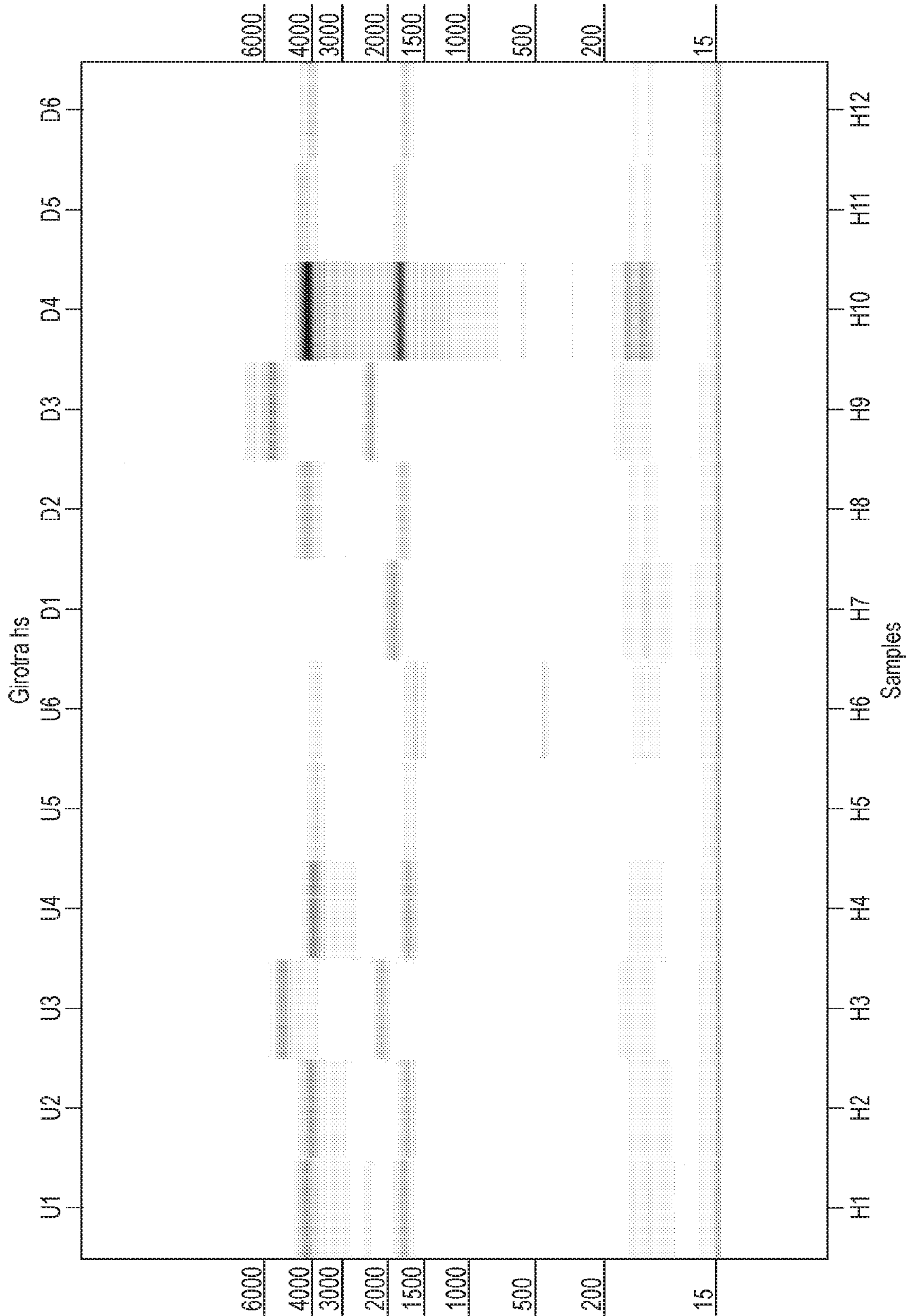


FIG. 6B

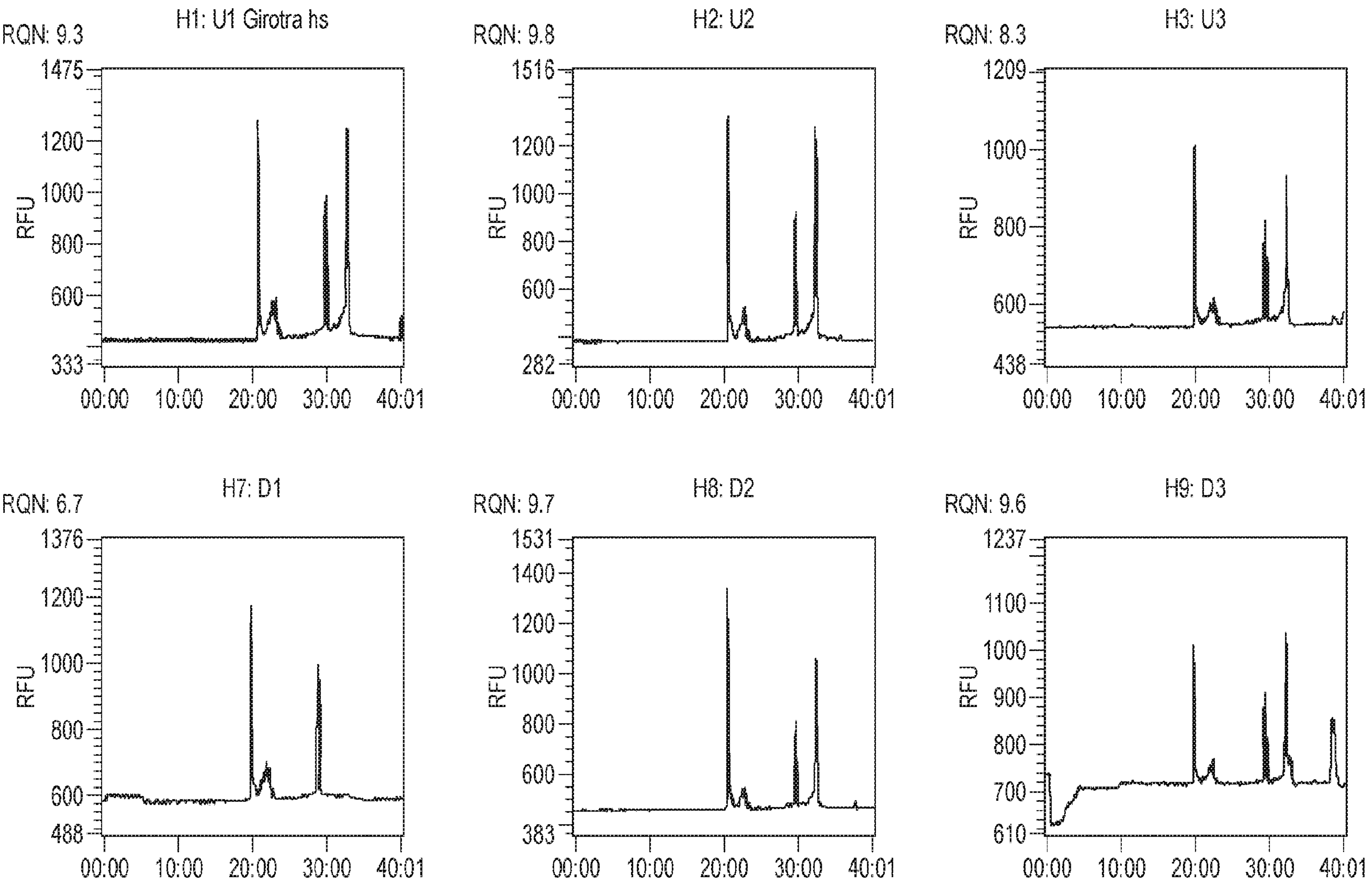


FIG. 6B (Cont.)

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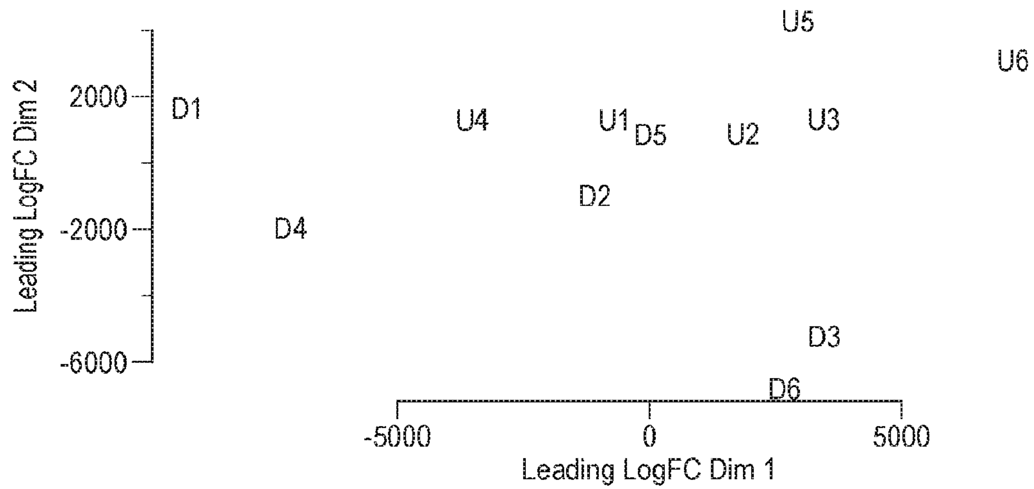


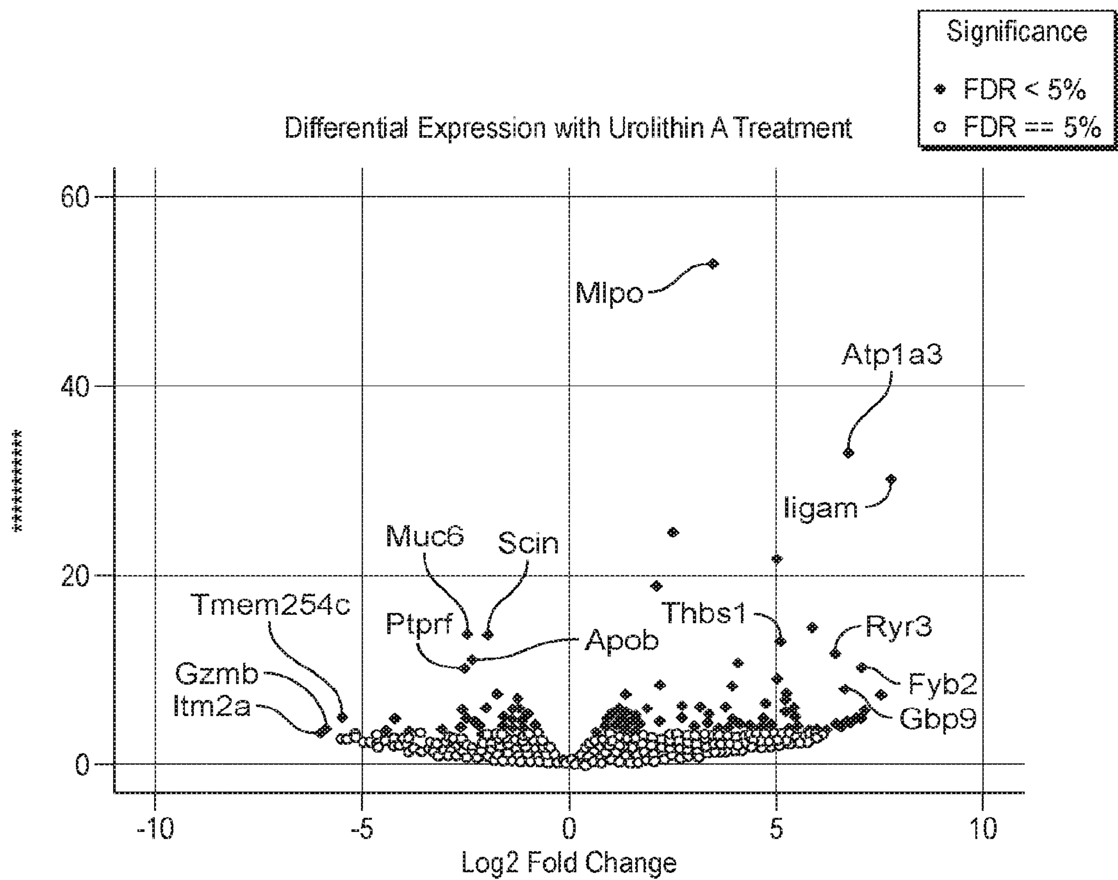


FIG. 6C

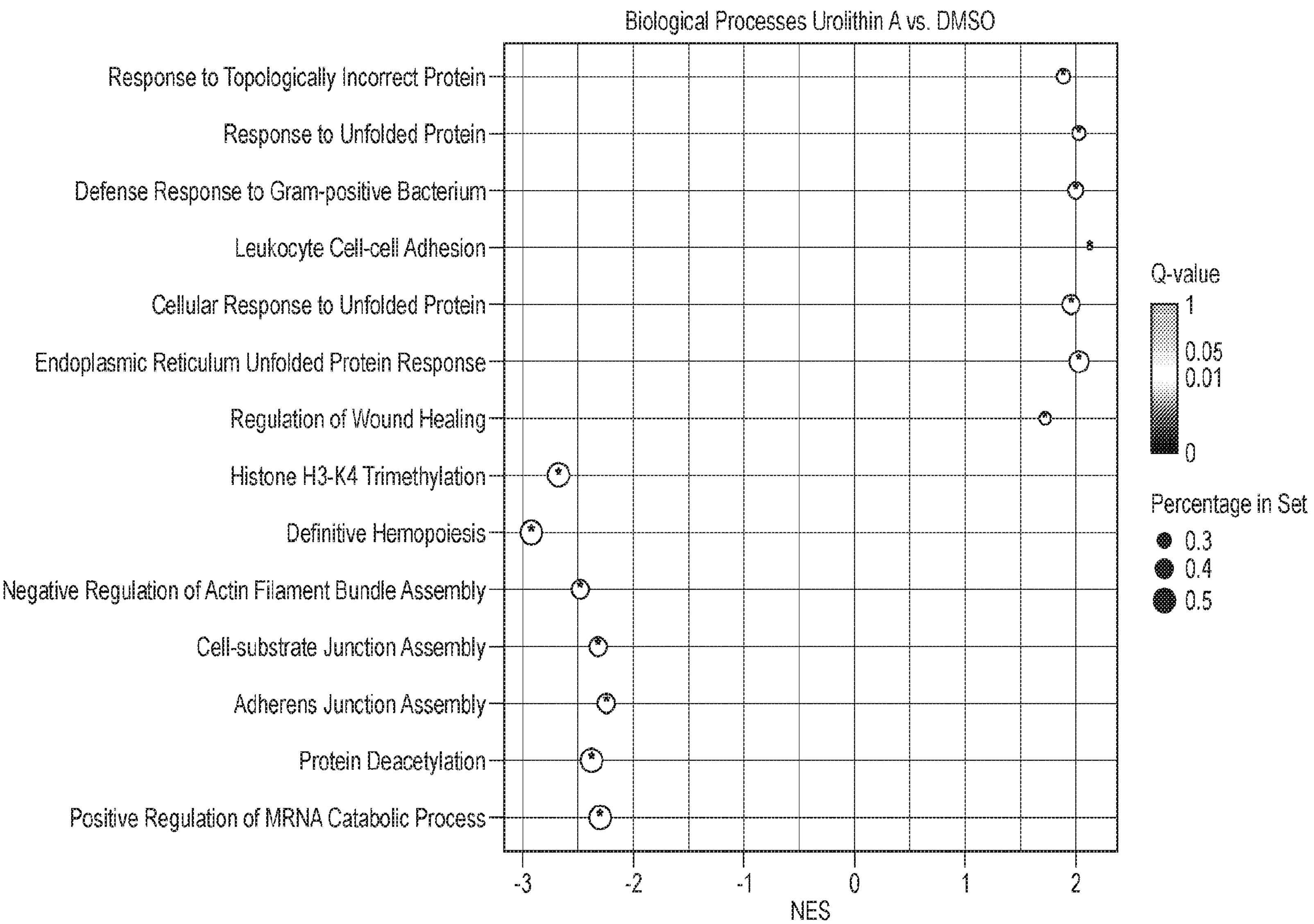


FIG. 6D

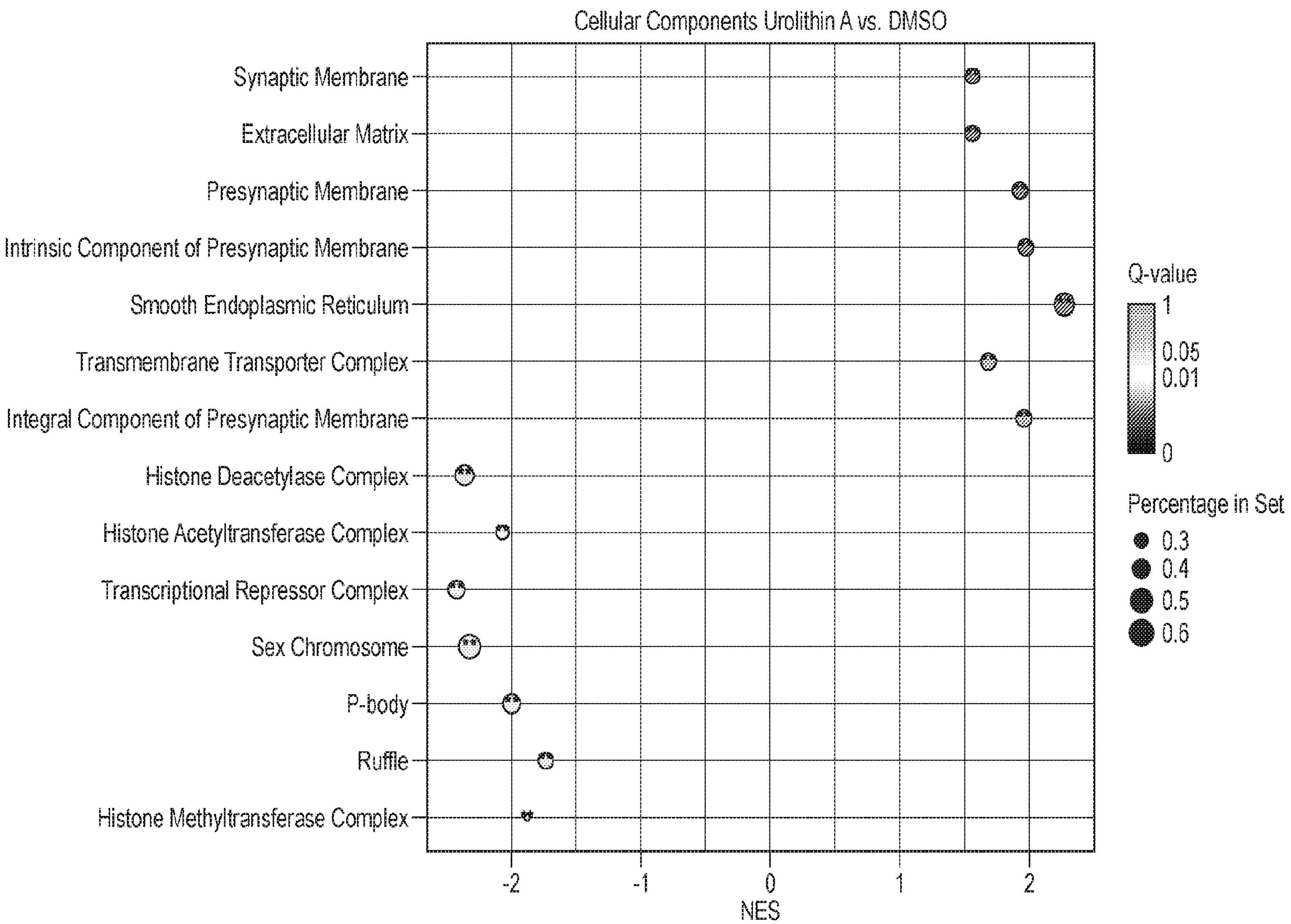
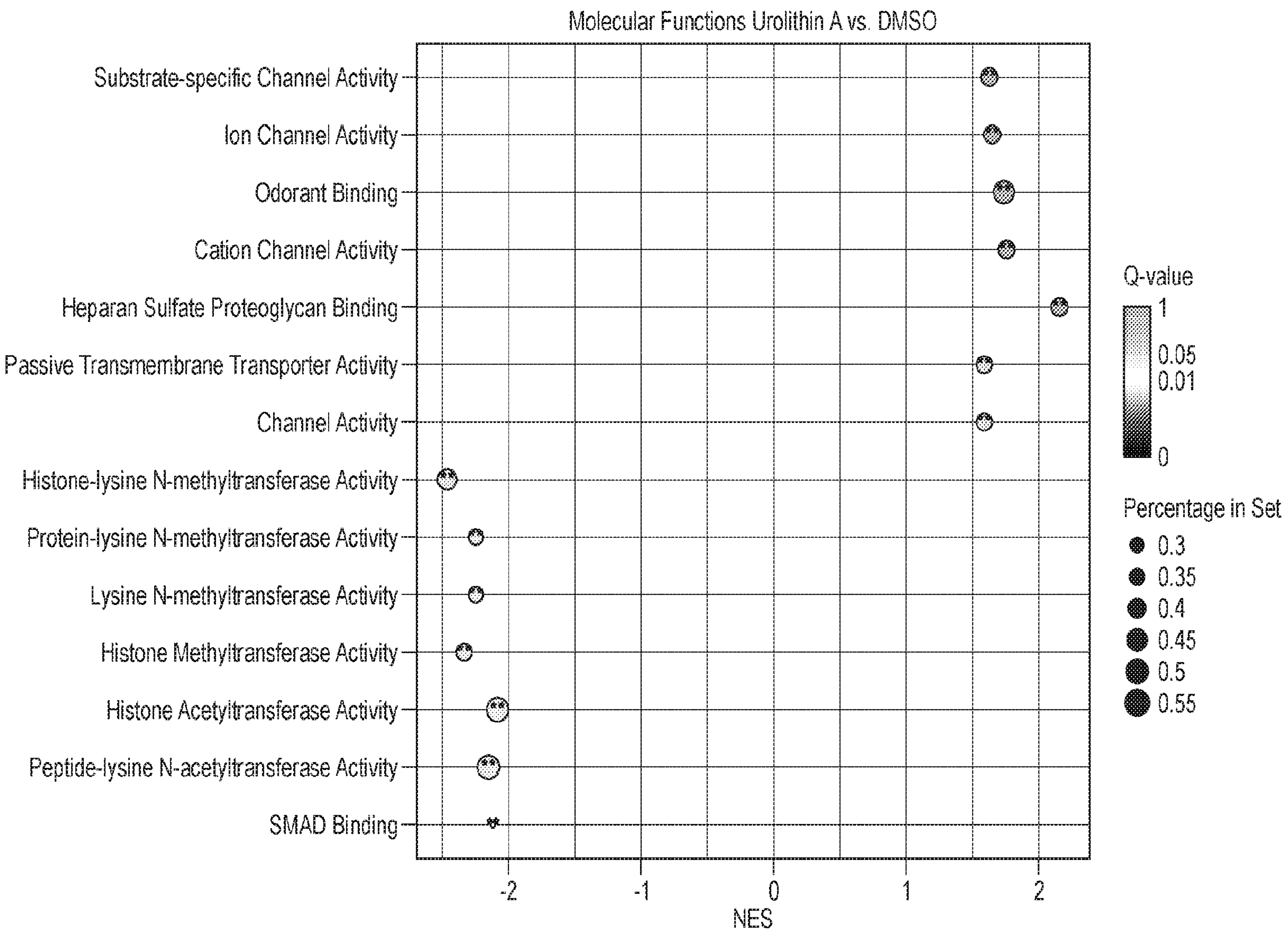


FIG. 6D (Cont.)



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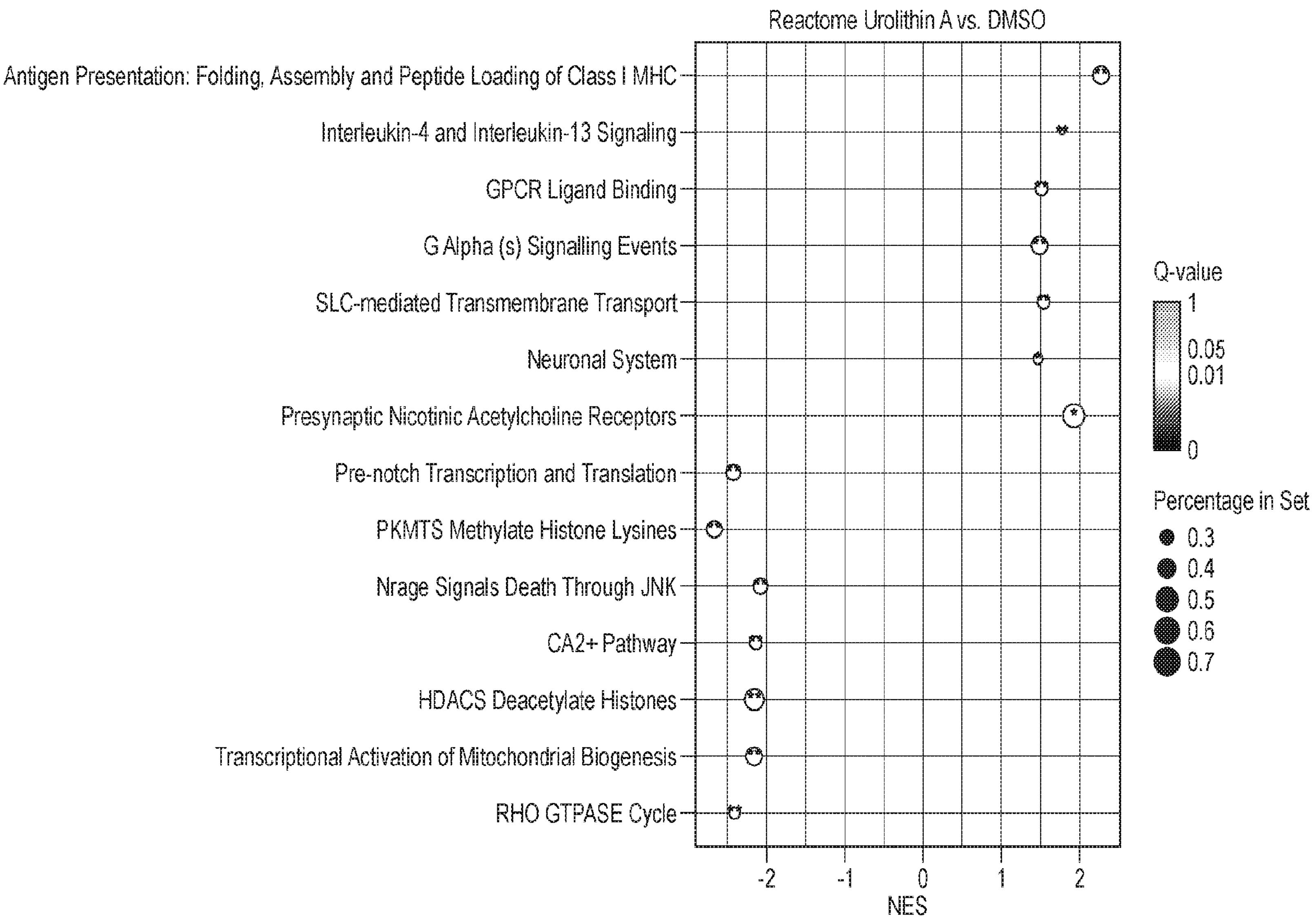

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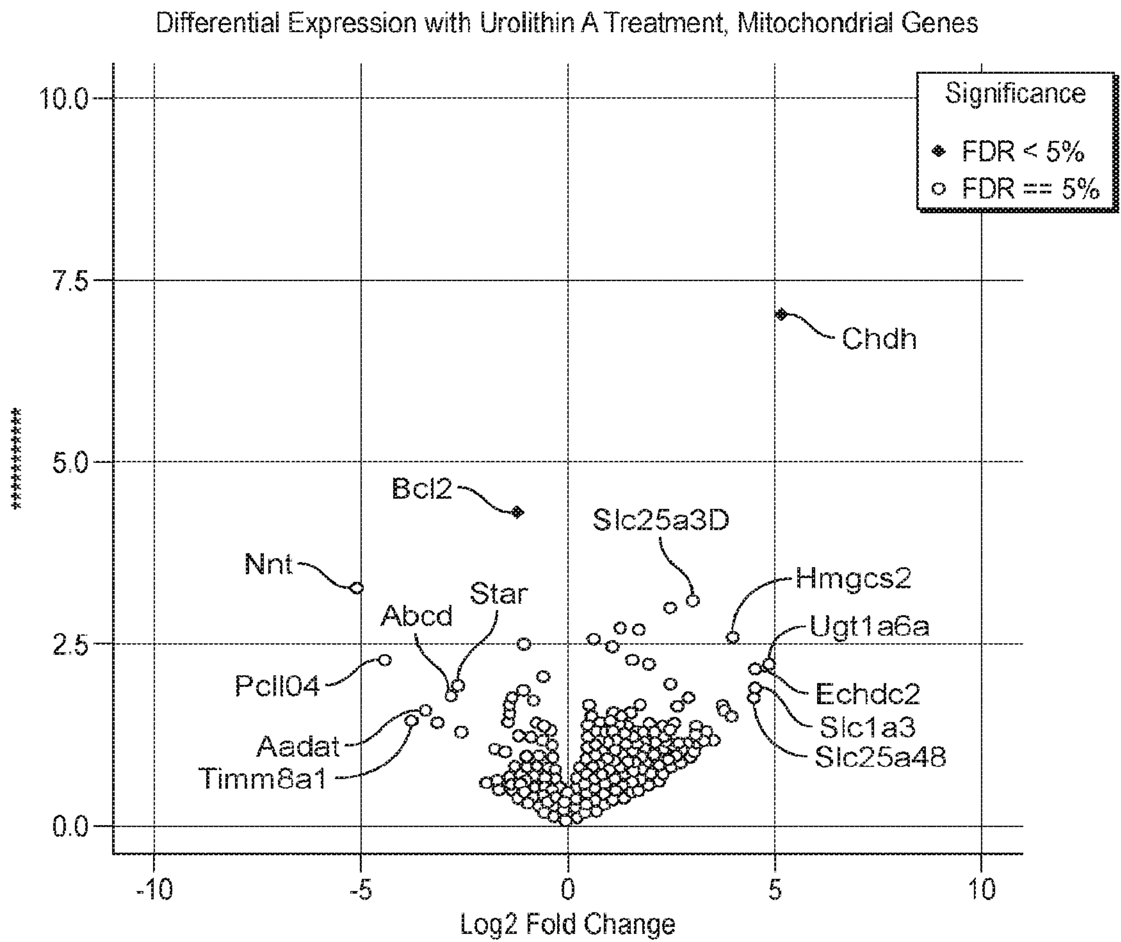

FIG. 6E