(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 September 2016 (22.09.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/149672 A1

(51) International Patent Classification:
C12N 5/0789 (2010.01)

(21) International Application Number:
PCT/US2016/023270

(22) International Filing Date:
18 March 2016 (18.03.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/135,134 18 March 2015 (18.03.2015) US
62/151,684 23 April 2015 (23.04.2015) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th Floor, Oakland, CA 94607-5200 (US).

(72) Inventor: **CHEN, Danica**; 645 Stockton Street #904, San Francisco, CA 94108 (US).

(74) Agents: **WARD, Michael R.** et al.; Morrison & Foerster LLP, 425 Market Street, San Francisco, CA 94105-2482 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2016/149672 A1

(54) Title: METHODS OF PREVENTING AND REVERSING STEM CELL AGING

(57) Abstract: Methods of preventing and reversing stem cell aging including the step of activating the mitochondrial unfolded protein response in a stem cell are described. Further described are methods of promoting stem cell maintenance and methods of preventing and/or reversing tissue degeneration or injury including the step of activating the mitochondrial unfolded protein response.

METHODS OF PREVENTING AND REVERSING STEM CELL AGING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Prov. App. No. 62/135,134, filed March 18, 2015, and U.S. Ser. App. No. 62/151,684, filed April 23, 2015, both of which are hereby incorporated by reference in their entireties.

FIELD

[0002] The present disclosure relates to methods of preventing and/or reversing aging of stem cells, methods for promoting stem cell maintenance, and methods for preventing and/or reversing tissue degeneration or injury.

BACKGROUND

[0003] Despite its central role in the life cycle of a human being, aging remains one of the least understood processes of human biology. Aging is characterized by physiological decline and increased susceptibility to pathologies and mortality. The rate of aging is controlled by evolutionarily conserved genetic pathways (1, 2). The general cause of aging is thought to be the chronic accumulation of cellular damage (2, 3).

[0004] One aspect of aging involves a diminished capacity to maintain tissue homeostasis or to repair tissues after injury. This diminished capacity is evident in certain conditions that occur with aging, such as anemia, sarcopenia (loss of muscle mass), and osteoporosis,. Deterioration or aging of adult stem cells accounts for much of aging-associated compromised tissue maintenance. Adult stem cells mostly reside in a metabolically inactive quiescent state to preserve their integrity, but convert to a metabolically active proliferative state to replenish the tissue (4-6). The signals that trigger stem cells to exit the cell cycle and re-enter quiescence, and the signal transduction leading to the transition remain elusive.

[0005] By 2050 the number of people in the world that will be aged 65 or older is expected to nearly triple to about 1.5 billion, representing 16% of the world's population (NIH, National Institute on Aging, 2011). Yet in spite of this approaching burden on the world's healthcare systems, a basic understanding of the mechanisms of aging and basic treatments to combat aging are lacking. Methods are needed to alleviate aging, and in particular, methods are needed to treat the tissue degeneration and lack of tissue repair that accompanies aging.

[0006] Citation of the above documents and studies is not intended as an admission that any of the foregoing is pertinent prior art. All statements as to the contents of these documents are based on the information available to the applicants and do not constitute any admission as to the correctness of the contents of these documents.

BRIEF SUMMARY

[0007] Accordingly, there is a need for methods to prevent or reverse aging of stem cells. The present disclosure provides methods of reversing aging of stem cells, methods of preventing aging of stem cells, methods of promoting stem cell maintenance, and methods of preventing and/or reversing tissue degeneration or injury, where the methods include a step of activating the mitochondrial unfolded protein response. The teachings herein demonstrate the surprising result that the mitochondrial unfolded protein response plays a critical role in aging of stem cells. Previously, aging of stem cells was thought to be caused primarily by an accumulation of cellular damage.

[0008] Accordingly, certain aspects of the present disclosure relate to a method of reversing aging of stem cells including a step of activating the mitochondrial unfolded protein response in a stem cell, where aging of the stem cell is reversed. Other aspects of the present disclosure relate to a method of preventing aging of stem cells including a step of activating the mitochondrial unfolded protein response in a stem cell, where aging of the stem cell is prevented. Other aspects of the present disclosure relate to a method of promoting stem cell maintenance including a step of activating the mitochondrial unfolded protein response in a stem cell, where the stem cell continues to self-renew. Other aspects of the present disclosure relate to a method of preventing and/or reversing tissue degeneration or injury including a step of activating the

mitochondrial unfolded protein response in a stem cell, where the stem cell is in an animal and where degeneration or injury of a tissue in the animal is prevented and/or reversed.

[0009] In some embodiments of any of the above aspects, the mitochondrial unfolded protein response is activated by activating SIRT7 in the stem cell. In some embodiments the mitochondrial unfolded protein response is activated by activating a mitochondrial stress protein. In some embodiments the mitochondrial stress protein is selected from the group consisting of mtDnaJ, HSP60, HSP10, and ClpP in the stem cell.

[0010] In certain embodiments where the mitochondrial unfolded protein response is activated by activating SIRT7 in the stem cell, SIRT7 is activated by increasing the transcription of the sirt7 gene in the stem cell. In some embodiments SIRT7 is activated by increasing the translation of SIRT7 protein in the stem cell. In some embodiments SIRT7 is activated by delivering an exogenous copy of the sirt7 gene to the stem cell wherein the exogenous sirt7 gene is expressed in the stem cell. In some embodiments the exogenous copy of sirt7 gene is delivered to the stem cell with a viral vector. In some embodiments SIRT7 is activated by a small molecule. In some embodiments SIRT7 is activated by increasing intracellular NAD levels. In some embodiments intracellular NAD levels are increased by delivering small molecules that activate NAD synthesis enzymes to the stem cell. In some embodiments intracellular NAD levels are increased by increasing the level of a NAD precursor in the stem cell. In some embodiments the NAD precursor is selected from the group consisting of nicotinamide mono nucleotide (NMN) and nicotinamide riboside (NR). In some embodiments intracellular NAD levels are increased by increasing the level of a NAD biosynthesis enzyme in the stem cell.

[0011] In some embodiments of aspects of the present disclosure related to a method of reversing aging of stem cells, the stem cell exhibits one or more of the characteristics selected from the group consisting of reduced occurrence of cell death, increased quiescence, increased occurrence of self-renewal, increased ability to repopulate its organ or tissue of origin, increased homing ability, and a change in differentiation profile.

[0012] In some embodiments of any of the aspects of the present disclosure, the stem cell is in an animal. In certain embodiments the animal is a human. In certain embodiments the stem cell is a hematopoietic stem cell. In some embodiments the stem cell originated in an organ or tissue selected from the group consisting of brain, spinal cord, peripheral blood, blood vessels, skeletal muscle, skin, teeth, hair follicle, heart, gut, liver, ovarian epithelium, and testis. In embodiments where the stem cell is a hematopoietic stem cell, the hematopoietic stem cell exhibits improved performance in an assay selected from the group consisting of HSC engraftment, bone marrow reconstitution, and competitive transplantation. In embodiments where the stem cell is in an animal and the animal is a human, blood drawn from the human does not exhibit one or more characteristics selected from the group consisting of increased myeloid differentiation, fewer lymphoid cells, and anemia. In some embodiments the stem cell is an aging stem cell.

[0013] In some embodiments of aspects of the disclosure related to a method of preventing aging of stem cells, aging of the stem cell is delayed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **Figures 1A-1D** depict the stabilization of SIRT7 by NRF1 at the promoters of mitochondrial translational machinery components. **Figure 1A** depicts co-immunoprecipitation of transfected Flag-tagged SIRT7 with endogenous NRF1 from 293T cells. **Figure 1B** depicts co-immunoprecipitation of transfected endogenous SIRT7 with endogenous NRF1 from 293T cells. **Figure 1C** depicts chromatin immunoprecipitation followed by quantitative real-time PCR (ChIP-qPCR) showing SIRT7 occupancy at gene promoters. **Figure 1D** depicts chromatin immunoprecipitation followed by quantitative real-time PCR (ChIP-qPCR) showing NRF1 occupancy at gene promoters. Error bars represent standard error (SE). **: $p<0.01$. ***: $p<0.001$. ns: $p>0.05$. Student's t test.

[0015] **Figures 2A-2C** depict co-occupation by SIRT7 and NRF1 of the same genomic regions at the promoters of mitochondrial translation machinery components. **Figure 2A** depicts SIRT7 bound to the promoters of mRPs but not other mitochondrial genes. **Figure 2B** depicts NRF1 bound to the promoters of mRPs and other mitochondrial genes, but not NME1, a known

target of SIRT7. SIRT7 and NRF1 occupancy at gene promoters in 293T cells was determined by ChIP-PCR compared to IgG negative control samples. All samples were normalized to input DNA. **Figure 2C** depicts a schematic representation of NRF1 and NRF2 consensus binding sequences, and SIRT7 binding sites at gene promoters. SIRT7 binding sites were determined in a ChIP-seq study (Barber et al. Nature 2012). Error bars represent SE. **: $p<0.01$. ***: $p<0.001$.

[0016] **Figures 3A-3B** depict knockdown of NRF1 with siRNA in cells. **Figure 3A** depicts an immunoblot showing reduced expression of NRF1 protein in 293T cells that were transfected with NRF1 siRNA. **Figure 3B** depicts results of qPCR showing a 40% reduction in NRF1 expression in 293T cells that were transfected with NRF1 siRNA. Error bars represent SE. *: $p<0.05$.

[0017] **Figures 4A-4J** depict limitation of mitochondrial activity, proliferation, and PFS^{mt} by SIRT7. **Figure 4A** depicts increased expression of GFM2 and MRPL24 in SIRT7 KD cells. **Figure 4B** depicts the abrogation of increased expression of GFM2 and MRPL24 in SIRT7 KD cells by NRF1 siRNA. **Figure 4C** depicts increased mitochondrial mass in SIRT7 KD cells determined by MTG staining. n=3. **Figure 4D** depicts increased mitochondrial mass in SIRT7 KD cells determined by mitochondrial DNA quantification. n=3. **Figure 4E** depicts increased proliferation in SIT7 KD cells. n=3. **Figure 4F** depicts SIRT7 expression induced by PFS^{mt}. Dox: doxycycline. EB: ethidium bromide. **Figure 4G** depicts increased accumulation of misfolded ΔOTC in SIRT7 KD cells and rescue by NRF1 siRNA. OTC was used as a control. **Figure 4H** depicts increased apoptosis in SIRT7 KD cells treated with ethidium bromide. n=3. **Figure 4I** depicts abrogation of increased PFS^{mt} in SIRT7 KD cells by NRF1 siRNA. **Figure 4J** depicts a proposed model of SIRT7's role in mediating the UPR^{mt}. Error bars represent SE. *: $p<0.05$. **: $p<0.01$. Student's *t* test.

[0018] **Figures 5A-5B** depict repression of NRF1 transcription by SIRT7. **Figure 5A** depicts repression of the expression of mRPs by SIRT7. SIRT7 was knocked down in 293T cells via shRNA. Gene expression in SIRT7 KD cells and control cells was determined by qPCR. **Figure 5B** depicts the repression of the expression of mRPs by SIRT7 via NRF1. SIRT7 KD

293T cells and control cells were treated with control or NRF1 siRNA as indicated. Gene expression was determined by qPCR. NRF1 KD abrogated SIRT7-mediated transcriptional repression of mRPs in cells. Error bars represent SE **: $p<0.01$. ***: $p<0.001$. ns: $p>0.05$.

[0019] **Figures 6A-6J** depict the limitation of mitochondrial activity and of cell proliferation by SIRT7. **Figures 6A – 6D** depict increased mitochondrial activity in SIRT7 KD cells. **Figure 6A** depicts a comparison of SIRT7 KD 293T cells and control cells for citrate synthase activity quantification, **Figure 6B** depicts cellular ATP quantification using a luminescent assay, and **Figures 6C** and **6D** depict Seahorse analyses. **Figures 6E-6G** depict OCR (oxygen consumption rate) and ECAR (extracellular acidification rate). Overexpression of WT but not a catalytically inactive mutant (HY) SIRT7 reduced mitochondrial activity and proliferation. **Figure 6E** depicts a comparison of 293T cells overexpressing WT and mutant SIRT7 and control cells for MTG staining. **Figure 6F** depicts a comparison of 293T cells overexpressing WT and mutant SIRT7 and control cells for Seahorse analyses. **Figure 6G** depicts a comparison of 293T cells overexpressing WT and mutant SIRT7 and control cells for cell proliferation analyses. Cells were counted using a Vi-Cell analyzer. **Figures 6H – 6J** depict restoration of increased mitochondrial activity and proliferation in SIRT7 KD cells by NRF1 siRNA. **Figure 6H** depicts SIRT7 KD 293T cells and control cells treated with control or NRF1 siRNA and analyzed for MTG staining. **Figure 6I** depicts cellular ATP quantification. **Figure 6J** depicts cell proliferation analyses. Error bars represent SE *: $p<0.05$. **: $p<0.01$. ***: $p<0.001$. ns: $p>0.05$.

[0020] **Figures 7A-7F** depict that SIRT7 promotes nutritional stress resistance. **Figure 7A** depicts that SIRT7 expression is increased upon glucose starvation. qPCR is shown comparing SIRT7 expression in 293T cells growing in medium containing 25mM glucose and without glucose. **Figures 7B-7D** depict SIRT7 causing an increase in nutrient starvation stress resistance. **Figure 7B** depicts SIRT7 OE in 293T cells and control cells that were deprived of glucose for 68 hours. **Figures 7C and 7D** depict SIRT7 KD 293T cells and control cells that were deprived of glucose (**Fig. 7C**) and glutamine (**Fig. 7D**) for 48 hours. Cells were counted using a Vi-Cell analyzer. **Figures 7E** and **7F** depict that NRF1 KD attenuated the sensitivity of SIRT7 deficient cells to glucose (**Fig. 7E**) or glutamine (**Fig. 7F**) starvation. SIRT7 KD 293T cells and control cells were treated with control or NRF1 siRNA. Cells were deprived of glucose

or glutamine for 48 hours. Cells were counted using a Vi-Cell analyzer. Error bars represent SE *: $p<0.05$. **: $p<0.01$.

[0021] **Figures 8A-8C** depict that SIRT7 represses NRF1 activity to suppress PFS^{mt}. **Figures 8A** and **8B** depict SIRT7 repression of PFS^{mt}. SIRT7 KD 293T cells and control cells were treated with or without EB for 7 days. The expression of UPR^{mt} genes (ClpP and HSP60) were determined by immunoblots (Fig. 8A) or qPCR (Fig. 8B). **Figure 8C** depicts SIRT7 repression of NRF1 activity to suppress PFS^{mt}. SIRT7 KD 293T cells and control cells were treated with control or NRF1 siRNA. The expression of UPR^{mt} (ClpP, HSP10, HSP60, mtDnaJ) and UPR^{ER} genes (Grp78) was determined by qPCR.

[0022] **Figures 9A-9C** depict SIRT7 expression in various tissues and cellular compartments. **Figure 9A** depicts that SIRT7 is highly expressed in the bone marrow. The expression of SIRT7 in various tissues was compared by qPCR. **Figure 9B** depicts that SIRT7 is ubiquitously expressed in various hematopoietic cellular compartments in the bone marrow. Various cell populations in the bone marrow were isolated via cell sorting based on cell surface markers. HSC, Lin-c-Kit+Sca1+CD150+CD48-; multipotent progenitors (MPPs), Lin-c-Kit+Sca1+CD150-CD48-; CD48+, Lin-c-Kit+Sca1+CD48+; myeloid progenitors (MPs), Lin-c-Kit+Sca1-; and differentiated blood cells, Lin+. The expression of SIRT7 was determined by qPCR. **Figure 9C** depicts the gating strategy for sorting HSCs. Error bars represent SE.

[0023] **Figures 10A-10I** depict that SIRT7 ensures HSC maintenance. **Figure 10A** depicts qPCR showing increased PFS^{mt} in $SIRT7^{-/-}$ HSCs. n=4. **Figures 10B** and **10C** depict MTG staining and mitochondrial DNA quantification showing increased mitochondrial mass in $SIRT7^{-/-}$ HSCs. n=4. MP: myeloid progenitor cells. Lin-: lineage negative cells. **Figure 10D** depicts in vivo BrdU incorporation showing increased proliferation of $SIRT7^{-/-}$ HSCs. n=4. **Figure 10E** depicts increased propensity of $SIRT7^{-/-}$ HSCs to cycle upon ex vivo culture with cytokines, indicated by BrdU pulse. n=4. **Figure 10F** depicts that mice reconstituted with $SIRT7^{-/-}$ BMCs have increased sensitivity to 5-Fluorouracil. n=12. Log-rank test. **Figure 10G** depicts competitive transplantation using $SIRT7^{+/+}$ and $SIRT7^{-/-}$ BMCs as donors, which shows the reduced reconstitution capacity of $SIRT7^{-/-}$ HSCs. n=15. Representative of 5 transplants. **Figure**

10H depicts reduced white blood cell count in SIRT7$^{-/-}$ mice. **Figure 10I** depicts myeloid-biased differentiation in the peripheral blood (PB) of SIRT7$^{-/-}$ mice. MNCs: mononuclear cells. n=7. Error bars represent SE. *: $p<0.05$. ***: $p<0.001$. ns: $p>0.05$. Student's *t* test.

[0024] **Figure 11** depicts that SIRT7 suppresses mitochondrial number in HSCs. Electron microscopy of SIRT7$^{+/+}$ and SIRT7$^{-/-}$ HSCs is depicted.

[0025] **Figures 12A-12G** depict that SIRT7 ensures HSC maintenance. **Figure 12A** depicts competitive transplantation using HSCs isolated from SIRT7$^{+/+}$ and SIRT7$^{-/-}$ mice as donors, which shows reduced reconstitution capacity of SIRT7$^{-/-}$ HSCs. n=15. **Figure 12B** depicts the frequency of HSCs in the bone marrow of SIRT7$^{+/+}$ and SIRT7$^{-/-}$ mice, as determined via flow cytometry. n = 4. **Figure 12C** depicts SIRT7$^{+/+}$ or SIRT7$^{-/-}$ HSCs transduced with NRF1 KD lentivirus or control lentivirus that were used as donors in a competitive transplantation assay. Data shown are the frequency of donor-derived HSCs in the bone marrow of recipient mice. n=6. **Figure 12D** depicts Annexin V staining showing increased apoptosis in SIRT7$^{-/-}$ HSCs under transplantation stress. n=7. **Figure 12E** depicts competitive transplantation using HSCs isolated from SIRT7$^{+/+}$ and SIRT7$^{-/-}$ mice as donors. Data shown are the distribution of donor-derived myeloid or lymphoid lineage in the peripheral blood of transplant recipients. n=15. **Figures 12F** and **12G** depict SIRT7$^{+/+}$ or SIRT7$^{-/-}$ HSCs transduced with SIRT7 lentivirus or control lentivirus that were used as donors in a competitive transplantation assay. Data shown are the percentage of total donor-derived contribution (**Fig. 12F**) and donor-derived mature hematopoietic subpopulations (**Fig. 12G**) in the peripheral blood of recipients. n=7. Error bars represent SE. *: $p<0.05$. **: $p<0.01$. ***: $P<0.001$. ns: $p>0.05$.

[0026] **Figures 13A-13D** depict repression of NRF1 activity by SIRT7 to ensure HSC maintenance. SIRT7$^{+/+}$ or SIRT7$^{-/-}$ HSCs transduced with NRF1 KD lentivirus or control lentivirus were used as donors in a competitive transplantation assay. Data shown are qPCR analyses of UPR^{mt} gene expression (**Fig. 13A**) and cell cycle analysis with Ki67 staining (**Fig. 13B**) of donor-derived HSCs, the percentage of total donor-derived contribution (**Fig. 13C**) and donor-derived mature hematopoietic subpopulations (**Fig. 13D**) in the peripheral blood of recipients. n=7. Error bars represent SE. *: $p<0.05$. **: $p<0.01$.

[0027] **Figures 14A-14E** depict that HSC aging is regulated by SIRT7. **Figure 14A** depicts qPCR showing reduced SIRT7 expression in aged HSCs. n=3. **Figures 14B** and **14C** depict qPCR showing that increased PFS^{mt} in aged HSCs is rescued by SIRT7 overexpression. n=3. **Figures 14D** and **14E** depict competitive transplantation using aged HSCs transduced with SIRT7 or control lentivirus as donors. SIRT7 overexpression increased reconstitution capacity and reversed myeloid-biased differentiation of aged HSCs. n=7. Error bars represent SE. *: $p<0.05$. **: $p<0.01$. Student's *t* test.

[0028] **Figures 15A-B** depict HSC aging regulated by NRF1. **Figures 15A** and **15B** depict competitive transplantation using aged HSCs transduced with NRF1 KD virus or control virus as donors, which shows that NRF1 inactivation increased reconstitution capacity and reversed myeloid-biased differentiation of aged HSCs. Data shown are the percentage of donor-derived cells in the peripheral blood of the recipients (**Fig. 15A**) and the percentage of lymphoid and myeloid cells in donor-derived cells in the peripheral blood of the recipients (**Fig. 15B**). n=7. Error bars represent SE *: $p<0.05$.

DETAILED DESCRIPTION

[0029] In one aspect, the present disclosure provides methods of reversing aging of stem cells where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell and where aging of the stem cell is reversed. In another aspect, the present disclosure provides methods of preventing aging of stem cells where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell and where aging of the stem cell is prevented. In another aspect, the present disclosure provides methods of promoting stem cell maintenance where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell and where the stem cell continues to self-renew. In another aspect, the present disclosure provides methods of preventing and/or reversing tissue degeneration or injury where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell, where the stem cell is in an animal, and where degeneration or injury of a tissue in the animal is prevented and/or reversed.

Activating the Mitochondrial Unfolded Protein Response

[0030] The methods of the present disclosure include a step of activating the mitochondrial unfolded protein response in a stem cell. The mitochondrial unfolded protein response is a retrograde signaling pathway leading to transcriptional upregulation of mitochondrial chaperones and stress relief. Perturbation of mitochondrial proteostasis, a form of mitochondrial stress, is one way by which the mitochondrial unfolded protein response is activated.

Activating SIRT7 in the Stem Cell

[0031] In some embodiments, the mitochondrial unfolded protein response is activated by activating SIRT7 in the stem cell. SIRT7 may be activated in the stem cell by any methods known in the art. For example, in some embodiments, SIRT7 is activated by increasing the transcription of the sirt7 gene in the stem cell. In other embodiments, SIRT7 is activated by increasing the translation of SIRT7 protein in the stem cell. Methods of increasing the transcription of the sirt7 gene or of increasing the translation of SIRT7 protein in the stem cell include, without limitation, increasing the gene copy number of sirt7 in the stem cell and expressing sirt7 with a promoter that gives higher levels of expression of sirt7 than that of its native promoter. In some embodiments, SIRT7 is activated by delivering an exogenous copy of the sirt7 gene to the stem cell such that the exogenous sirt7 gene is expressed in the stem cell. In certain embodiments, the exogenous copy of the sirt7 gene is delivered to the stem cell with a viral vector. The viral vector may be, for example, derived from a lentivirus.

[0032] In some embodiments SIRT7 is activated by a small molecule. A small molecule may be, for example, an organic molecule. Organic molecules relate or belong to the class of chemical compounds having a carbon basis, the carbon atoms linked together by carbon-carbon bonds. The original definition of the term organic related to the source of chemical compounds, with organic compounds being those carbon-containing compounds obtained from plant or animal or microbial sources, whereas inorganic compounds were obtained from mineral sources. Organic compounds can be natural or synthetic. Alternatively the compound may be an inorganic compound. Inorganic compounds are derived from mineral sources and include all compounds without carbon atoms (except carbon dioxide, carbon monoxide and carbonates). Small molecule activators of SIRT7 may be identified, for example, by high throughput screening, such

as a forward chemical genetic screen. Small molecules that activate SIRT7 may be delivered to stem cells by any method known in the art. For example, small molecule activators of SIRT7 may be delivered to stem cells with nanoparticles that are targeted to a particular type of stem cell. See, *e.g.*, WO2007133750 A2 and WO2006033679 A2.

[0033] SIRT7 activity is dependent on NAD. Accordingly, in some embodiments SIRT7 is activated by increasing intracellular NAD levels. In some embodiments intracellular NAD levels are increased by delivering small molecules to the stem cell that activate NAD biosynthesis enzymes. NAD biosynthesis enzymes include nicotinamide phosphoribosyltransferase (NamPRT), nicotinic acid phosphoribosyltransferase (NAPRT), quinolinic acid phosphoribosyltransferase (QAPRT), nicotinamide riboside kinase (NRK), nicotinamide adenylyltransferase (NMNAT), and NAD synthetase (NADS). Small molecules that activate NAD biosynthesis enzymes may be delivered to stem cells by any method known in the art. For example, small molecule activators of NAD biosynthesis enzymes may be delivered to stem cells with nanoparticles that are targeted to a particular type of stem cell. See, *e.g.*, WO2007133750 A2 and WO2006033679 A2.

[0034] In other embodiments intracellular NAD levels are increased by increasing the level of a NAD precursor in the stem cell. The NAD precursor may be, for example, nicotinamide mono nucleotide (NMN), nicotinamide riboside (NR), or nicotinamide. The level of NAD precursors in the stem cell may be increased by any method known in the art. For example, NAD precursors may be ingested as nutritional supplements.

[0035] In some embodiments, intracellular NAD levels are increased by increasing the level of a NAD biosynthesis enzyme in the stem cell. Increasing the level of a NAD biosynthesis enzyme in the stem cell may be achieved, for example, by increasing the transcription of the gene encoding such an enzyme or by increasing the translation of the enzyme in the stem cell. An exogenous copy of a gene encoding a NAD biosynthesis enzyme may also be delivered to the stem cell such that the exogenous gene is expressed in the stem cell. The exogenous copy of the gene encoding a NAD biosynthesis enzyme may be delivered to the stem cell with a viral vector such as a vector derived from a lentivirus.

Activating a Mitochondrial Stress Protein

[0036] In some embodiments, the mitochondrial unfolded protein response is activated by activating a mitochondrial stress protein in the stem cell. Mitochondrial stress proteins are encoded by nuclear genes that are transcriptionally upregulated in response to the accumulation of unfolded protein within the mitochondrial matrix (Zhao et al., *EMBO J* 2002, 21(17): 4411-4419). Mitochondrial stress proteins include, without limitation, mtDnaJ, HSP60, HSP10, and ClpP. Mitochondrial stress proteins may be activated, for example, by increasing the level of a mitochondrial stress protein in the stem cell. Increasing the level of a mitochondrial stress protein in the stem cell may be achieved, for example, by increasing the transcription of the gene encoding such a protein or by increasing the translation of such a protein in the stem cell. An exogenous copy of a gene encoding a mitochondrial stress protein may also be delivered to the stem cell such that the exogenous gene is expressed in the stem cell. The exogenous copy of the gene encoding a mitochondrial stress protein may be delivered to the stem cell with a viral vector such as a vector derived from a lentivirus.

Methods of Reversing Aging of Stem Cells

[0037] In one aspect of the present disclosure, a method of reversing stem cell aging where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell and where aging of the stem cell is reversed is provided. The aging of a stem cell is reversed where any property of an aging stem cell changes to be characteristic of a non-aging stem cell. Aging stem cells may exhibit one or more of the following properties or characteristics: decreased per cell repopulating activity, decreased self-renewal and homing abilities, myeloid skewing of differentiation, and increased apoptosis with stress (Janzen et al., *Nature*, 2006, v. 443, p. 421). Thus, in some embodiments, aging of the stem cell is reversed where the stem cell exhibits one or more of the following characteristics: reduced occurrence of cell death, increased quiescence, increased occurrence of self-renewal, increased ability to repopulate its organ or tissue of origin, increased homing ability, a change in differentiation profile, decreased transcriptional activity, decreased cell size, decreased mitochondrial metabolic activity, and a change in chromatin profile. Any methods known in the art to evaluate these properties may be

used to identify whether the aging of a stem cell is reversed. For example, assays such as competitive repopulation and transplantation, engraftment, colony-forming unit assays, long-term culture assays, cell proliferation assays such as BrdU or EdU incorporation, transcriptional profiling by microarray analysis, chromatin immunoprecipitation sequencing (ChIP-seq), cellular ATP measurements, secondary neurophore formation assays, secondary neurophore differentiation assays, and caspase activity assays may be used. Description of these assays and others may be found, for example, in the following publications: Mohrin *et al.*, *Science* 347, 1374 (March 20, 2015); Tang, Rando, *EMBO J.* 33, 2782 (Dec. 1, 2014); Rodgers *et al.*, *Nature* 510, 393 (June 19, 2014); Liu, *et al.*, *Cell Rep.* 4, 189 (Jul. 11, 2013); Renault *et al.*, *Cell Stem Cell.* 5, 527 (November 6, 2009); Webb *et al.*, *Cell Rep.* 4, 477 (Aug. 15, 2013); Keyes *et al.*, *PNAS* 110, E4950 (Dec. 17, 2013); Hsu *et al.*, *Nat. Med.* 20, 847 (Aug. 2014).

[0038] In some embodiments where the stem cell is a hematopoietic stem cell, aging of the stem cell is reversed where the hematopoietic stem cell exhibits improved performance in an assay such as HSC engraftment, bone marrow reconstitution, or competitive transplantation. These assays measure the ability of transplanted stem cells to gain access to the bone marrow of an irradiated recipient animal, take up residence in the bone marrow, undergo self-renewing cell division to produce a larger pool of hematopoietic stem cells, and differentiate to generate different cell types (See, *e.g.*, *J. Clin Invest.* 2002; 110(3): 303-304). In some embodiments where the stem cell is in a human and where the stem cell is a hematopoietic stem cell, reversal of stem cell aging is detected by drawing and testing blood. Blood drawn from a human where stem cell aging has been reversed does not exhibit one or more of the following characteristics typical of aging, such as increased myeloid differentiation, fewer lymphoid cells, or anemia. Myeloid differentiation, lymphoid cells, and anemia may be measured with standard assays known in the art (*See*, *e.g.*, Pang et al., PNAS 108, 20012 (Dec. 13, 2011).

Methods of Preventing Aging of Stem Cells

[0039] In one aspect of the present disclosure, a method of preventing stem cell aging where the method includes a step of activating the mitochondrial unfolded protein response in a stem cell and where aging of the stem cell is prevented is provided. In some embodiments, aging of a

stem cell is prevented where aging of the stem cell is delayed. Aging of a stem cell is delayed where, for example, characteristics or properties of an aging stem cell occur later than those characteristics or properties would occur for an unmanipulated stem cell. A delay in aging of a stem cell may be apparent when stem cells from an older adult have similar characteristics to stem cells from a young adult. Characteristics or properties of an aging stem cell and of stem cells from an older adult include, without limitation, decreased per cell repopulating activity, decreased self-renewal and homing abilities, myeloid skewing of differentiation, and increased apoptosis with stress.

Methods of Promoting Stem Cell Maintenance

[0040] In one aspect of the present disclosure, a method of promoting stem cell maintenance where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell and where the stem cell continues to self-renew is provided. Self-renewal of stem cells is division of the stem cell with maintenance of the undifferentiated state. Assays to measure the self-renewal capabilities of stem cells include, without limitation, a serial dilution assay, a colony-forming unit assay, a long-term culture assay, and a secondary neurophore formation assay.

Methods of Preventing and/or Reversing Tissue Degeneration or Injury

[0041] Deterioration or aging of adult stem cells accounts for much of aging-associated compromised tissue maintenance. Certain conditions that occur with aging reflect compromised tissue maintenance. These conditions include, for example, anemia, sarcopenia, and osteoporosis. In addition, aging of adult stem cells contributes to a diminished capacity to repair tissues after injury. This diminished capacity to repair is exemplified in poor wound healing of skin, reduced angiogenesis in damaged organs, reduced remyelination in the central nervous system in response to demyelinating conditions, and fibrous scarring instead of the formation of new muscle fibers following a necrotic injury (*Cell Cycle* 4:3, 407-410; 2005). Accordingly, in one aspect of the present disclosure, a method of preventing and/or reversing tissue degeneration or injury where the method includes the step of activating the mitochondrial unfolded protein response in a stem cell, where the stem cell is in an animal, and where degeneration or injury of a

tissue in the animal is prevented and/or reversed is provided. The degenerated or injured tissue may be, without limitation, brain, spinal cord, peripheral blood, blood vessels, skeletal muscle, skin, teeth, hair follicle, heart, gut, liver, ovarian epithelium, or testis.

Stem Cells

[0042] The methods described herein may be performed with a stem cell from any animal and from any tissue or organ, without limitation. A stem cell is generally defined as a cell that is capable of renewing itself and can give rise to more than one type of cell through asymmetric cell division. Stem cells exist in many tissues of embryos and adult mammals. Pluripotent stem cells have the ability to differentiate into almost any cell type, and multipotent stem cells have the ability to differentiate into many cell types. In certain embodiments, the stem cell originated in brain, spinal cord, peripheral blood, blood vessels, skeletal muscle, skin, teeth, hair follicle, heart, gut, liver, ovarian epithelium, or testis. In certain embodiments of the methods of the present disclosure, the stem cell is in an animal. In some embodiments, the animal is a human.

Hematopoietic Stem Cells (HSCs)

[0043] In certain embodiments, the stem cell is a hematopoietic stem cell. Hematopoietic stem cells may be isolated from blood (i.e. hematopoietic tissue). Possible sources of human hematopoietic tissue include, but are not limited to, embryonic hematopoietic tissue, fetal hematopoietic tissue, and post-natal hematopoietic tissue. Embryonic hematopoietic tissue can be yolk sac or embryonic liver. Fetal hematopoietic tissue can come from fetal liver, fetal bone marrow and fetal peripheral blood. The post-natal hematopoietic can be cord blood, bone marrow, normal peripheral blood, mobilized peripheral blood, hepatic hematopoietic tissue, or splenic hematopoietic tissue.

[0044] HSCs suitable for use with the methods of the present disclosure may be obtained by any suitable technique known in the art. For example, HSCs may be found in the bone marrow of a donor, which includes femurs, hip, ribs, sternum, and other bones. Any method known in the art for extracting or harvesting bone marrow cells may be used. In one non-limiting example, HSCs may be obtained directly from the marrow cavity of the hip using a needle and syringe to

aspirate cells from the marrow cavity. Rich marrow may be obtained from the hip by performing multiple small aspirations.

[0045] Alternatively, suitable HSCs may be obtained from peripheral blood cells found in the blood of a donor, optionally following pre-treatment with cytokines, such as G-CSF (granulocyte colony-stimulating factors), that induce HSCs to be released from the bone marrow compartment of the donor. HSCs may also be obtained from peripheral blood that has undergone an apheresis procedure to enrich for HSCs. Any apheresis procedure known in the art may be used. In certain embodiments, the apheresis procedure is a leukapheresis procedure.

[0046] Additionally, suitable HSCs may be obtained from umbilical cord blood, placenta, and mobilized peripheral blood. For experimental purposes, fetal liver, fetal spleen, and AGM (Aorta-gonad-mesonephros) of animals are also useful sources of HSCs. Additionally, HSCs may be procured from a source that obtained HSCs from the bone marrow, peripheral blood, umbilical cord, or fetal tissue of a donor.

[0047] In some embodiments, HSCs are obtained from a human umbilical cord or placenta. Another source of HSCs that may be utilized is the developing blood-producing tissues of fetal animals. In humans, HSCs may be found in the circulating blood of a human fetus by about 12 to 18 weeks.

[0048] In some embodiments, human HSCs are obtained from any source, e.g., the bone marrow, umbilical cord, peripheral blood, or fetal tissue of blood, of type A+, A-, B+, B-, O+, O-, AB+, and AB- donors. In other embodiments, human HSCs are obtained from any source, e.g., the bone marrow, umbilical cord, peripheral blood, or fetal tissue of blood, of universal donors or donors having a rare blood type.

[0049] In other embodiments, human HSCs are obtained from any source, e.g., the bone marrow, umbilical cord, peripheral blood, or fetal tissue of blood, of donors having an aging disorder or aging-associated condition that would benefit from a transplantation of HSCs and/or transfusion of blood. Such donors may also be the recipients. Advantageously, HSCs obtained from such donor may be used for personalized HSC and/or blood therapy.

[0050] In one non-limiting example, human HSCs may be obtained by anesthetizing the stem cell donor, puncturing the posterior superior iliac crest with a needle, and performing aspiration of bone marrow cells with a syringe. In another non-limiting example, HSCs may be obtained from the peripheral blood of a donor, where a few days prior to harvesting the stem cells form the peripheral blood, the donor is injected with G-CSF in order to mobilize the stem cells to the peripheral blood.

[0051] Cells obtained from, for example, bone marrow, peripheral blood, or cord blood, are typically processed after extraction or harvest. Any method known in the art for processing extracted or harvested cells may be used. Examples of processing steps include, without limitation, filtration, centrifugation, screening for hematopathologies, screening for viral and/or microbial infection, erythrocyte depletion, T-cell depletion to reduce incidence of graft-versus-host disease in allogenic stem cell transplant recipients, volume reduction, cell separation, resuspension of cells in culture medium or a buffer suitable for subsequent processing, separation of stem cells from non-stem cells (e.g., stem cell enrichment), *ex vivo* or *in vitro* stem cell expansion with growth factors, cytokines, and/or hormones, and cryopreservation.

[0052] Any suitable method for stem cell enrichment known in the art may be used. Examples of stem cell enrichment methods include, without limitation, fluorescence activated cell sorting (FACS) and magnetic activated cell sorting (MACS).

[0053] Accordingly, in certain embodiments, HSCs suitable for use in the methods of the present disclosure are human HSCs.

Identification and Targeting of HSCs

[0054] HSCs obtained from a donor may be identified and/or enriched by any suitable method of stem cell identification and enrichment known in the art, such as by utilizing certain phenotypic or genotypic markers. For example, in some embodiments, identification of HSCs includes using cell surface markers associated with HSCs or specifically associated with terminally differentiated cells of the system. Suitable surface markers may include, without limitation, one or more of c-kit, Sca-1, CD4, CD34, CD38, Thy1, CD2, CD3, CD4, CD5, CD8,

CD43, CD45, CD59, CD90, CD105, CD133, CD135, ABCG2, NK1.1, B220, Ter-119, Flk-2, CDCP1, Endomucin, Gr-1, CD46, Mac-1, Thy1.1, and the signaling lymphocyte activation molecule (SLAM) family of receptors. Examples of SLAM receptors include, without limitation, CD150, CD48, and CD244.

[0055] In some embodiments of the present disclosure, small molecules are delivered to stem cells. Cell surface markers associated with HSCs, such as c-kit, Sca-1, CD4, CD34, CD38, Thy1, CD2, CD3, CD4, CD5, CD8, CD43, CD45, CD59, CD90, CD105, CD133, CD135, ABCG2, NK1.1, B220, Ter-119, Flk-2, CDCP1, Endomucin, Gr-1, CD46, Mac-1, Thy1.1, and the signaling lymphocyte activation molecule (SLAM) family of receptors, may be helpful in targeting small molecules to HSCs where the HSC is in an animal. For example, nanoparticles carrying small molecules for delivery to a stem cell may be targeted to an HSC based on its expression of cell-surface molecules.

[0056] Additionally, HSCs obtained from a donor may be separated from non-stem cells by any suitable method known in the art including, without limitation, fluorescence activated cell sorting (FACS) and magnetic activated cell sorting (MACS).

[0057] In one non-limiting example, human peripheral blood cells are incubated with antibodies recognizing c-kit, Sca-1, CD34, CD38, Thy1, CD2, CD3, CD4, CD5, CD8, CD43, CD45, CD59, CD90, CD105, CD133, ABCG2, NK1.1, B220, Ter-119, Flk-2, CDCP1, Endomucin, or Gr-1. Antibodies for CD2, CD3, CD4, CD5, CD8, NK1.1, B220, Ter-119, and Gr-1 are conjugated with magnetic beads. The cells expressing CD2, CD3, CD4, CD5, CD8, NK1.1, B220, Ter-119, or Gr-1 are retained in the column equipped to trap magnetic beads and cells attached to magnetic bead conjugated antibodies. The cells that are not captured by the MACS column are subjected to FACS analysis. Antibodies for c-kit, Sca-1, CD34, CD38, Thy1, are conjugated with fluorescent materials known in the art. The cells that are $CD34^{+}$, $CD38^{low/-}$, $c\text{-}kit^{-/low}$, $Thy1^{+}$ are separated from the rest of sample by virtue of the types of fluorescent antibodies associated with the cells. These cells are provided as human long-term HSCs suitable for use with any of the methods of the present disclosure.

[0058] In another non-limiting example, cells obtained from a subject are labeled with the same set of magnetic bead conjugated antibodies as described above (antibodies against one or more of CD2, CD3, CD4, CD5, CD8, NKl.1, B220, Ter-1l9, or Gr-l) and fluorescent conjugated CD150, CD244 and/or CD48 antibodies. After removing cells captured by the magnetic bead conjugated antibodies from the sample, the sample is analyzed by FACS and $CD150^{+}$, $CD244^{-}$ and $CD48^{-}$ cells are retained as long-term HSCs.

[0059] In some embodiments, HSCs utilized in the methods of the present disclosure contain one or more of the markers: c-kit^{+}, Sca-l^{+}, $CD34^{low/-}$, $CD38^{+}$, $Thy1^{+/low}$, $CD34^{+}$, $CD38^{low/-}$, c-$kit^{-/low}$, and/or $Thy1^{+}$. In some embodiments, the HSCs utilized in the methods of the present disclosure lack one or more of the markers: CD2, CD3, CD4, CD5, CD8, NK1.1, B220, Ter-1l9, and/or Gr-l. In certain embodiments, the HSCs utilized in the methods of the present disclosure are of an A+, A-, B+, B-, O+, O-, AB+, or AB- type.

[0060] Alternatively, suitable HSCs may be obtained from a non-human source. Suitable non-human HSCs may be isolated from, femurs, hip, ribs, sternum, and other bones of a non-human animal, including, without limitation, laboratory/research animals, rodents, pets, livestock, farm animals, work animals, pack animals, rare or endangered species, racing animals, and zoo animals. Further examples of suitable non-human animals include, without limitation, monkeys, primates, mice, rats, guinea pigs, hamsters, dogs, cats, horses, cows, pigs, sheep, goats, and chickens. For example, HSCs may be obtained from murine bone marrow cells, by incubating the bone marrow cells with antibodies recognizing cell surface molecules such as one or more of c-kit, Sca-1, CD34, CD38, Thy1, CD2, CD3, CD4, CD5, CD8, CD43, CD45, CD59, CD90, CD105, CD133, ABCG2, NK1.1, B220, Ter-119, Flk-2, CDCP1, Endomucin, or Gr-1. Antibodies for CD2, CD3, CD4, CD5, CDS, NK1.I, B220, Ter-119, and Gr-1 are conjugated with magnetic beads. In MACS equipment, the cells harboring CD2, CD3, CD4, CD5, CD8, NK1.1, B220, Ter-119, or Gr-1 on their surface are retained in the column equipped to trap magnetic beads and the cells attached to magnetic bead conjugated antibodies. The cells that are not captured by MACS column are subjected to FACS analysis. For FACS analysis, Antibodies for surface molecules such as c-kit, Sca-1, CD34, CD38, Thy1, are conjugated with fluorescent materials. The cells that are c-kit^{+}, Sca-1^{+}, $CD341^{ow/-}$, $CD38^{+}$, $Thy1^{+/low}$ are separated from the

rest of the sample by virtue of the types of fluorescent antibodies associated with the cells. These cells are provided as murine long-term HSCs suitable for use with any of the methods of the present disclosure. In other embodiments, different sets of marker are used to separate murine long-term HSCs from cells of bone marrow, umbilical cord blood, fetal tissue, and peripheral blood.

[0061] In some embodiments, obtaining HSCs from bone marrow includes first injecting the HSC donor, such as a mouse or other non-human animal, with 5-fluorouracil (5-FU) to induce the HSCs to proliferate in order to enrich for HSCs in the bone marrow of the donor.

[0062] Moreover, HSCs suitable for use with any of the methods of the present disclosure, whether obtained from, or present in, cord blood, bone marrow, peripheral blood, or other source, may be grown or expanded in any suitable, commercially available or custom defined medium (e.g., Hartshorn et al., *Cell Technology for Cell Products*, pages 221-224, R. Smith, Editor; Springer Netherlands, 2007). For example, serum free medium may utilize albumin and/or transferrin, which have been shown to be useful for the growth and expansion of $CD34^+$ cells in serum free medium. Also, cytokines may be included, such as Flt-3 ligand, stem cell factor (SCF), and thrombopoietin (TPO), among others. HSCs may also be grown in vessels such as bioreactors (e.g., Liu et al., *Journal of Biotechnology* 124:592-601, 2006). A suitable medium for *ex vivo* expansion of HSCs may also contain HSC supporting cells, such as stromal cells (e.g., lymphoreticular stromal cells), which can be derived, for example, from the disaggregation of lymphoid tissue, and which have been shown to support the *in vitro*, *ex vivo*, and i*n vivo* maintenance, growth, and differentiation of HSCs, as well as their progeny.

[0063] HSC growth or expansion may be measured *in vitro* or *in vivo* according to routine techniques known in the art. For example, WO 2008/073748, describes methods for measuring *in vivo* and *in vitro* expansion of HSCs, and for distinguishing between the growth/expansion of HSCs and the growth/expansion of other cells in a potentially heterogeneous population (e.g., bone marrow), including for example intermediate progenitor cells.

HSC cell lines

[0064] In other embodiments, HSCs suitable for use in any of the methods of the present disclosure may also be derived from an HSC cell line. Suitable HSC cell lines include any cultured hematopoietic stem cell line known in the art. Non-limiting examples include the conditionally immortalized long-term stem cell lines described in U.S. Patent Application Publication Nos. US 2007/0116691 and US 2010/0047217.

Aging Stem Cells

[0065] In certain embodiments of the methods of the present disclosure, the stem cell is an aging stem cell. Aging stem cells are typically present in older animals and may be isolated from older animals using the techniques described above. In certain embodiments, the older animal is an older human. Aging stem cells may exhibit one or more of the following properties: decreased per cell repopulating activity, decreased self-renewal and homing abilities, myeloid skewing of differentiation, and increased apoptosis with stress (Janzen et al., *Nature*, 2006, v. 443, p. 421). Any methods known in the art to evaluate these properties may be used to identify aging stem cells. For example, assays such as competitive repopulation and transplantation, engraftment, colony-forming unit assays, and long-term culture assays may be used.

[0066] Citation of documents and studies referenced herein is not intended as an admission that any of the foregoing is pertinent prior art. All statements as to the contents of these documents are based on the information available to the applicants and do not constitute any admission as to the correctness of the contents of these documents.

[0067] The following Examples are merely illustrative and are not meant to limit any aspects of the present disclosure in any way.

EXAMPLES

[0068] The following examples describe the identification of a regulatory branch of the mitochondrial unfolded protein response (UPR^{mt}), which is mediated by the interplay of SIRT7 and NRF1, and is coupled to cellular energy metabolism and proliferation. SIRT7 inactivation

caused reduced quiescence, increased mitochondrial protein folding stress (PFS^{mt}), and compromised regenerative capacity of hematopoietic stem cells (HSCs). SIRT7 expression was reduced in aged HSCs and SIRT7 upregulation improved the regenerative capacity of aged HSCs. These findings define the deregulation of a UPR^{mt}-mediated metabolic checkpoint as a reversible contributing factor for HSC aging and are the first evidence of a role for the UPR^{mt}-mediated metabolic checkpoint in aging.

Example 1: SIRT7 Represses Transcription through NRF1

[0069] SIRT7 is a histone deacetylase that is recruited to its target promoters via interactions with transcription factors for transcriptional repression (7). A proteomic approach was taken to identify SIRT7-interacting transcription factors. 293T cells were transfected with Flag-tagged SIRT7, affinity-purified the Flag-tagged SIRT7 interactome, and identified SIRT7-interacting proteins by mass spectrometry. Among the potential SIRT7-interacting proteins was Nuclear Respiratory Factor 1 (NRF1), a master regulator of mitochondria (8). Transfected Flag-SIRT7 and endogenous SIRT7 interacted with NRF1 in 293T cells (Fig. 1, A and B).

Transcriptional Repression of Mitochondrial Translational Machinery

[0070] SIRT7 bound the proximal promoters of mitochondrial ribosomal proteins (mRPs) and mitochondrial translation factors (mTFs), but not other NRF1 targets (Fig. 1C and Fig. 2A and (7)). NRF1 bound the same regions as SIRT7 at the proximal promoters of mRPs and mTFs, but not RPS20 (Fig. 1D and Fig. 2B), where SIRT7 binding is mediated through Myc (9). SIRT7 binding sites were found adjacent to NRF1 consensus binding motifs at the promoters of mRPs and mTFs (Fig. 2C). NRF1 knockdown (KD) using siRNA reduced SIRT7 occupancy at the promoters of mRPs and mTFs, but not RPS20 (Fig. 1C, and Fig. 3). SIRT7 KD using short hairpin RNAs led to increased expression of mRPs and mTFs, which was abrogated by NRF1 siRNA (Fig. 4, A and B, and Fig. 5). Thus, NRF1 targets SIRT7 specifically to the promoters of mRPs and mTFs for transcriptional repression.

Suppression of Mitochondrial Activity and Proliferation

[0071] Transcriptional repression of mitochondrial and cytosolic (7, 9) translation machinery by SIRT7 suggests that SIRT7 might suppress mitochondrial activity and proliferation. SIRT7 KD cells had increased mitochondrial mass, citrate synthase activity, ATP levels, respiration, and proliferation, while cells overexpressing wild type (WT) but not a catalytically inactive SIRT7 mutant (H187Y) showed reduced mitochondrial mass, respiration, and proliferation (Fig. 4, C to E, and Fig. 6, A to G, and (10, 11)). NRF1 siRNA abrogated the increased mitochondrial activity and proliferation of SIRT7 KD cells (Fig. 6, H to J). Thus, SIRT7 represses NRF1 activity to suppress mitochondrial activity and proliferation.

Promotion of Nutritional Stress Resistance

[0072] Sirtuins are increasingly recognized as stress resistance genes (12-14). Nutrient deprivation induced SIRT7 expression (Fig. 7A). Upon nutrient deprivation stress, cells reduce mitochondrial activity, growth, and proliferation to prevent cell death (15, 16). When cultured in nutrient-deprived medium, cells overexpressing SIRT7 showed increased survival, while SIRT7 KD cells showed reduced survival, which was improved by NRF1 siRNA (Fig. 7). Thus, SIRT7 suppresses NRF1 activity to promote nutritional stress resistance.

Example 2: SIRT7 Mediates a Regulatory Branch of the UPR^{mt}

[0073] Perturbation of mitochondrial proteostasis, a form of mitochondrial stress, activates the UPR^{mt}, a retrograde signaling pathway leading to transcriptional upregulation of mitochondrial chaperones and stress relief (17, 18). Mitochondrial dysfunction results in attenuated translation, which helps restore mitochondrial homeostasis (19). SIRT7-mediated transcriptional repression of the translation machinery suggests that SIRT7 may alleviate PFS^{mt}. PFS^{mt} induced SIRT7 expression (Fig. 4F). Induction of PFS^{mt} via overexpression of an aggregation-prone mutant mitochondrial protein ornithine transcarbamylase (ΔOTC) results in UPR^{mt} activation and efficient clearance of misfolded ΔOTC (18). In SIRT7 KD cells, misfolded ΔOTC accumulated to a higher level (Fig. 4G). SIRT7 KD cells displayed increased apoptosis upon PFS^{mt} (Fig. 4H), but are not prone to general apoptosis (9). Thus, SIRT7 alleviates PFS^{mt} and promotes PFS^{mt} resistance. Consistently, mitochondrial dysfunction is manifested in the metabolic tissues of SIRT7 deficient mice (20).

[0074] PFS^{mt} induced the expression of canonical UPR^{mt} genes in SIRT7 deficient cells (Fig. 8, A and B), indicating that induction of SIRT7 and canonical UPR^{mt} genes are in separate branches of the UPR^{mt}. Untreated SIRT7 KD cells displayed increased expression of canonical UPR^{mt} genes (Fig. 8, A and B), but SIRT7 did not bind to their promoters (Fig. 2A and (7)), suggesting that SIRT7 deficiency results in constitutive PFS^{mt} and compensatory induction of canonical UPR^{mt} genes. NRF1 siRNA abrogated increased PFS^{mt}, but not endoplasmic reticulum stress, in SIRT7 KD cells (Fig. 4I, and Fig. 8C). Thus, the interplay between SIRT7 and NRF1 constitutes a regulatory branch of UPR^{mt}, functioning as the nexus of reduced mitochondrial translation for homeostasis reestablishment, and repressed energy metabolism and proliferation (Fig. 4J).

Example 3: SIRT7 is Critical for HSC Maintenance and Can Reverse Aging of HSCs

HSCs Require SIRT7 to Limit PFS^{mt}, Mitochondrial Mass, and Proliferation

[0075] What is the physiological relevance of the SIRT7-mediated UPR^{mt}? In C. elegans, the UPR^{mt} is activated during a developmental stage when a burst of mitochondrial biogenesis takes place and is attenuated when mitochondrial biogenesis subsides (17). Thus, the SIRT7-mediated UPR^{mt} may be important for cells that experience bursts of mitochondrial biogenesis and convert between growth states with markedly different bioenergetic demands and proliferative potentials, such as stem cells. Quiescent adult stem cells have low mitochondrial content, but mitochondrial biogenesis increases during proliferation and differentiation (4).

[0076] Because SIRT7 is highly expressed in the hematopoietic system, focus was given to HSCs isolated from $SIRT7^{+/+}$ and $SIRT7^{-/-}$ mice (Fig. 9). $SIRT7^{-/-}$ HSCs had increased expression of UPR^{mt} genes, indicative of increased PFS^{mt} (Fig. 10A). Mitotracker Green (MTG) staining, mitochondrial DNA quantification, and electron microscopy revealed increased mitochondrial numbers in $SIRT7^{-/-}$ HSCs (Fig. 10, B and C, and Fig. 11). In vivo BrdU incorporation showed increased proliferation of $SIRT7^{-/-}$ HSCs (Fig. 10D). $SIRT7^{-/-}$ HSCs also exhibited an increased propensity to enter the cell cycle upon ex vivo culture with cytokines (Fig. 10E). However, there was no difference in mitochondrial number or proliferation in the differentiated subpopulations of these two genotypes (Fig. 10, B, D, and E). Animals with loss of

HSC quiescence are sensitive to the myeloablative drug 5-Fluorouracil (21). Upon 5-Fluorouracil treatment, mice reconstituted with SIRT7$^{-/-}$ bone marrow cells (BMCs) died sooner than SIRT7$^{+/+}$ controls (Fig. 10F). Thus, HSCs require SIRT7 to limit PFS^{mt}, mitochondrial mass, and proliferation.

SIRT7 Promotes HSC Maintenance, and Its Downregulation in Aged HSCs Contributes to Their Functional Decline

[0077] Reduced HSC quiescence causes compromised regenerative function (21). SIRT7$^{-/-}$ BMCs or purified HSCs displayed a 40% reduction in long term reconstitution of the recipients' hematopoietic system compared to their SIRT7$^{+/+}$ counterparts (Fig. 10G, and Fig. 12A). SIRT7$^{-/-}$ mice also had reduced numbers of white blood cells (Fig. 10H). Although SIRT7$^{+/+}$ and SIRT7$^{-/-}$ mice had comparable HSC frequency in the bone marrow under steady-state conditions, there was a 50% reduction in the frequency of SIRT7$^{-/-}$ HSCs upon transplantation (Fig. 12, B and C). SIRT7$^{-/-}$ HSCs showed increased apoptosis upon transplantation (Fig. 12D), which may account for compromised HSC engraftment. HSCs differentiate into lymphoid and myeloid lineages. Myeloid-biased differentiation was apparent in SIRT7$^{-/-}$ mice or in mice reconstituted with SIRT7$^{-/-}$ HSCs (Fig. 10I, and Fig. 12E). Thus, SIRT7 promotes HSC maintenance and prevents myeloid-biased differentiation.

[0078] Reintroduction of SIRT7 in SIRT7$^{-/-}$ HSCs improved reconstitution capacity and rescued myeloid-biased differentiation (Fig. 12, F and G), indicating that SIRT7 regulates HSC maintenance cell-autonomously. NRF1 inactivation in SIRT7$^{-/-}$ HSCs reduced PFS^{mt}, improved HSC quiescence, engraftment, reconstitution, and rescued myeloid-biased differentiation (Fig. 12C, and 13). Thus, SIRT7 represses NRF1 activity to alleviate PFS^{mt} and ensure HSC maintenance.

[0079] SIRT7 expression was reduced in aged HSCs (Fig. 14A and (22)). Notably, defects manifested in SIRT7$^{-/-}$ HSCs (increased PFS^{mt} and apoptosis, loss of quiescence, decreased reconstitution capacity, and myeloid-biased differentiation) resemble aspects of aged HSCs ((23-25) and Fig. 14B). SIRT7 overexpression or NRF1 inactivation in aged HSCs reduced PFS^{mt}, improved reconstitution capacity, and rescued myeloid-biased differentiation (Fig. 14, C to E,

and fig. 15). Thus, SIRT7 downregulation results in increased PFS^{mt} in aged HSCs, contributing to their functional decline.

Example 4: Summary of Findings

[0080] Collectively, the results highlight PFS^{mt} as a trigger of a metabolic checkpoint that regulates HSC quiescence, and establish the deregulation of UPR^{mt} as a contributing factor for HSC aging. Using a stress signal as a messenger to return to quiescence may ensure the integrity of HSCs, which persist throughout the entire lifespan for tissue maintenance. The interplay between SIRT7, which is induced upon PFS^{mt}, and NRF1, a master regulator of mitochondria, is uniquely positioned to integrate PFS^{mt} to metabolic checkpoint regulation.

[0081] SIRT7 represses NRF1 activity to reduce the expression of the mitochondrial translation machinery and to alleviate PFS^{mt} (Fig. 1 and 4). In vivo gene expression studies cannot distinguish direct versus indirect effects. In this regard, ChIP-seq studies are informative in identifying direct SIRT7 targets (7). While gene expression changes in the metabolic tissues of $SIRT7^{-/-}$ mice are likely reflective of severe mitochondrial and metabolic defects (20), transiently knocking down SIRT7 in cultured cells can capture the direct effect of SIRT7 on its targets ((7, 9) and Fig. 4) and may account for different gene expression changes. In contrast to the severe defects in metabolic tissues of $SIRT7^{-/-}$ mice, $SIRT7^{-/-}$ HSCs have increased mitochondria number and proliferation under homeostatic conditions, but do not fare well upon transplantation stress (Fig. 10). These observations are consistent with the notion that while metabolic tissues have a large number of mitochondria, HSCs have very few mitochondria under homeostatic conditions and increase mitochondrial biogenesis upon transplantation. The combined power of biochemistry, cell culture, and mouse genetics is necessary to tease out direct and indirect effects of SIRT7 under various physiological conditions. The proposed model (Fig. 4J) is consistent with the functions of SIRT7 in chromatin remodeling and gene repression (7), stress responses ((9) and Fig. 4), and mitochondrial maintenance ((20) and Fig. 4).

[0082] Reintroduction of SIRT7 in aged HSCs reduces PFS^{mt} and improves their regenerative capacity. Thus, PFS^{mt}-induced HSC aging is reversible. It appears that HSC aging is not due to passive chronic accumulation of cellular damage over the lifetime, but the regulated

repression of cellular protective programs. The dysregulated UPR^{mt} cellular protective program may be targeted to reverse HSC aging and rejuvenate tissue homeostasis.

Example 5: Materials and Methods

[0083] The following sections describe the materials and methods used in the experiments described in Examples 1-4.

Cell Culture and RNAi

[0084] 293T cells were acquired from the ATCC. Cells were cultured in advanced DMEM (Invitrogen) supplemented with 1% penicillin-streptomycin (Invitrogen) and 10% FBS (Invitrogen). For inducing PFS^{mt}, cells were treated with doxycycline (30 μg/mL) for 48 hrs, or ethidium bromide (50 ng/mL) for 7 days. Alternatively, cells were transfected with a construct expressing an aggregation prone mutant mitochondrial OTC protein from the Hoogenraad lab (18). For nutrient deprivation, cells were cultured in glucose free medium (Invitrogen) or glutamine free medium (Invitrogen) for 48-68 hours. Cell proliferation and survival were scored using a Vi-Cell Analyzer (Beckman Coulter).

[0085] SIRT7 knockdown target sequences are as follows, as previously described (9):

S7KD1, 5'-CACCTTTCTGTGAGAACGGAA-3';

S7KD2, 5'-TAGCCATTTGTCCTTGAGGAA-3',

NRF1 knockdown target sequences are as follows:

NRF1 KD (mouse), 5'-GAAAGCTGCAAGCCTATCT-3'

NRF1 KD (human), 5'-CACCGTTGCCCAAGTGAATTA-3'

Double-stranded siRNAs were purchased from Thermo Scientific and were transfected into cells via RNAiMax (Invitrogen) according to manufacturer's instructions. Generation of SIRT7 knockdown and overexpressing cells was described previously (9). After puromycin selection, cells were recovered in puromycin free medium for 2-3 passages before analyses.

Measurements of mitochondrial mass, ATP, citrate synthase activity, and oxygen consumption

[0086] To measure mitochondrial mass, cells were stained with 100nM MitoTracker Green (Invitrogen) for 30 minutes in at 37°C, and analyzed with flow cytometry (BD Fortessa).

[0087] For oxygen consumption, 3X105 cells were plated using CellTak (BD) and the oxygen consumption rate (OCR) was measured using a Seahorse XF24 instrument following the manufacturer's instructions (Seahorse Biosciences). OCR was measured under basal conditions, in the presence of the mitochondrial inhibitor oligomycin A (1 μM), mitochondrial uncoupler FCCP (1 μM) and respiratory chain inhibitor antimycin and rotenone (1 μM).

[0088] Citrate synthase activity was measured following the manufacturer's instruction (Biovision Citrate Synthase Activity Colorimetric Assay Kit #K318-100). To measure ATP, cells in suspension were mixed with an equal volume of CellTiterGlo in solid white luminescence plates (Grenier Bio-One) following the manufacturer's instructions (Promega). Luminescence was measured using a luminometer (LMAX II 384 microplate reader, Molecular Devices) to obtain relative luciferase units (RLU).

Co-immunoprecipitations

Co-immunoprecipitations were performed as previously described (*14*) with Flag-resin (Sigma) or Protein A/G beads (Santa Cruz) for SIRT7 IP. Elution was performed with either Flag peptide (Sigma) or 100mM Glycine solution (pH 3) for SIRT7 IP. Antibodies are provided in Table 1, below.

Table 1. Antibodies used.

Antibodies	Source	Catalog #
SIRT7	Abnova	H00051547
Beta Actin	Sigma	A2066
Flag	Sigma	F1804
IgG	Santa Cruz	SC-2027
RPS20	Abcam	Ab74700
NRF-1	Proteintech	12482-1-AP
MRPL24	Proteintech	16224-1-AP
GFM2	Proteintech	16941-1-AP
ClpP	Proteintech	15698-1-AP

HSP60	Cell Signaling	12165S	
OTC	Santa Cruz	SC-102051	
FACS Antibodies & Reagents	**Source**	**Catalog #**	**Clone #**
CD45.1 PerCP	Biolegend	110726	A20
Streptavidin PerCP	Biolegend	405213	
Mac1 PerCP	Biolegend	101230	M1/70
CD3 Pacific Blue	Biolegend	100214	17A2
Sca1 Pacific Blue	Biolegend	108120	D7
Streptavidin APC-Cy7	Biolegend	405208	
c-Kit APC-Cy7	Biolegend	105826	2B8
CD45.2 Cy7-PE	Biolegend	109830	104
CD150 Cy7-PE	Biolegend	115914	TC15-12F12.2
Gr1 Cy7-PE	Biolegend	108416	RB6-8C5
c-Kit Cy7-PE	Biolegend	105813	2B8
Streptavidin Cy7-PE	Biolegend	405206	
CD3 Biotin	Biolegend	100304	145-2C11
B220 Biotin	Biolegend	103204	RA3-6B2
Gr1 Biotin	Biolegend	108404	RB6-8C5
CD8a Biotin	Biolegend	100704	53-6.8
Mac1 Biotin	Biolegend	101204	M1/70
Ter119 Biotin	Biolegend	116204	TER-119
CD4 Biotin	Biolegend	100404	GK1.5
CD48 FITC	Biolegend	103404	HM48-1
Gr1 FITC	Biolegend	108406	RB6-8C5
CD45.2 FITC	eBioscience	11-0454-85	104
AnnexinV FITC	Biolegend	640906	
Ki67 A488	Biolegend	350508	Ki-67
CD150 PE	Biolegend	115904	TC15-12F12.2
CD45.1 PE	Biolegend	110708	A20
Mac1	Biolegend	101208	M1/70
c-Kit APC	Biolegend	105812	2B8
B220 APC	Biolegend	103212	RA3-6B2
Ki67 APC	Biolegend	350514	Ki-67
AnnexinV APC	Biolegend	640920	
CD48 A647	Biolegend	103416	HM48-1
Fixation buffer	Biolegend	420801	
Permeabilization wash buffer	Biolegend	421002	
AnnexinV binding buffer	Biolegend	422201	
7AAD	Biolegend	420404	
BrdU labeling reagent	Invitrogen	000103	

ChIP and mRNA Analysis

[0089] Cells were prepared for ChIP as previously described (*26*), with the exception that DNA was washed and eluted using a PCR purification kit (Qiagen) rather than by phenolchloroform extraction. RNA was isolated from cells or tissues using Trizol reagent (Invitrogen) and purified using the RNeasy Mini Kit (Qiagen). cDNA was generated using the

qScript™ cDNA SuperMix (Quanta Biosciences). Gene expression was determined by real time PCR using Eva qPCR SuperMix kit (BioChain Institute) on an ABI StepOnePlus system. All data were normalized to ActB or GAPDH expression. Antibodies and PCR primer details are provided in Tables 1-3.

Table 2. Primers used for qPCR analysis.

Gene	Primer	Sequence
SIRT7 (human)	Forward	CGCCAAATACTTGGTCGTCT
	Reverse	CCCTTTCTGAAGCAGTGTCC
ClpP (human)	Forward	CTCTTCCTGCAATCCGAGAG
	Reverse	GGATGTACTGCATCGTGTCG
Hsp10 (human)	Forward	CAGTAGTCGCTGTTGGATCG
	Reverse	TGCCTCCATATTCTGGGAGA
Hsp60 (human)	Forward	TGACCCAACAAAGGTTGTGA
	Reverse	CATACCACCTCCCATTCCAC
mtDnaJ(human)	Forward	CGAAATGGCAGAAGAAGAGG
	Reverse	TGCATGCACTACAGAGCACA
Grp78 (human)	Forward	TCATCGGACGCACTTGGAA
	Reverse	CAACCACCTTGAATGGCAAGA
ClpP (mouse)	Forward	CTGCCCAATTCCAGAATCAT
	Reverse	TGTAGGCTCTGCTTGGTGTG
Hsp10 (mouse)	Forward	CCAAAGGTGGCATTATGCTT
	Reverse	TGACAGGCTCAATCTCTCCA
Hsp60 (mouse)	Forward	ACCTGTGACAACCCCTGAAG
	Reverse	TGACACCCTTTCTTCCAACC
mtDnaJ (mouse)	Forward	GAGCTGAAGAAGGCATACCG
	Reverse	CAGCTCTCGCTTCTCTGGAT
ND4 (mtDNA) (mouse)	Forward	GGAACCAAACTGAACGCCTA
	Reverse	ATGAGGGCAATTAGCAGTGG
b2 microglobulin (nDNA) (Mouse)	Forward	TCATTAGGGAGGAGCCAATG
	Reverse	ATCCCCTTTCGTTTTTGCTT
SIRT7 (mouse)	Forward	CCATGGGAAGTGTGATGATG
	Reverse	TCCTACTGTGGCTGCCTTCT
MRPL16	Forward	ACATACGGGGACCTTCCACT
	Reverse	AAACATGTTCTTGGGGTCCA
MRPL20	Forward	GAACATGAGGACCCTCTGGA
	Reverse	CCGCTAGGACTTTCCTGTTG
MRPL24	Forward	GGGGAACCATGATCCCTAGT
	Reverse	AATTCTCCCTGATCGTGTGG
MRPS31	Forward	GAGGAAGAGTCAAGGGCACA
	Reverse	CTGAATCCGAAGCTCTGGTC
MRPS33	Forward	ATATGCCTTCCGCATGTCTC
	Reverse	GCCAAGGGCAGTTCACTAAA
CytC	Forward	AAGTGTTCCCAGTGCCACA
	Reverse	GTTCTTATTGGCGGCTGTGT
POLRMT	Forward	AAAGCCCAACACACGTAAGC
	Reverse	GTGCACAGAGACGAAGGTCA

TFAM	Forward	TGGCAAGTTGTCCAAAGAAA
	Reverse	ACGCTGGGCAATTCTTCTAA
TFB1M	Forward	CTCCCTTGATACAGCCCAAG
	Reverse	TGCGCTTCAGGGAATAACAT
TFB2M	Forward	AGATCCCGGAAATCCAGACT
	Reverse	CTACGCTTTGGGTTTTCCAG
TIMM17A	Forward	AGGGCTGTTTTCCATGATTG
	Reverse	CCACTGGTCCATTTCTTGCT
NRF1	Forward	CCCAGGCTCAGCTTCGGGCA
	Reverse	GCTCTTCTGTGCGGACATCAC
tRNALeu (mtDNA) (human)	Forward	CACCCAAGAACAGGGTTTGT
	Reverse	TGGCCATGGGTATGTTGTTA
β2-microglobulin (nDNA) (human)	Forward	TGCTGTCTCCATGTTTGATGTATCT
	Reverse	TCTCTGCTCCCCACCTCTAAGT
16S rRNA (mtDNA) (human)	Forward	GCCTTCCCCCGTAAATGATA
	Reverse	TTATGCGATTACCGGGCTCT

Table 3. PCR primers used for ChIP analysis

Target	**Primer**	**Sequence**
γ-tubulin	Forward	ACGGGTTTCATCATGTTTGTT
	Reverse	GGCAGATCCCCTGAGGTC
RPS20	Forward	AAGTTCTTTCTTTTTGAGGAAGACG
	Reverse	GAACAGCGGTGAGTCAGGA
GFM2	Forward	CGGGACAGGAAAGAGTCACC
	Reverse	CGGAAAACAGAGGCTCGGAA
mRPL24	Forward	TGAACAGGAAGCCACAACCA
	Reverse	GAGGCCGCTGGGAATTGTAG
NME1	Forward	CCGTAATACTTGGCTCTCGAA
	Reverse	GAATAGACCTGCATGAAGTGAGG
HSP60	Forward	CAGCGACTACTGTTGCTTGC
	Reverse	ACAGGCAGGACAAGCGTTTA
TFB1M	Forward	CCTAGTCCACCCGGCTCT
	Reverse	GAGGAACCTGCGAGACCTAA
TFB2M	Forward	ACGGTCCACTCACAATCCTC
	Reverse	CCCACGTGGAACATTTTCTG
Cytc	Forward	CCGTACACCCTAACATGCTC
	Reverse	TGGCACAACGAACACTCC
mRPL16	Forward	TCTTCTGGGGAAAGACTGGA
	Reverse	TGAGTTCCTGCGGTCAAAG
mRPL20	Forward	CGAGTTCAGGAGCACAACTG
	Reverse	GTCAGCCCCTGCGATACTT
ClpP	Forward	ATGTGGCCCGGAATATTGGT
	Reverse	CAGGCCGTTCTGGAGTGTC
HSP10	Forward	AGAGGAGGAAGGCCCACTC
	Reverse	CTGCACTCTGTCCCTCACTC

Mice

[0090] SIRT7$^{-/-}$ mice have been described previously (*9*). All mice were housed on a 12:12 hr light:dark cycle at 25°C. For 5-Fluorouricil treatment study, 1x106 BMCs from SIRT7$^{+/+}$ or SIRT7$^{-/-}$ mice were transplanted into lethally irradiated recipient mice. Four months posttransplantation, 5-Fluorouricil was administrated to mice intraperitoneally at a dose of 150 mg/kg once per week, and the survival of the mice was monitored daily. All animal procedures were in accordance with the animal care committee at the University of California, Berkeley.

Flow Cytometry and Cell Sorting

[0091] BMCs were obtained by crushing the long bones with sterile PBS without calcium and magnesium supplemented with 2% FBS. Lineage staining contained a cocktail of biotinylated anti-mouse antibodies to Mac-1 (CD11b), Gr-1 (Ly-6G/C), Ter119 (Ly-76), CD3, CD4, CD8a (Ly-2), and B220 (CD45R) (BioLegend). For detection or sorting, we used streptavidin conjugated to APC-Cy7, c-Kit-APC, Sca-1-Pacific blue, CD48-FITC, and CD150-PE (BioLegend). For congenic strain discrimination, anti-CD45.1 PerCP and anti-CD45.2 PE-Cy7 antibodies (BioLegend) were used. For assessment of apoptosis and cell cycle analysis, AnnexinV and Ki-67 (BioLegend) staining were performed respectively according to the manufacturer's recommendation after cell surface staining. For in vivo cell-cycle analysis, BrdU (Invitrogen) was incorporated over a 16-hour period. For ex vivo proliferation analysis, cultured BMCs were pulsed with BrdU (Invitrogen) for one hour before flow cytometry analysis. For mitochondrial mass, BMCs were incubated with 100nM MitoTracker Green (Invitrogen) for 30 min at 37°C in the dark after cell surface staining. All data were collected on a Fortessa (Becton Dickinson), and data analysis was performed with FlowJo (TreeStar). For cell sorting, lineage depletion or c-kit enrichment was performed according to the manufacturer's instructions (Miltenyi Biotec). Cells were sorted using a Cytopeia INFLUX Sorter (Becton Dickinson). Antibody details are provided in Table 1.

Lentiviral Transduction of HSCs

[0092] As previously described (*27*), sorted HSCs were prestimulated for 5-10 hrs in a 96 well U bottom dish in StemSpan SFEM (Stem Cell Technologies) supplemented with 10% FBS (Stem Cell Technologies), 1% Penicillin/Streptomycin (Invitrogen), IL3 (20ng/ml), IL6 (20ng/ml), TPO (50ng/ml), Flt3L (50ng/ml), and SCF (100ng/ml) (Peprotech).

[0093] SIRT7 was cloned into the pFUGw lentiviral construct. NRF1 shRNA was cloned into pFUGw-H1 lentiviral construct. Lentivirus was produced as described (*14*), concentrated by centrifugation, and resuspended with supplemented StemSpan SFEM media. The lentiviral media were added to HSCs in a 24 well plate, spinoculated for 90 min at 270g in the presence of 8ug/ml polybrene. This process was repeated 24 hr later with a fresh batch of lentiviral media.

mtDNA/nDNA

[0094] The mitochondrial DNA/nuclear DNA (mtDNA/nDNA) ratio was determined by isolating DNA from cells with Trizol (Invitrogen), as described previously (*28*). The ratio of mtDNA/nDNA was calculated as previously described (*29*).

Electron Microscopy

[0095] 40,000 HSCs were pelleted at 150g. Samples were fixed with 2% gluaraldehyde for 10 minutes at room temperature while rocking. Samples were pelleted at 600g and further fixed with 2% glutaraldehyde / 0.1M NaCacodylate at 4°C and were submitted to the UC Berkeley Electron Microscope Core Facility for standard transmission electron microscopy ultrastructure analyses.

Transplantation Assays

[0096] For transplantations, $5x10^5$ BMCs from $SIRT7^{+/+}$ or $SIRT7^{-/-}$ CD45.2 littermates was mixed with $5x10^5$ CD45.1 B6.SJL (Jackson Laboratory) competitor BMCs and injected into lethally irradiated (950 Gy) CD45.1 B6.SJL recipient mice. Alternatively, 250 sorted HSCs from $SIRT7^{+/+}$ or $SIRT7^{-/-}$ mice were mixed with $5x10^5$ CD45.1 B6.SJL competitor BMCs and injected into lethally irradiated B6.SJL recipient mice. To assess multilineage reconstitution of transplanted mice, peripheral blood was collected every month for 4 months by retroorbital

bleeding. Red blood cells were lysed and the remaining blood cells were stained with CD45.2 FITC, CD45.1 PE, Mac1 PerCP, Gr1 Cy7PE, B220 APC, and CD3 PB (Biolegend). Antibody details are provided in Table 1.

Statistical Analysis

[0097] The number of mice chosen for each experiment is based on the principle that the minimal number of mice is used to have sufficient statistical power and is comparable to published literature for the same assays performed. Mice were randomized to groups and analysis of mice and tissue samples were performed by investigators blinded to the treatment of genetic background of the animals. Transplant experiments have been repeated 5 times. HSC characterizations in SIRT7$^{-/-}$ mice have been repeated in 10 different cohorts of mice. Analyses in SIRT7 KD cells have been repeated 2-5 times. Experiments are repeated by at least 2 different scientists. Statistical analysis was performed with Excel (Microsoft) and Prism 5.0 Software (GraphPad Software). Means between two groups were compared with two-tailed, unpaired Student's t-test. Error Bars represent standard errors. In all corresponding Figures, * represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.001$, and ns represents $p>0.05$.

[0098] References

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[0099] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

What is claimed is:

1. A method of reversing aging of stem cells comprising activating the mitochondrial unfolded protein response in a stem cell, wherein aging of the stem cell is reversed.

2. The method of claim 1 wherein the mitochondrial unfolded protein response is activated by activating SIRT7 in the stem cell.

3. The method of claim 1 wherein the mitochondrial unfolded protein response is activated by activating a mitochondrial stress protein.

4. The method of claim 3 wherein the mitochondrial stress protein is selected from the group consisting of mtDnaJ, HSP60, HSP10, and ClpP in the stem cell.

5. The method of claim 2 wherein SIRT7 is activated by increasing the transcription of the sirt7 gene in the stem cell.

6. The method of claim 2 wherein SIRT7 is activated by increasing the translation of SIRT7 protein in the stem cell.

7. The method of claim 2 wherein SIRT7 is activated by delivering an exogenous copy of the sirt7 gene to the stem cell wherein the exogenous sirt7 gene is expressed in the stem cell.

8. The method of claim 7 wherein the exogenous copy of sirt7 gene is delivered to the stem cell with a viral vector.

9. The method of claim 2, wherein the SIRT7 is activated by a small molecule.

10. The method of claim 2, wherein the SIRT7 is activated by increasing intracellular NAD levels.

11. The method of claim 10 wherein intracellular NAD levels are increased by delivering small molecules that activate NAD synthesis enzymes to the stem cell.

12. The method of claim 10 wherein intracellular NAD levels are increased by increasing the level of a NAD precursor in the stem cell.

13. The method of claim 12 wherein the NAD precursor is selected from the group consisting of nicotinamide mono nucleotide (NMN) and nicotinamide riboside (NR).

14. The method of claim 10 wherein intracellular NAD levels are increased by increasing the level of a NAD biosynthesis enzyme in the stem cell.

15. The method of any of the above claims wherein the stem cell exhibits one or more of the characteristics selected from the group consisting of reduced occurrence of cell death, increased quiescence, increased occurrence of self-renewal, increased ability to repopulate its organ or tissue of origin, increased homing ability, and a change in differentiation profile.

16. The method of any of the above claims wherein the stem cell is in an animal.

17. The method of claim 16 wherein the animal is a human.

18. The method of any of the above claims, wherein the stem cell is a hematopoietic stem cell.

19. The method of claim 18 wherein the hematopoietic stem cell exhibits improved performance in an assay selected from the group consisting of HSC engraftment, bone marrow reconstitution, and competitive transplantation.

20. The method of claim 17 wherein blood drawn from the human does not exhibit one or more characteristics selected from the group consisting of increased myeloid differentiation, fewer lymphoid cells, and anemia.

21. The method of any one of claims 1-17 wherein the stem cell originated in an organ or tissue selected from the group consisting of brain, spinal cord, peripheral blood, blood vessels, skeletal muscle, skin, teeth, hair follicle, heart, gut, liver, ovarian epithelium, and testis.

22. The method of any of the above claims, wherein the stem cell is an aging stem cell.

23. A method for preventing aging of stem cells comprising activating the mitochondrial unfolded protein response in a stem cell, wherein aging of the stem cell is prevented.

24. The method of claim 23 wherein aging of the stem cell is delayed.

25. A method for promoting stem cell maintenance comprising activating the mitochondrial unfolded protein response in a stem cell, wherein the stem cell continues to self-renew.

26. A method for preventing and/or reversing tissue degeneration or injury comprising activating the mitochondrial unfolded protein response in a stem cell, wherein the stem

cell is in an animal and wherein degeneration or injury of a tissue in the animal is prevented and/or reversed.

FIG 1A

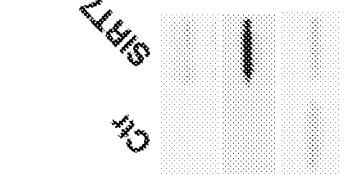


FIG 1B

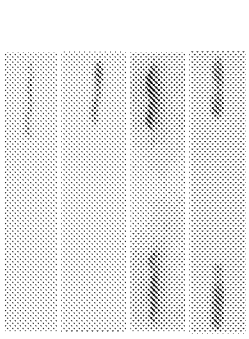


FIG 1C

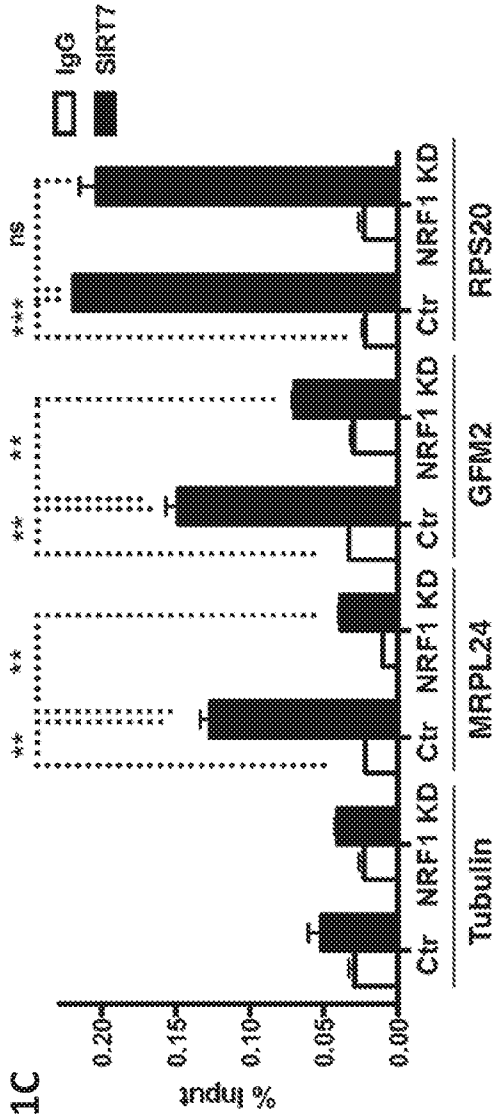


FIG 1D

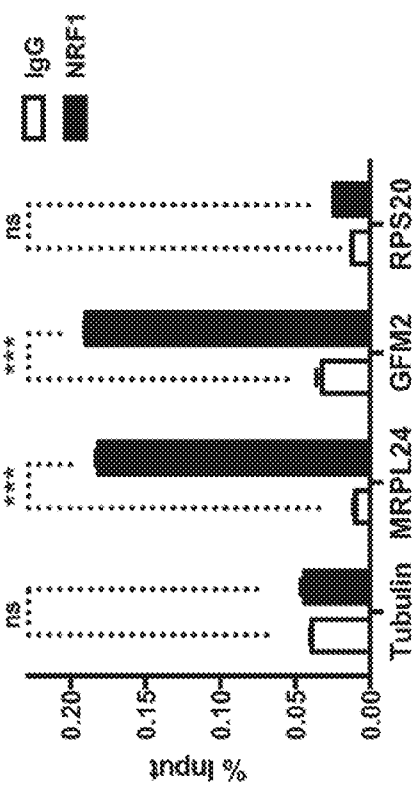


FIG 2A

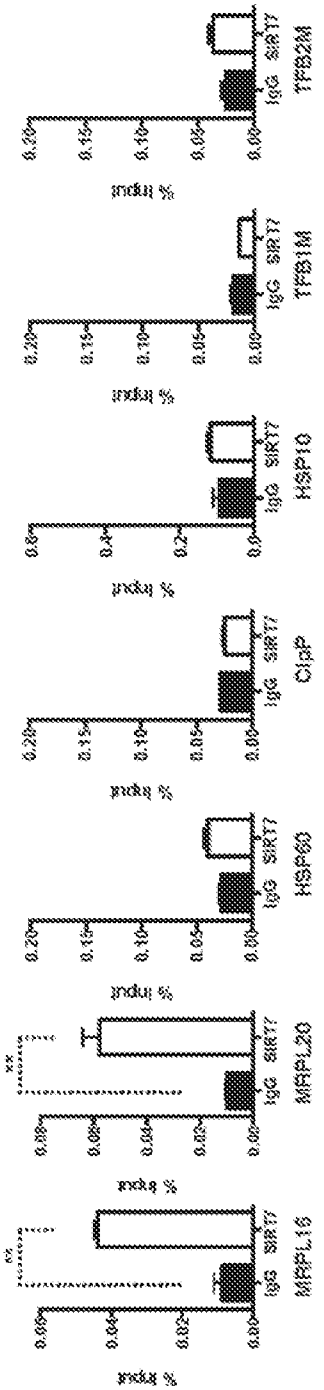


FIG 2B

FIG 2C

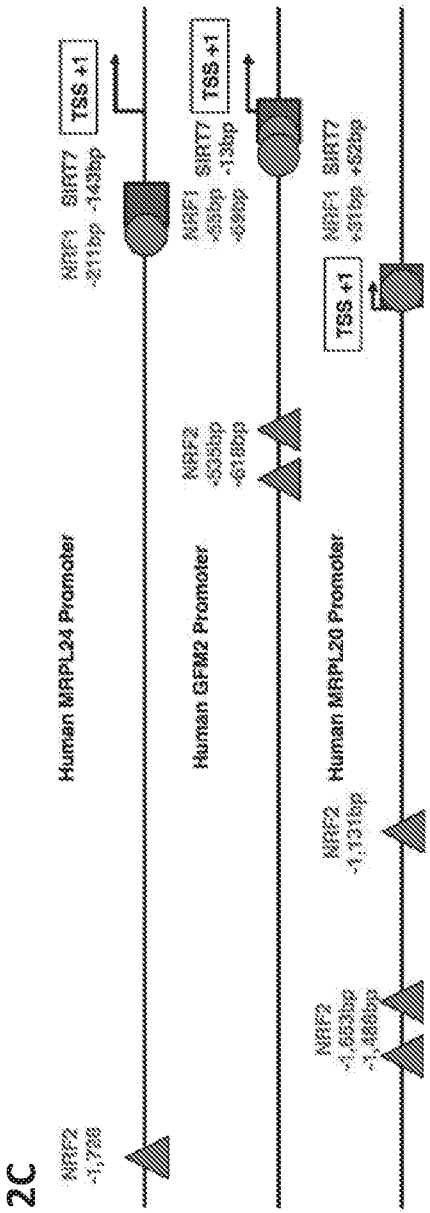


FIG 3A

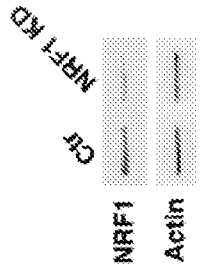
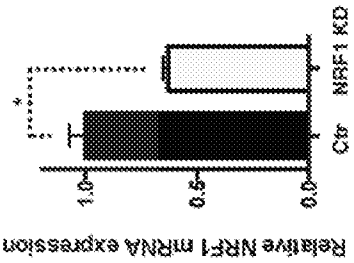

NRF1 KD
Ctr
NRF1
Actin

FIG 3B


Relative NRF1 mRNA expression
1.0
0.5
0.0
Ctr
NRF1 KD

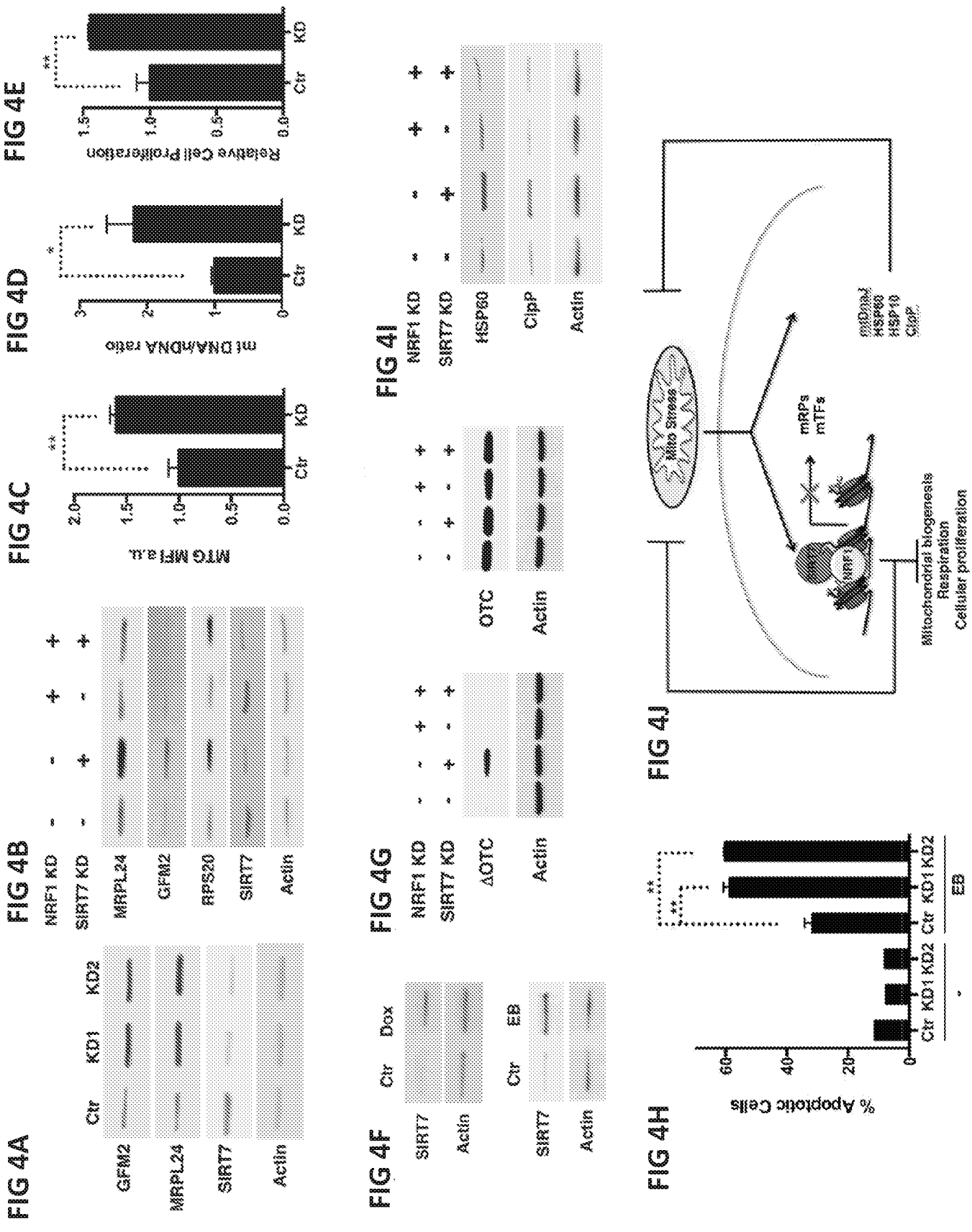

FIG 4A
Ctr
KD1
KD2
GFM2
MRPL24
SIRT7
Actin
FIG 4B
NRF1 KD
SIRT7 KD
MRPL24
GFM2
RPS20
SIRT7
Actin
FIG 4C
MTG MFI a.u.
Ctr
KD
FIG 4D
mt DNA/nDNA ratio
Ctr
KD
FIG 4E
Relative Cell Proliferation
Ctr
KD
FIG 4F
Ctr
Dox
SIRT7
Actin
Ctr
EB
SIRT7
Actin
FIG 4G
NRF1 KD
SIRT7 KD
ΔOTC
Actin
FIG 4I
NRF1 KD
SIRT7 KD
HSP60
ClpP
Actin
FIG 4J
Mito Stress
mRPs
mTFs
NRF1
mtDNA
HSP60
HSP10
ClpP
Mitochondrial biogenesis
Respiration
Cellular proliferation
FIG 4H
% Apoptotic Cells
Ctr
KD1
KD2
Ctr
KD1
KD2
-
EB

FIG 5A

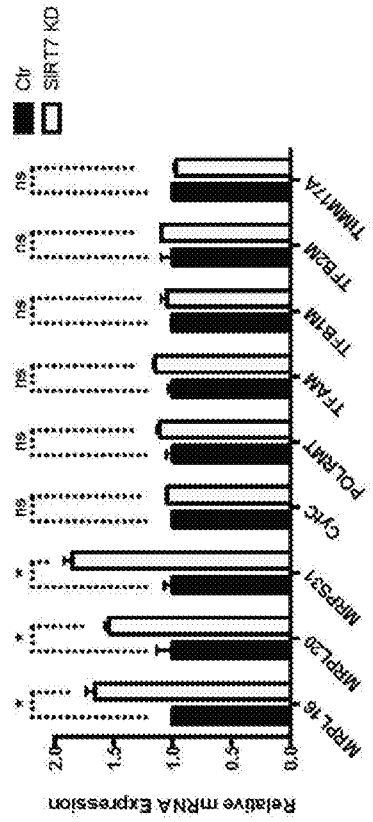
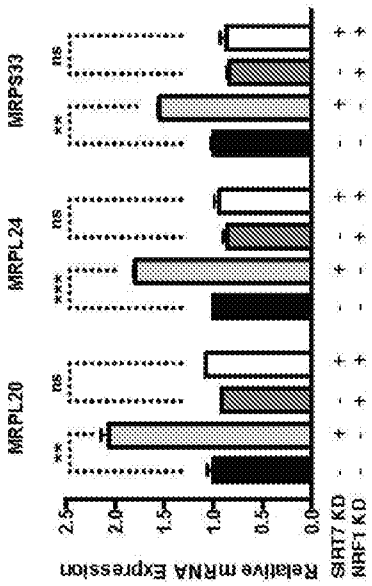
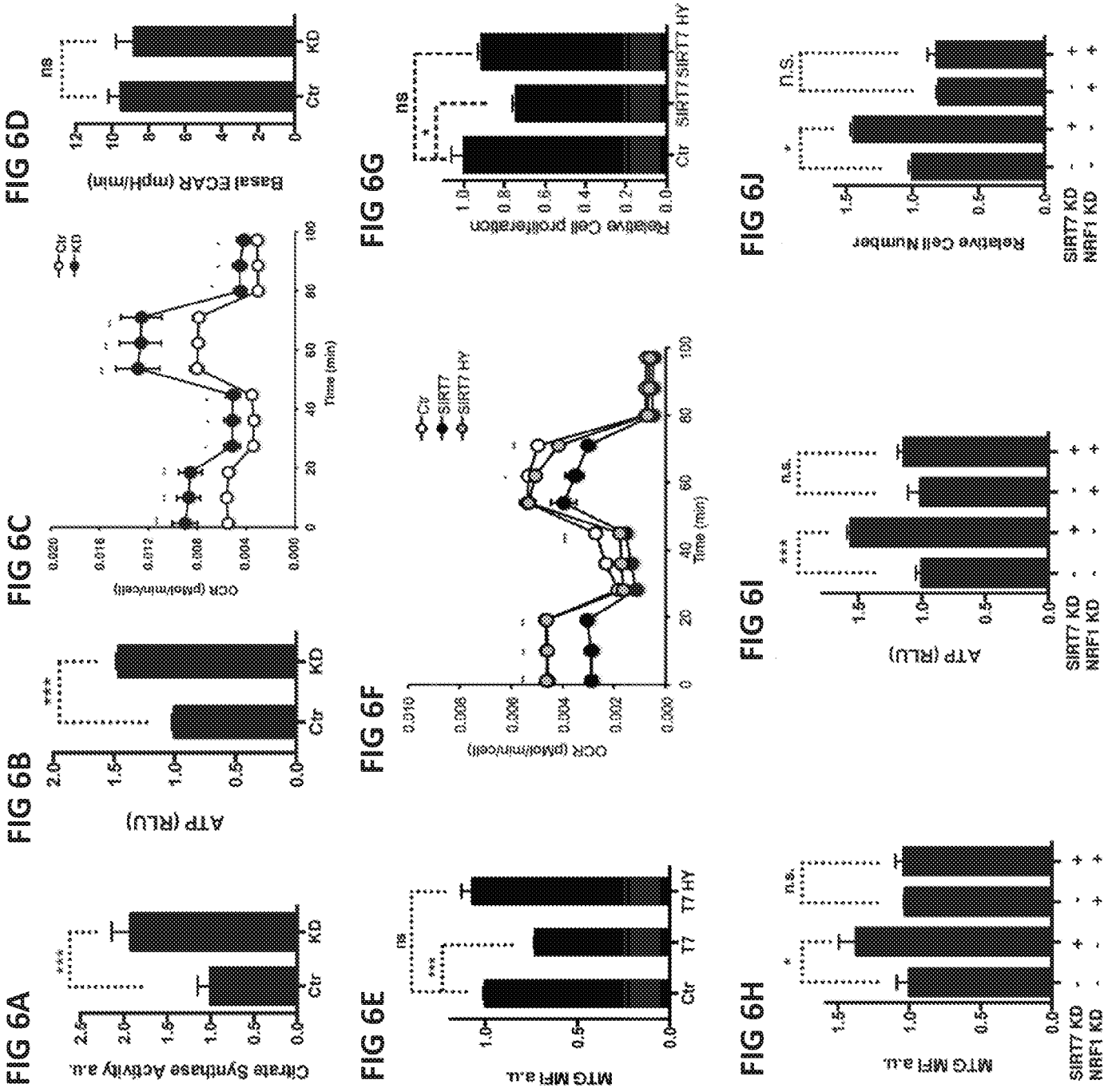

Ctr
SIRT7 KD
Relative mRNA Expression
2.0
1.5
1.0
0.5
0.0
MRPL16
MRPL20
MRPS31
CYTC
POLRMT
TFAM
TFB1M
TFB2M
TMEM17A
*
*
*
ns
ns
ns
ns
ns
ns

FIG 5B


MRPL20
MRPL24
MRPS33
Relative mRNA Expression
2.5
2.0
1.5
1.0
0.5
0.0
**
ns
***
ns
**
ns
SIRT7 KD - + - + - + - + - + - +
NRF1 KD - - + + - - + + - - + +


FIG 6A
Citrate Synthase Activity a.u.
Ctr
KD
FIG 6B
ATP (RLU)
Ctr
KD
FIG 6C
OCR (pMol/min/cell)
Time (min)
Ctr
KD
FIG 6D
Basal ECAR (mpH/min)
Ctr
KD
FIG 6E
MTG MFI a.u.
Ctr
T7
T7 HY
FIG 6F
OCR (pMol/min/cell)
Time (min)
Ctr
SIRT7
SIRT7 HY
FIG 6G
Relative Cell proliferation
Ctr
SIRT7
SIRT7 HY
FIG 6H
MTG MFI a.u.
SIRT7 KD
NRF1 KD
FIG 6I
ATP (RLU)
SIRT7 KD
NRF1 KD
FIG 6J
Relative Cell Number
SIRT7 KD
NRF1 KD

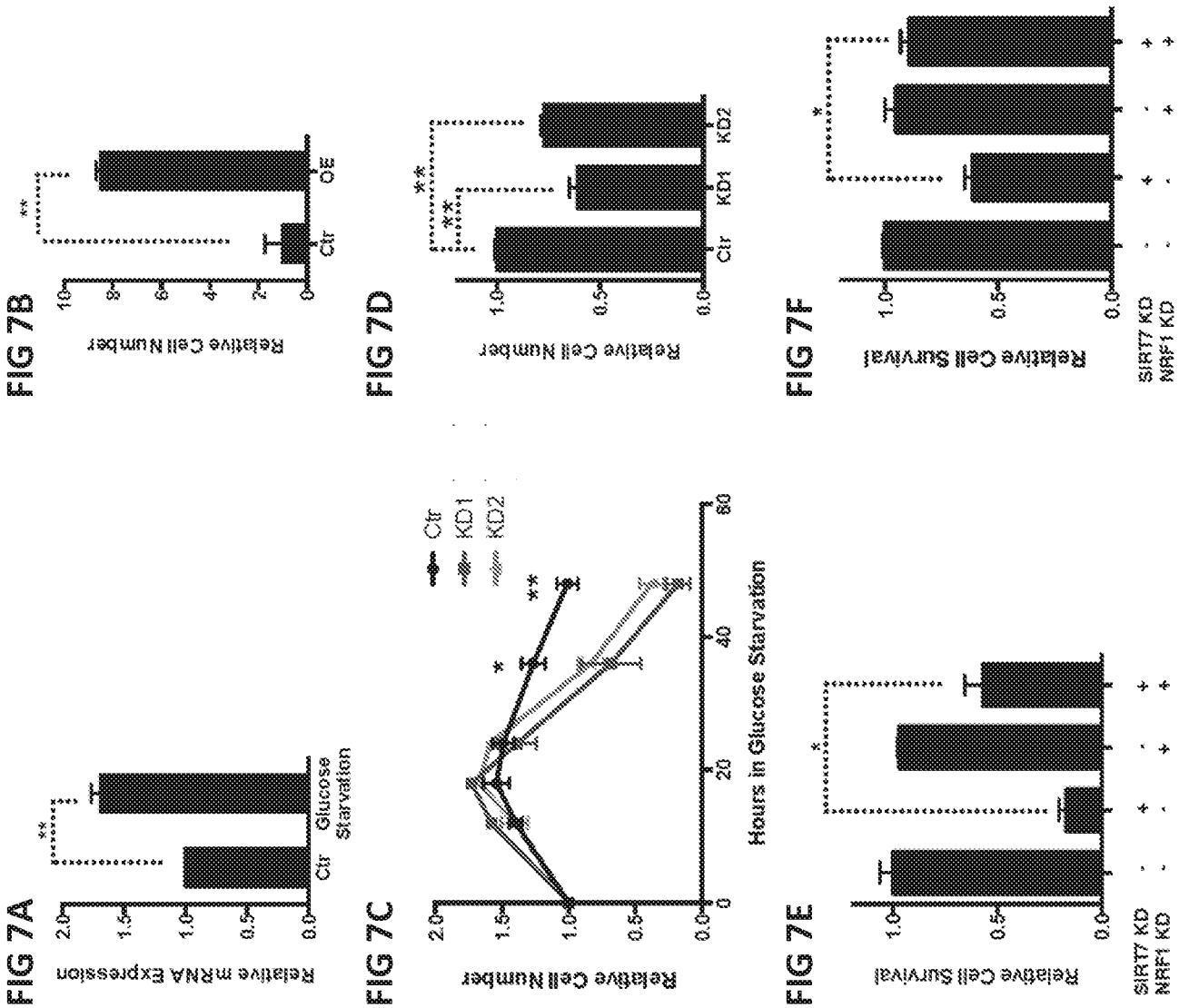

FIG 7A
Relative mRNA Expression
Ctr
Glucose Starvation
FIG 7B
Relative Cell Number
Ctr
OE
FIG 7C
Ctr
KD1
KD2
Relative Cell Number
Hours in Glucose Starvation
FIG 7D
Relative Cell Number
Ctr
KD1
KD2
FIG 7E
Relative Cell Survival
SIRT7 KD
NRF1 KD
FIG 7F
Relative Cell Survival
SIRT7 KD
NRF1 KD

FIG 8A

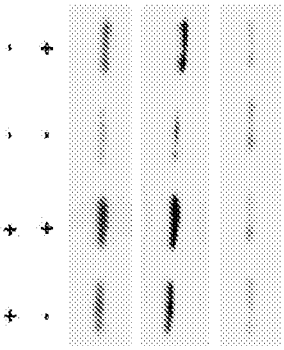


FIG 8B

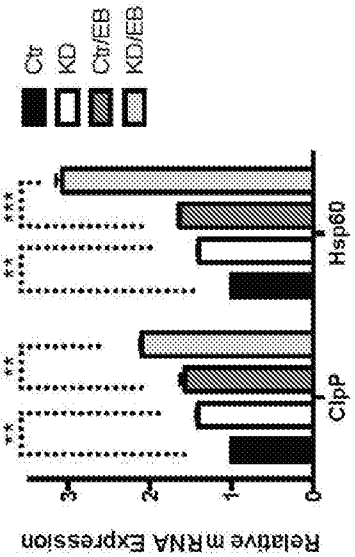


FIG 8C

FIG 9A

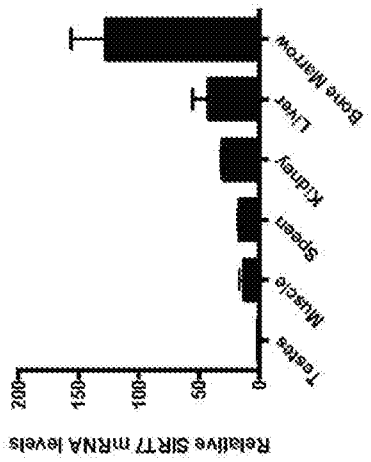


FIG 9B

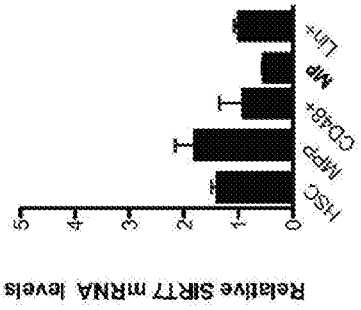
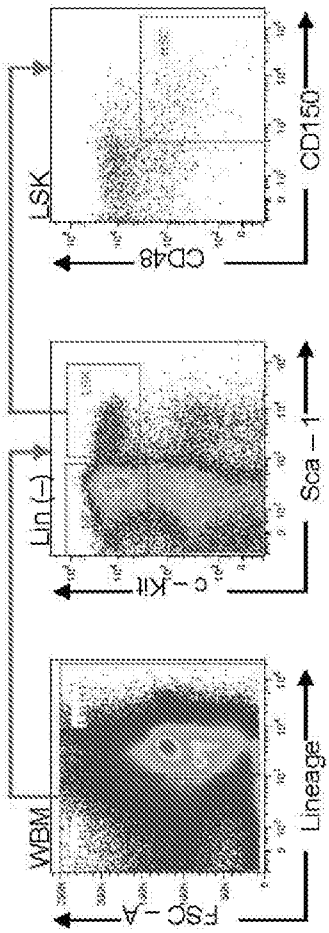


FIG 9C



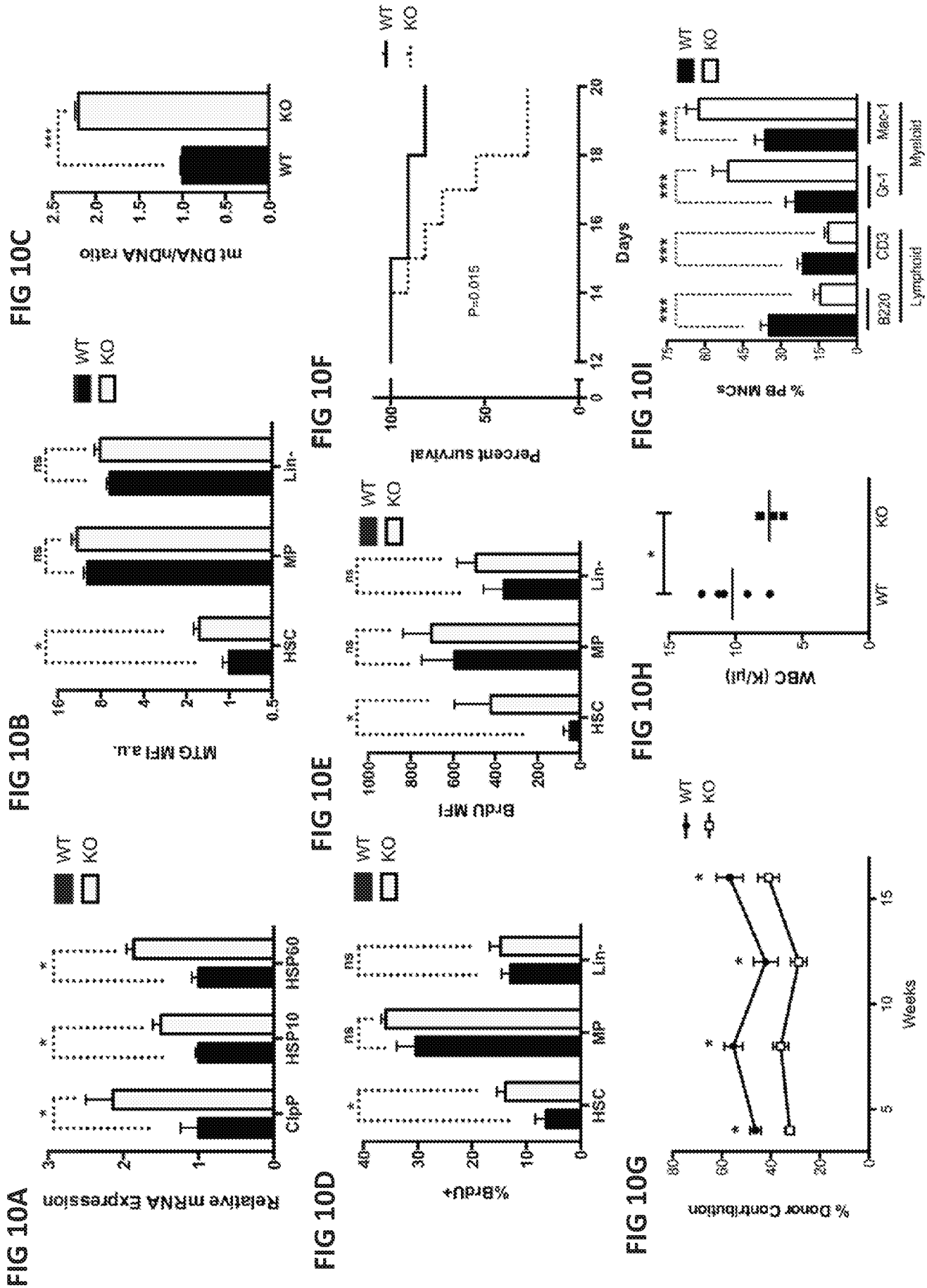

FIG 10A
Relative mRNA Expression
WT
KO
ClpP
HSP10
HSP60
FIG 10B
MTG MFI a.u.
HSC
MP
Lin-
ns
FIG 10C
mt DNA/nDNA ratio
WT
KO
FIG 10D
%BrdU+
FIG 10E
BrdU MFI
FIG 10F
Percent survival
P=0.016
FIG 10G
% Donor Contribution
Weeks
FIG 10H
WBC (K/µl)
FIG 10I
% PB MNCs
Days
B220
CD3
Gr-1
Mac-1
Lymphoid
Myeloid

FIG 11

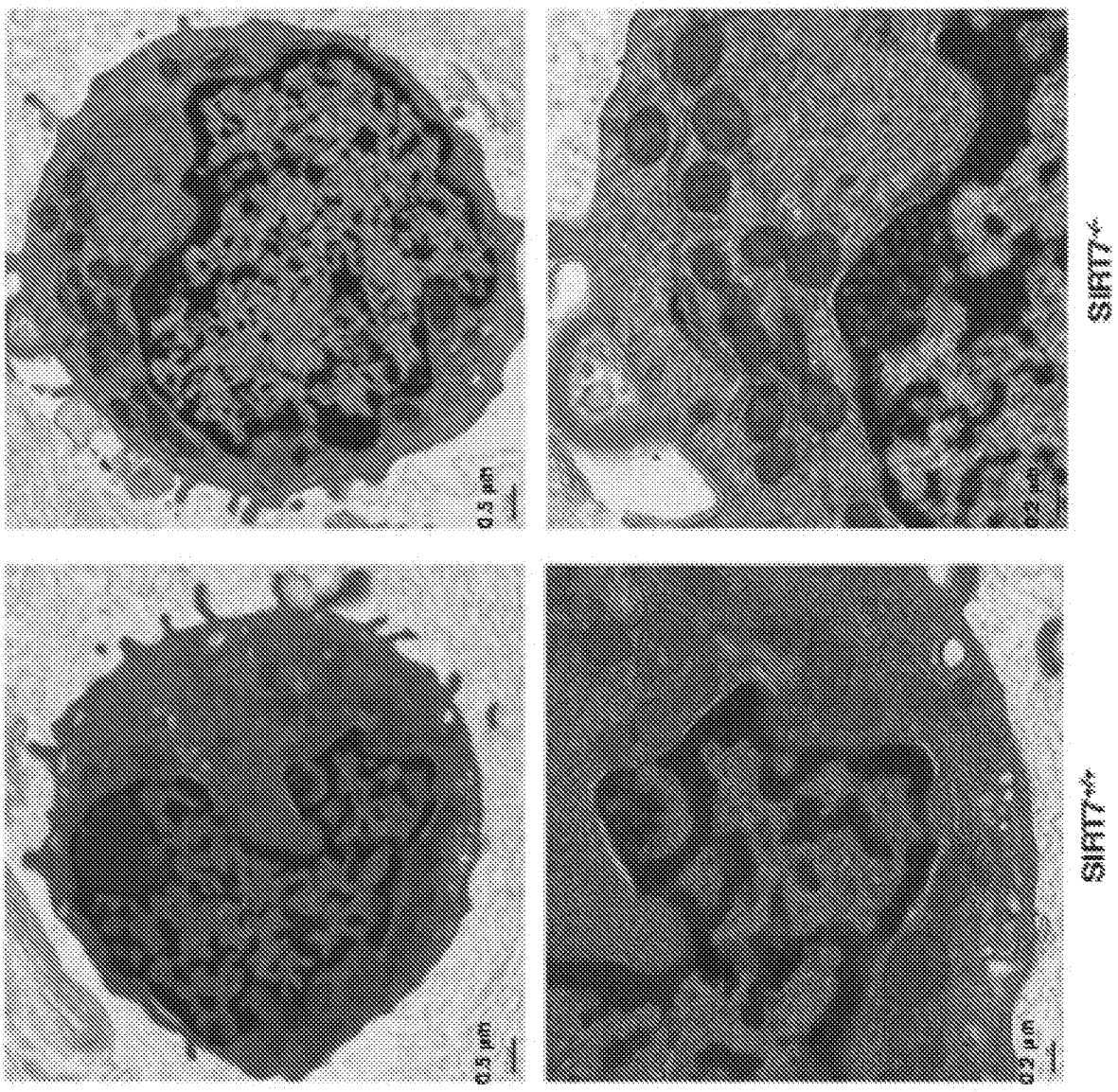


FIG 12A

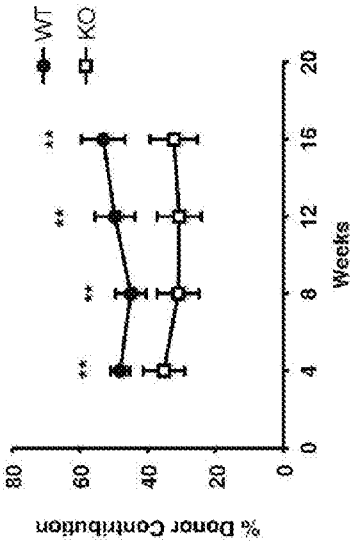


FIG 12B

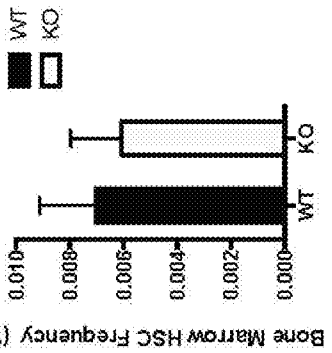


FIG 12C

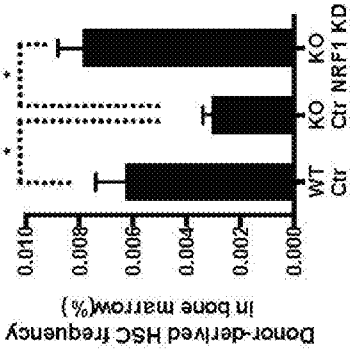


FIG 12D

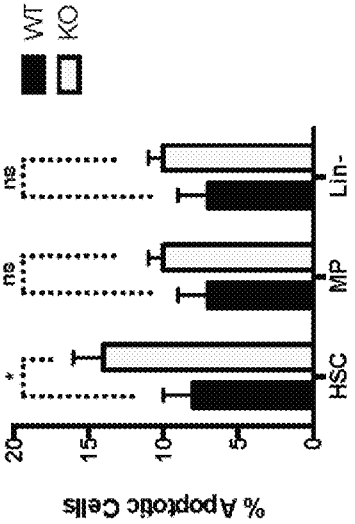


FIG 12E

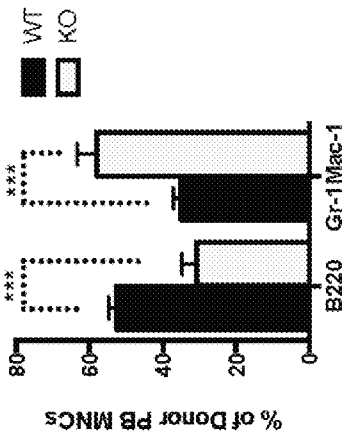


FIG 12F

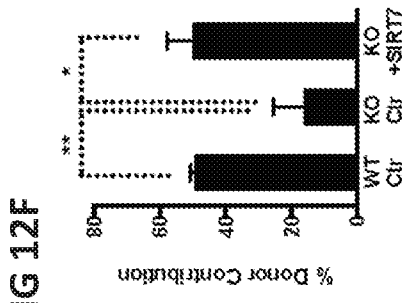


FIG 12G

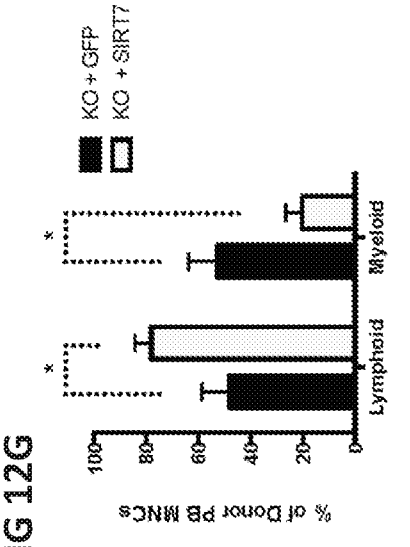


FIG 13A

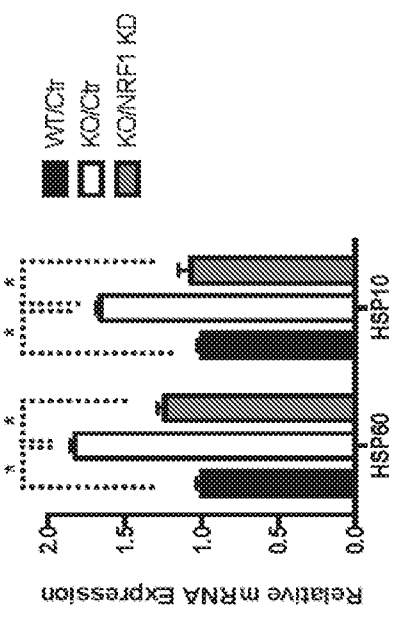


FIG 13B

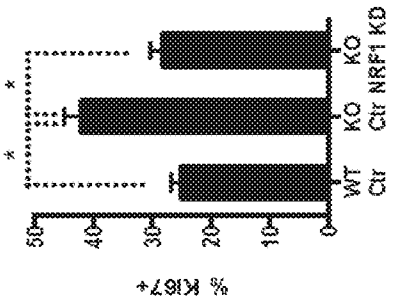


FIG 13C

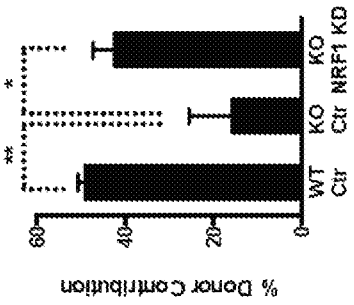


FIG 13D

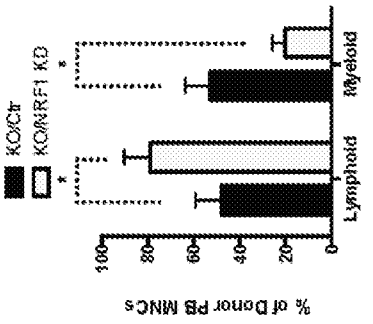


FIG 14A

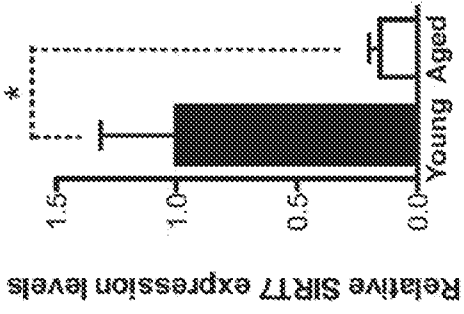


FIG 14B

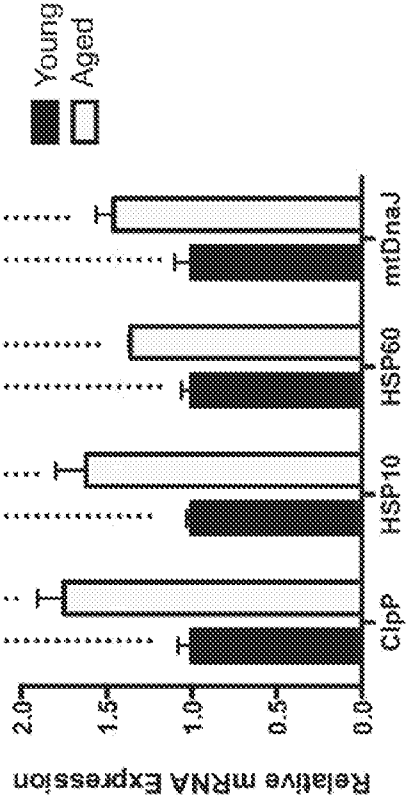


FIG 14C

FIG 14D

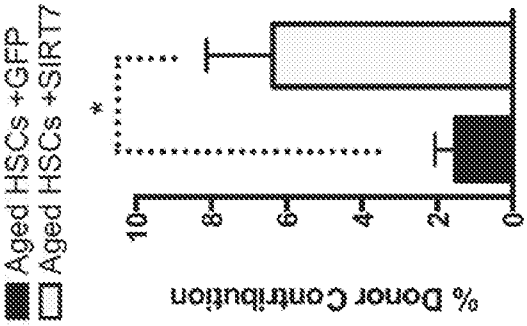


FIG 14E

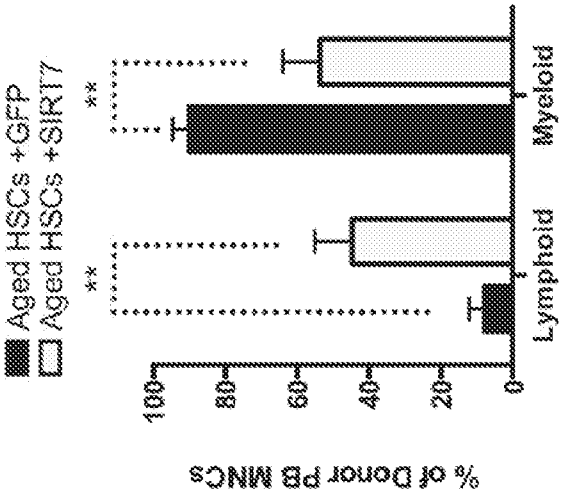


FIG 15A

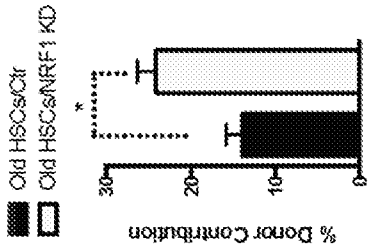
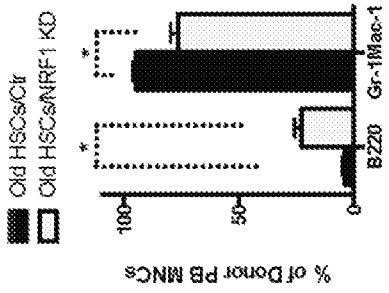


FIG 15B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/023270

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0789
ADD.

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)
C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Barbara Bailus: "Interview with Dr. Danica Chen: Metabolic Regulation of Stem Cell Aging and Rejuvenation ¦ SAGE", , 27 January 2015 (2015-01-27), XP055284388, Retrieved from the Internet: URL:http://sage.buckinstitute.org/interview-with-dr-danica-chen-metabolic-regulation-of-stem-cell-aging-and-rejuvenation/ [retrieved on 2016-06-29]	1-26
Y	the whole document	1-26

-/--

[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 2016	12/07/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Martin

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/023270

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous: "Molecular Mechanism of Dietary Restriction's Protection Against Aging and Diseases of Aging - UNIVERSITY OF CALIFORNIA, BERKELEY", , 30 September 2014 (2014-09-30), XP055284532, Retrieved from the Internet: URL:http://www.reeis.usda.gov/web/crisprojectpages/0218571-molecular-mechanism-of-dietary-restrictions-protection-against-aging-and-diseases-of-aging.html [retrieved on 2016-06-29]	1-26
Y	the whole document	1-26

Y	MENDEL ROTH ET AL: "Sirtuins in Hematological Aging and Malignancy", CRITICAL REVIEWS IN ONCOGENESIS, vol. 18, no. 6, 12 March 2014 (2014-03-12), pages 531-547, XP055284597, ISSN: 0893-9675, DOI: 10.1615/CritRevOncog.2013010187 the whole document	1-26

X,P	M. MOHRIN ET AL: "A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging", SCIENCE, vol. 347, no. 6228, 20 March 2015 (2015-03-20), pages 1374-1377, XP055284412, US ISSN: 0036-8075, DOI: 10.1126/science.aaa2361 the whole document	1-26
