



(51) International Patent Classification:

A61K 41/00 (2020.01)

(21) International Application Number:

PCT/US2023/066096

(22) International Filing Date:

22 April 2023 (22.04.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/334,022 22 April 2022 (22.04.2022) US

(71) Applicant: SENS RESEARCH FOUNDATION
[US/US]; 110 Pioneer Way, Suite J, Mountain View, California 94041 (US).

(72) Inventors: ADMASU, Teshahun Dessale; SENS RESEARCH FOUNDATION, 110 Pioneer Way, Suite J, Mountain View, California 94041 (US). SHARMA, Amit; SENS RESEARCH FOUNDATION, 110 Pioneer Way, Suite J, Mountain View, California 94041 (US).

(74) Agent: ENTRALTA PLLC et al.; 4500 Williams Drive, Suite 212-511, Georgetown, Texas 78633 (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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: SENOLYTIC TARGETS OF SENESCENT CELLS

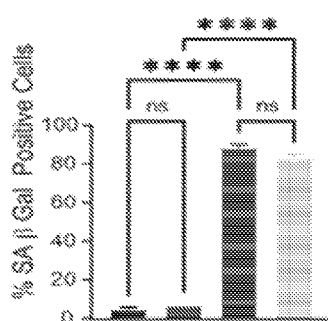


FIG. 1A

(57) Abstract: Senescent cells are implicated in aspects of age-related decline in health and contribute to many diseases. Cells can be transformed into a senescent state upon prolonged exposure to the Senescence-Associated Secretory Phenotype (SASP) factors produced by primary senescent cells (SCs). The transformed cells are known as Secondary Senescent Cells (SCCs). Applicants discovered phenotypic differences between SCCs and SCs. SCCs can present distinct pro-survival pathways and resistance to conventional senolytic drugs. Embodiments include methods of killing SCCs and SCs by inducing ferroptosis with an agent such as erastin. The methods described herein have therapeutic uses for senescence-associated diseases and disorders.



SENOLYTIC TARGETS OF SENESCENT CELLS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application serial number 63/334,022 filed on April 22, 2022. The contents of the aforementioned application are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention relates to therapeutics, and more specifically, it relates to therapeutic methods for senescence-associated diseases and disorders and for removing high iron accumulating cells such as senescent cells, macrophages and cancer cells.

BACKGROUND

[0003] Aging can be defined as the process of becoming older. In humans, aging represents the accumulation of changes over time and can encompass physical, psychological and social changes. Advanced age is the greatest risk factor for many chronic diseases. More than 90% of adults aged 65 or older experience at least one chronic disease such as cancer, diabetes or cardiovascular disease. Aging phenotypes and pathologies, including diverse age-associated diseases and disorders, are causally linked to the accumulation of senescent cell burden with age. According to the estimate released by US Census Bureau in June 2020, the numbers of individuals over the age 65 years has grown over 34.2% in last decade. This increasingly aging population is expected to put an unprecedented burden on the healthcare system as aging is linked to various diseases. Thus, a better understanding of the common mechanisms influencing the age-related phenotypes has become a priority for many researchers.

[0004] Cellular senescence can be considered a state of permanently arrested cell cycle. Senescent cells are characterized by irreversible cell-cycle arrest of proliferation-competent cells, morphological and metabolic changes, altered gene expression, chromatin reorganization, and a unique pro-inflammatory Senescence-Associated

Secretory Phenotype (SASP). Senescent cells in older adults contribute to chronic inflammation and damage to surrounding tissues. It has been demonstrated that removal of senescent cells via genetic manipulation in transgenic mouse models can prevent or delay tissue dysfunction, improve age-related pathologies, and extend health span. This suggests that removal of senescent cell burden in aging adults, merits further study as a therapeutic target of interest for the treatment and prevention of disease of aging.

[0005] Recent efforts have focused on developing senolytic agents to treat senescence-associated diseases or disorders. Due to their causal link with various age-related diseases in animal models and human studies, selective killing of senescent cells (SCs) by senolytics has shown great promise. Eliminating SCs to reduce senescence burden in with age presents promising therapeutic potential for several age-related pathologies.

[0006] Recent studies have identified substantial variations among SCs. For example, cellular senescence caused by different stresses such as replicative arrest, expression of oncogenes and genotoxic stress have differences in molecular signature. Further, the senescent phenotype can vary depending on the tissue of origin. Accordingly, there is a need for greater understanding of molecular regulatory pathways of cellular senescence. Applicants have characterized secondary senescence as an aspect of the present invention.

[0007] Unlike primary senescent cells (SCs) that undergo permanent cell cycle arrest upon exposure to insult (e.g., exposure to DNA damaging agents, telomer loss, mitochondrial dysfunction, oncogene activation, etc.) some cells are known to undergo a senescent state upon prolonged exposure to the SASP factors produced by SCs or similar pro-inflammatory factors. These cells are referred to as secondary senescent cells (SSCs). The phenomenon of secondary senescence has been demonstrated both in cell culture and *in vivo* and is known to drive the accumulation of SCs and subsequent functional defects with aged mice. However, investigations in differences

between SCs and secondary SCs has been hampered due to lack of adequate model systems. For example, *in vitro* studies, when secondary senescence is induced by exposing non-senescent cells (NS) to SASP-containing conditioned medium from SCs, the resulting population is highly heterogeneous, and less than half of treated cells undergo senescence.

[0008] Because of these shortcomings, there is a need for further characterization of cellular senescence. The present invention includes methods of identifying and selective targeting of primary and secondary senescent cells. Also included are methods of treating age-related diseases and conditions by selective elimination of senescent cells.

SUMMARY OF THE INVENTION

[0009] The inventions described and claimed herein have many attributes and embodiments including, but not limited to, those set forth or described or referenced in this brief summary. The inventions described and claimed herein are not limited to, or by, the features or embodiments identified in this summary, which is included for purposes of illustration only and not restriction.

[0010] Embodiments include pharmaceutical formulations for identifying and eliminating senescent (i.e., aging) cells.

[0011] Embodiments also include pharmaceutical formulations for treating a senescence-associated disease or disorder. The formulation can include an agent that selectively kills senescent cells, and/or cancer cells with high iron level by inducing ferroptosis.

[0012] Embodiments also include methods of identifying and eliminating cells with high iron content (e.g., macrophages and cancer cells) by, for example, inducing ferroptosis.

[0013] Embodiments also include pharmaceutical formulations for identifying and eliminating cells with high iron content (e.g., macrophages and cancer cells).

[0014] Embodiments also include methods of treating a senescence-associated disease or disorder that includes administering a senolytic agent (e.g., a small molecule), wherein the senolytic agent selectively kills senescent cells over non-senescent cells.

[0015] Embodiments also include methods of slowing the aging process or reducing signs of aging by inducing ferroptosis in senescent cells of a subject.

[0016] Embodiments also include methods of slowing the aging process or reducing signs of aging that include administering an agent (e.g., a small molecule medicament) that induces ferroptosis.

[0017] Embodiments also include methods of slowing the aging process or reducing signs of aging that include administering a senolytic agent (e.g., a small molecule), wherein the senolytic agent selectively kills senescent cells over non-senescent cells.

[0018] Embodiments also include methods of identifying senescent cells for targeted therapy. Embodiments also include methods of removing senescent cells from an affected tissue of a subject.

[0019] Embodiments include methods of treating an ailment. The method can include administering a therapeutic amount of a senolytic agent to a subject. The senolytic agent (e.g., erastin) can selectively target senescent cells (primary senescent cells, secondary senescent cells and/or tertiary senescent cells). The ailment can be a senescence-associated disease or disorder such as atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis or glaucoma.

[0020] Embodiments also include methods of treating a senescence-associated disease or disorder by inducing ferroptosis in senescent cells of a subject. Ferroptosis can be induced by administering a small molecule medicament such as erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162 and/or ML210. Embodiments also include methods of targeting cells that have high iron accumulation such as cancer

cells, senescent cells and macrophages.

[0021] In other embodiments, ferroptosis is induced by modulating one or more proteins or pathways in senescent cells which can include IRP1, IRP2, transferrin receptor 1 (TfR1), divalent metal transporter 1 (DMT1), ferroportin and ferritin.

[0022] Embodiments also include methods of slowing the aging process or reducing signs of aging by inducing ferroptosis in senescent cells of a subject.

[0023] Embodiments also include methods of identifying senescent cells and/or a senescence-associated disease or disorder. In one embodiment, dipeptidyl peptidase-4 (DPP4) is used as a biomarker. Further embodiments include methods of treating a senescence-associated disease or disorder by (a) identifying the senescence-associated disease or disorder based on altered expression levels of at least one biomarker and (b) treating the subject by administering a therapeutic amount of a senolytic agent. In one embodiment, the senolytic agent induces ferroptosis.

[0024] Embodiments also include methods of identifying a therapeutic target in senescent cells. The method can include steps of (a) inducing senescence in a population of cells (e.g., with UV light or DOXO) and (b) comparing levels of protein expression between senescent and non-senescent cells. The method can include a step of isolating senescent cells using a surface biomarker such as DPP4. The therapeutic target can be, for example, a pathway or a protein that is upregulated in senescent cells.

[0025] Embodiments also include methods of identifying a therapeutic target in secondary senescent cells. The method can include steps of (a) inducing primary senescence in a first population of cells, (b) inducing secondary senescence in a second population of cells and (c) comparing levels of protein expression between the first population and the second population of cells. The therapeutic target can be, for example, a pathway or a protein that is upregulated in senescent cells.

[0026] Embodiments also include methods of treating a senescence-associated disease or disorder. The method can include a step of modulating a pathway that

induces senescence. The pathway can be, for example, the p53 pathway, a protein secretion pathway, apoptosis, mTORC1 signaling and/or proinflammatory pathways such as interferon alpha (IFN- α) and interferon gamma (IFN- γ).

[0027] Embodiments also include methods of slowing the aging process or reducing signs of aging. The method can include a step of modulating a pathway that induces senescence. The pathway can be, for example, the p53 pathway, a protein secretion pathway, apoptosis, mTORC1 signaling and/or proinflammatory pathways such as interferon alpha (IFN- α) and interferon gamma (IFN- γ).

[0028] Embodiments also include methods of selectively removing or killing senescent cells from a subject. The method can include modulating protein expression or protein activity in senescent cells. The protein can be, for example, GDF7, WNT5B, IGFBP5, CST1, IGFBP3, PAPP2, EPHA7 and/or TNC. In one embodiment, the protein is a Metallothionein such as MT1A, MT1F, MT1G, MT1H and MT1M.

[0029] Embodiments also include methods of identifying senescent cells, a senescence-associated disease or disorder based on the expression (i.e., upregulation and/or downregulation) of one or more genes identified in Table IA – ID.

[0030] Embodiments also include methods of identifying senescent cells, a senescence-associated disease or disorder based on the expression (i.e., upregulation and/or downregulation) of one or more noncoding RNAs identified in Table IIA – IID.

[0031] Embodiments also include methods of identifying senescent cells, a senescence-associated disease or disorder based on the expression (i.e., upregulation and/or downregulation) of one or more pro-survival genes identified in Table IIIA – IIID.

[0032] Embodiments also include methods of identifying senescent cells, a senescence-associated disease or disorder based on the expression (i.e., upregulation and/or downregulation) of one or more surface proteins identified in Table IVA – IVD.

[0033] Embodiments also include methods of identifying senescent cells, a senescence-associated disease or disorder based on the expression (i.e., upregulation

and/or downregulation) of one or more secreted proteins identified in Table VA – VD.

[0034] Embodiments also include methods of killing senescent cells, slowing the aging process or reducing signs of aging by modulating activity of one or more genes identified in Table I – Table V.

[0035] Embodiments also include methods of identifying senescent cells, a senescence-associated disease or disorder based on the presence of one or more non-coding RNA genes. In embodiments, the non-coding RNA genes are one or more of those listed in Table IIA – IID.

[0036] Embodiments also include methods of killing senescent cells, slowing the aging process or reducing signs of aging by modulating activity of one or more non-coding RNA genes in a subject. In embodiments, the non-coding RNA genes are one or more of those listed in Table IIA – IID.

[0037] Embodiments also include methods of identifying senescent cell, a senescence-associated disease or disorder based on the presence of one or more biomarkers. In embodiments, the one or more biomarkers are selected from the surface proteins of Table IVA – IVD.

[0038] Embodiments also include methods of identifying senescence in cells based on the presence of one or more surface proteins. The methods can include measuring one or more of the surface proteins identified in Table IVA – IVD. The senescence can be primary senescence or secondary senescence.

[0039] Embodiment also include targeting drugs towards senescent cells using the surface proteins, such as senolytics or other drug, or molecules.

[0040] Embodiment include the use of natural killer (NK) cells or other immune cells. The cells can be modified to recognize the senescence cell surface proteins identified herein.

[0041] Embodiment for removal of senescent cells based on the identified

senescent cell surface proteins.

[0042] Embodiments also include methods of treating a senescence-associated disease or disorder by administering a senolytic agent such as vorinostat ("SAHA") or emetine.

[0043] Embodiments also include methods of treating a senescence-associated disease or disorder by modulating activity of one or more genes. The gene can include those identified in Table III.

[0044] Embodiments also include methods of slowing the aging process or reducing signs of aging by modulating one or more of the genes identified in Table III.

[0045] Embodiments also include methods of treating a senescence-associated disease or disorder by targeting cells (e.g., for selective removal) based on their iron content.

[0046] Embodiments also include methods of slowing the aging process or reducing signs of aging by targeting cells (e.g., for selective removal) based on their iron content.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] The accompanying drawings illustrate aspects of the present invention. In such drawings:

[0048] FIG. 1A is a graphical depiction of the percent of SA- β -gal positive cells in DPP4+ isolated primary and secondary senescent cells from three donors. Senescent cells (DPP4+) were isolated by magnetic beads using DPP4 as a surface marker. Isolated cells were replated, and SA- β -gal was conducted after 24 hours.

[0049] FIG. 1B shows the quantification of γ H2AX foci in DPP4+ isolated primary and secondary senescent cells. Cells with two or more foci per nucleus were defined as senescent cells.

[0050] FIG. 1C shows that HMGB1 relocalizes in senescent cells. The percentage

of cells expressing HMGB1 in the nucleus and nucleus + cytosol was scored. HMGB1 re-localizes to the cytosol in 80% of primary and 70% of DPP4+ isolated secondary senescent cells.

[0051] FIG. 1D shows mRNA expression of p16 Ink4a as determined by RT-qPCR in DPP4+ isolated primary and secondary senescent cells.

[0052] FIG. 1E shows mRNA expression of p21 Cip1 as determined by RT-qPCR in DPP4+ isolated primary and secondary senescent cells.

[0053] FIG. 1F shows mRNA expression of LMNB1 as determined by RT-qPCR in DPP4+ isolated primary and secondary senescent cells.

[0054] FIG. 1G shows IL-6 and IL-8 secretion as measured by ELISA in conditioned media from DPP4+ isolated senescent cells in three donors.

[0055] FIG. 1H shows the percent of SA- β -gal positive cells in cells treated with conditioned medium collected from unenriched and DPP4+ enriched secondary SCs.

[0056] FIG. 1I shows the percent of cells with two or more γ H2AX foci per nucleus in cells treated with conditioned medium collected from unenriched and DPP4+ enriched secondary SCs. A two-tailed t-test was used to analyze the data.

[0057] FIG. 1J depicts a comparison of previous method of comparing primary and secondary SCs. Values are presented as mean $\pm$ SEM. Comparison was made with one-way ANOVA. Error bars = SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

[0058] FIG. 2A is a graphical depiction of the expression levels of pro-survival BCL-2 family proteins in DPP4+ primary and secondary SCs. DPP4+ SCs were isolated 7 days after senescence induction and protein expression were determined by western blot from the whole cell lysate.

[0059] FIG. 2B depicts the experimental design for (subsequent experiments of FIG. 2C – 2F) and other senolytic studies. Cells were cultured in T175 flasks and 7 days after senescence induction DPP4 positive SCs were isolated and replated to 96

well plates. 24 hours after replating all suspended cells were removed and live attached cells were treated with senolytic drugs. After 72 hours of treatment viability was determined using calcein-AM assay unless otherwise specified.

[0060] FIG. 2C shows that ABT-199 has senolytic activity in DPP4+ primary but not secondary SCs. Quantification of viable endothelial non-senescent cells, cells treated with NSCM, DPP4+ primary SCs and DPP4+ secondary SCs 24 hours after treatment with increasing concentrations of ABT-199 (3 technical and 3 biological (3 different donors) replicates)

[0061] FIG. 2D shows that ABT-263 has senolytic activity in DPP4+ primary but not secondary SCs. Quantification of viable endothelial non-senescent cells, cells treated with NSCM, DPP4+ primary SCs and DPP4+ secondary SCs 72 hours after treatment with increasing concentrations of ABT-263 (3 technical and 3 biological (3 different donors) replicates).

[0062] FIG. 2E shows that Quercetin has senolytic activity in both DPP4+ primary and secondary SCs. Quantification of viable endothelial non-senescent cells, cells treated with NSCM, DPP4+ primary SCs and DPP4+ secondary SCs 72 hours after treatment with increasing concentrations of Quercetin (3 technical and 3 biological (3 different donors) replicates).

[0063] FIG. 2F shows that D and Q combination is toxic towards non senescent cells. Quantification of viable endothelial non-senescent cells, cells treated with NSCM, DPP4+ primary SCs and DPP4+ secondary SCs 72 hours after treatment with 10 μ M Q and increasing concentrations of D (3 technical and 3 biological (3 different donors) replicates). Comparison was made with one-way ANOVA. Error bars = mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

[0064] FIG. 3A is a graphical depiction of the transcriptome profile of DPP4+ primary and secondary SCs. The total number of differentially expressed genes (No. of DEF's) is indicated on the y-axis (LFC $\geq$ 1 and P-value < 0.05). Primary and secondary senescence were induced in endothelial cells from three young donors.

Seven days after the induction of senescence, DPP4+ primary and secondary SCs were collected and analyzed for gene expression by RNASeq.

[0065] FIG. 3B is a heat map of the top 30 DEG's for DPP4+ primary and secondary SCs. DPP4+ secondary SCs from three different donors were well separated from primary SCs and clustered together.

[0066] FIG. 3C is a two-dimensional principal component analysis ("2D PCA") of DPP4+ primary and secondary SCs from three donors. Samples from one donor (artery) were well separated from the other two donors (veins) by the first principal component followed by separation based on senescence types.

[0067] FIG. 3D is a 2D PCA of DPP4+ primary and secondary SCs and non-senescent controls from one donor. Secondary senescent samples were well separated from primary senescent and non-senescent samples.

[0068] FIG. 3E shows the gene ontology ("GO") terms enriched by the DEG's of DPP4+ primary SCs.

[0069] FIG. 3F shows the GO terms enriched by the DEG's of DPP4+ secondary SCs.

[0070] FIG. 3G is a graph showing RNASeq confirmation by RT-qPCR. RNA was isolated from NS, NSCM treated cells, DPP4+ primary and secondary SCs from three donors, reverse transcribed, and mRNAs encoding *GDF7* genes were measured by qPCR. Comparison was made with one-way ANOVA. Error bars = mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

[0071] FIG. 3H is a graph showing RNASeq confirmation by RT-qPCR. mRNAs encoding *WNT5B* genes were measured by qPCR.

[0072] FIG. 3I is a graph showing RNASeq confirmation by RT-qPCR. mRNAs encoding *IGFBP5* genes were measured by qPCR.

[0073] FIG. 3J is a graph showing RNASeq confirmation by RT-qPCR. mRNAs

encoding CST1 genes were measured by qPCR.

[0074] FIG. 3K is a graph showing RNASeq confirmation by RT-qPCR. mRNAs encoding IGFBP3 genes were measured by qPCR.

[0075] FIG. 3L is a graph showing RNASeq confirmation by RT-qPCR. mRNAs encoding PAPP2 genes were measured by qPCR.

[0076] FIG. 3M is a graph showing RNASeq confirmation by RT-qPCR. mRNAs encoding EPHA7 genes were measured by qPCR.

[0077] FIG. 3N is a graph showing RNASeq confirmation by RT-qPCR. mRNAs encoding TNC genes were measured by qPCR.

[0078] FIG. 4A is a volcano plot for differential expressed genes (DEG's) of secondary SCs. Metallothionines and solute carrier proteins are among the top DEG's.

[0079] FIG. 4B is a volcano plot for DEG's of primary SCs.

[0080] FIG. 4C is a heat map of iron metabolism related genes based on RNASeq analysis.

[0081] FIG. 4D is a graph showing levels of RNA isolated from NS, NSCM treated cells, DPP4+ primary and secondary SCs from three donors, reverse transcribed and mRNAs encoding *MT1G*.

[0082] FIG. 4E is a graph showing levels of RNA with mRNAs encoding *SLC7A11*.

[0083] FIG. 4F is a graph showing levels of RNA with mRNAs encoding *FTRC*.

[0084] FIG. 4G is a graph showing levels of RNA with mRNAs encoding *TGFBR1*.

[0085] FIG. 4H shows flow cytometry-based analysis of iron content in primary senescent cells and a non-senescent control.

[0086] FIG. 4I shows flow cytometry-based analysis of iron content in secondary senescent cells and its non-senescent control.

[0087] FIG. 4J shows quantification of SiRhoNox-1 + cells in primary and secondary SCs from three donors. Comparison was made with one-way ANOVA. Error bars = mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

[0088] FIG. 5A is a heat map of ferroptosis related genes based on RNASeq analysis.

[0089] FIG. 5B shows mRNA expression of ferroptosis related genes. RNA was isolated from NS, NSCM treated cells, DPP4+ primary and secondary SCs from three donors, reverse transcribed, and mRNAs encoding NFE2L2 were measured by RT-qPCR.

[0090] FIG. 5C shows mRNA expression of ferroptosis related genes using mRNAs encoding CHAC1 and PTGS2 were measured by RT-qPCR.

[0091] FIG. 5D shows mRNA expression of ferroptosis related genes using mRNAs encoding ACSL4 were measured by RT-qPCR.

[0092] FIG. 5E shows that erastin has senolytic activity in DPP4+ primary and secondary SCs. Quantification of viable endothelial non-senescent cells, cells treated with NSCM, DPP4+ primary SCs (Doxorubicin treated) and DPP4+ secondary SCs (treated with CM from Doxorubicin treated cells) 72 hours after treatment with increasing concentrations of erastin (3 technical and 3 biological (3 different donors) replicates).

[0093] FIG. 5F shows quantification of viable cells at the indicated times point after treatment of the cells with 10 μ M erastin (n = 3 donors).

[0094] FIG. 5G shows quantification of viable endothelial non-senescent cells, cells treated with NSCM, IR-induced DPP4+ primary senescent and secondary SCs 72 hours after treatment with 10 μ M erastin (n = 3 donors).

[0095] FIG. 5H shows quantification of viable IMR-90 non-senescent fibroblasts, cells treated with NSCM, Doxo-induced DPP4+ primary and secondary senescent

fibroblasts 72 hours after treatment with 10 μ M erastin (n = 3 donors).

[0096] FIG. 5I shows quantification of viable IMR-90 non-senescent fibroblasts, cells treated with NSCM, IR-induced DPP4+ primary and secondary senescent fibroblasts 72 hours after treatment with 10 μ M erastin (n = 3 donors).

[0097] FIG. 6A shows images of DPP4+ primary SCs viability over time (time 0, 24 hours, 36 hours, 48 hours and 72 hours) treated with erastin (10 μ M) and Pan caspase inhibitor VAD, Iron chelator deferoxamine (DFO, 100 μ M), or ferroptosis inhibitor Fer-1.

[0098] FIG. 6B shows quantification of viable DPP4+ primary senescent ECs (Doxorubicin treated) 72 hours after treatment with erastin alone or erastin + VAD (n=3 different donors).

[0099] FIG. 6C shows quantification of viable DPP4+ primary senescent ECs (Doxorubicin treated) 72 hours after treatment with erastin alone or erastin + DFO (n=3 different donors).

[00100] FIG. 6D shows quantification of viable DPP4+ primary senescent ECs (Doxorubicin treated) 72 hours after treatment with erastin alone or erastin + Fer-1 (n=3 different donors).

[00101] FIG. 6E shows RT-qPCR based mRNA expression level of *PTGS2* gene in S and NS cells 24 hours after erastin treatment.

[00102] FIG. 6F shows RT-qPCR based mRNA expression level of *CHAC1* gene in S and NS cells 24 hours after erastin treatment.

[00103] FIG. 6G shows MDA level in non-senescent and DPP4+ senescent cells. Cells were treated with erastin for 24 hours and MDA was measured from total cell lysate.

[00104] FIG. 6H shows MDA level in the CM of non-senescent and DPP4+ senescent cells treated with erastin. Cells were treated with erastin for 48 hours and MDA was measured from the CM. Comparison was made with one-way ANOVA.

[00105] FIG. 7A is a flow chart of primary and secondary senescence induction. Non senescent cells were treated with Doxorubicin for 24 hours. CM was collected from primary senescent cells 7 days after doxorubicin treatment. Proliferating cells were treated with CM collected from primary senescent or non-senescent cells. Multiple senescence markers were determined 7 days after CM treatment.

[00106] FIG. 7B shows the percent of SA- β -gal positive cells 7 days after doxorubicin or CM treatment. SA- β -gal quantification from multiple images of three donors.

[00107] FIG. 7C shows the quantification of γ H2AX foci, cells with two or more foci per nucleus were defined as senescent cells

[00108] FIG. 7D shows that HMGB1 relocalizes in senescent cells. The percentage of cells expressing HMGB1 in the nucleus (green) and nucleus + cytosol (red) was scored.

[00109] FIG. 7E shows a cell proliferation assay. The percentage of Click-it EdU Alexa Fluor 488 positive cells were determined in senescent and non-senescent cells

[00110] FIG. 7F shows mRNA expression of p16 Ink4a and p21 Cip1 as determined by RT-qPCR in untreated and doxorubicin-treated ECs.

[00111] FIG. 7G shows mRNA expression of the classical SASP factors *IL-6* and *IL-8* as determined by RT-qPCR.

[00112] FIG. 7H shows mRNA expression of p16 Ink4a, p21 Cip1. *il-6* and *il-8* was determined by RT-qPCR in secondary senescent ECs. Values were presented as mean $\pm$ SEM. Comparison was made with one-way ANOVA

[00113] FIG. 8A is an image of DPP4 immunofluorescence staining of senescent fibroblasts (IMR90).

[00114] FIG. 8B is an image of DPP4 immunofluorescence staining of non-senescent fibroblasts (IMR90).

- [00115]** FIG. 8C shows flow cytometry analysis of DPP4 expression in IMR90
- [00116]** FIG. 8D is an image of DPP4 immunofluorescence staining in mesenchymal stem cells (non-senescent).
- [00117]** FIG. 8E is an image of DPP4 immunofluorescence staining in mesenchymal stem cells (senescent).
- [00118]** FIG. 8F shows flow cytometry analysis of DPP4 expression in mesenchymal stem cells.
- [00119]** FIG. 8G shows DPP4 immunofluorescence staining of primary senescent ECs.
- [00120]** FIG. 8H shows DPP4 immunofluorescence staining of primary non-senescent ECs.
- [00121]** FIG. 8I shows flow cytometry analysis of DPP4 expression in primary senescent and non-senescent ECs.
- [00122]** FIG. 8J is an image of DPP4 immunofluorescence staining of non senescent ECs.
- [00123]** FIG. 8K is an image of DPP4 immunofluorescence staining of secondary senescent ECs.
- [00124]** FIG. 8L shows flow cytometry analysis of DPP4 expression in secondary senescent and non-senescent EC.
- [00125]** FIG. 8M shows the percent of DPP4+ ECs from flow analysis in three donors. Values were presented as mean $\pm$ SEM.
- [00126]** FIG. 9A is an image of SA β Gal staining in DPP4+ isolated non-senescent (NS) cells.
- [00127]** FIG. 9B is an image of SA β Gal staining in DPP4+ isolated non-senescent

cells treated with conditioned media (NSCM).

[00128] FIG. 9C is an image of SA β Gal staining in isolated DPP4+ primary senescent cells (S^{DPP4+}).

[00129] FIG. 9D is an image of SA β Gal staining in isolated DPP4+ secondary senescent cells (SS^{DPP4+}).

[00130] FIG. 9E is an image of γ H2AX and HMGB1 staining in DPP4+ isolated non-senescent (NS) cells. Both primary and secondary senescent cells were isolated based on DPP4 expression and replated. 24 hours after replating cells were stained for SA β Gal, or γ H2AX and HMGB1

[00131] FIG. 9F is an image of γ H2AX and HMGB1 staining in DPP4+ isolated non-senescent cells treated with conditioned media (NSCM).

[00132] FIG. 9G is an image of γ H2AX and HMGB1 staining in isolated DPP4+ primary senescent cells (S^{DPP4+}).

[00133] FIG. 9H is an image of γ H2AX and HMGB1 staining in isolated DPP4+ secondary senescent cells (SS^{DPP4+}).

[00134] FIG. 9I shows the percent of SA β Gal positive cells in DPP4+ isolated primary senescent cells (irradiated) and DPP4+ isolated secondary senescent cells treated with CM from irradiated ECs.

[00135] FIG. 9J shows the quantification of γ H2AX foci.

[00136] FIG. 9K shows the percent of SA β Gal positive cells in DPP4+ isolated primary senescent cells (irradiated) and DPP4+ isolated secondary senescent cells treated with CM from irradiated ECs.

[00137] FIG. 9L shows the quantification of γ H2AX foci.

[00138] FIG. 9M shows the percent of SA β Gal positive cells in DPP4- flowthrough secondary senescent ECs.

[00139] FIG. 9N shows the percent of cells with two or more γ H2AX foci in DPP4-flowthrough secondary senescent ECs.

[00140] FIG. 10A is a Venn diagram of DEG's of DPP4+ isolated primary and secondary senescent cells.

The same clustering was observed for the top 50 DEG's (see Figure FIG. 3B). Heat maps indicate the averages of 6 experiments (3 technical and 3 biological (different donors)).

[00141] FIG. 10B is a heat map of the top 50 DEG's for DPP4+ primary and secondary SCs. DPP4+ secondary SCs from three different donors were well separated from primary SCs and clustered together. Heat maps indicate the averages of 6 experiments (3 technical and 3 biological (different donors)).

[00142] FIG. 10C is a two-dimensional (2D) PCA chart of DPP4+ primary and secondary SCs and non-senescent controls from one donor. Secondary senescent samples were well separated from primary senescent and non-senescent samples

[00143] FIG. 10D is a 2D PCA chart of DPP4+ primary and secondary SCs and non-senescent controls from another donor. Secondary senescent samples were well separated from primary senescent and non-senescent samples

[00144] FIG. 10E is a graph showing GO terms enriched by the DEG's of DPP4+ primary SCs categorized by biological process, cellular component and molecular function categories.

[00145] FIG. 10F is a graph showing GO terms enriched by the DEG's of DPP4+ secondary SCs categorized by biological process, cellular component and molecular function categories

[00146] FIG. 11A is an image of side and forward scattering of DPP4+ primary and secondary SCs have high iron content.

[00147] FIG. 11B is an image of single cell gating strategy scattering of DPP4+

primary and secondary SCs have high iron content.

[00148] FIG. 11C shows flow cytometry-based analysis of iron content in primary senescent cells and its non-senescent control from one donor.

[00149] FIG. 11D shows flow cytometry-based analysis of iron content in secondary senescent cells and its non-senescent control from one donor.

[00150] FIG. 11E shows flow cytometry-based analysis of iron content in primary senescent cells and its non-senescent control from one donor.

[00151] FIG. 11F shows flow cytometry-based analysis of iron content in secondary senescent cells and its non-senescent control from one donor.

[00152] FIG. 12 shows MDA level in senescent and non-senescent epithelial cells (ECs).

[00153] FIG. 13 is chart that shows senolytic drugs and targets identified based on RNASeq analysis.

[00154] FIG. 14A shows the results of a cytotoxicity assay using homoharringtonine in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00155] FIG. 14B shows the results of a cytotoxicity assay using pelitinib in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00156] FIG. 14C shows the results of a cytotoxicity assay using vinorelbine in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00157] FIG. 14D shows the results of a cytotoxicity assay using vincristine in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00158] FIG. 15A shows the results of a cytotoxicity assay using YK-4-299 in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00159] FIG. 15B shows the results of a cytotoxicity assay using oxibendazole in NS,

NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00160] FIG. 15C shows the results of a cytotoxicity assay using mebendazole in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00161] FIG. 15D shows the results of a cytotoxicity assay using radicicol in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00162] FIG. 15E shows the results of a cytotoxicity assay using NVP-AUY922 in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00163] FIG. 15F shows the results of a cytotoxicity assay using docataxel in NS, NSCM, S^{DDP4+} and SS^{DDP4+} cells.

[00164] FIG. 16A shows senolytics optimization of Vorinostat using Xcellegence machine.

[00165] FIG. 16B shows senolytics optimization of Emetine using Xcellegence machine.

[00166] FIG. 17A shows a pathway analysis for DEGs of PS^{DPP4+}.

[00167] FIG. 17B shows protein-protein networks of interaction for genes responsible for the top three pathways in FIG. 5A.

[00168] FIG. 17C shows a heatmap of ferroptosis-related genes.

[00169] FIG. 17D is a graph showing confirmation of mRNA expression of ferroptosis-related genes with mRNAs encoding for NFE2L2.

[00170] FIG. 17E is a graph showing confirmation of mRNA expression of ferroptosis-related genes with mRNAs encoding for CHAC1 and PTGS2.

[00171] FIG. 17F is a graph showing confirmation of mRNA expression of ferroptosis-related genes with mRNAs encoding for ACSL4.

[00172] FIG. 18A shows the cytotoxicity of FIN56 in S^{DPP4+} and PS^{DPP4+} ECs 72 hours after treatment (N=9, in three donors).

[00173] FIG. 18B shows the cytotoxicity of FIN56 in S^{DPP4+} and PS^{DPP4+} IMR90 fibroblasts 72 hours after treatment (n = 3).

[00174] FIG. 18C shows mRNA expression of p16 and ferroptosis-related genes (GPX4, FDFT1, and PTGS2) before and after FIN56 treatment.

[00175] FIG. 18D shows quantification of viable DPP4+ primary senescent ECs (Doxorubicin treated) 72 hours after treatment with FIN56 alone or FIN56 + z-vad-fmk.

[00176] FIG. 18E shows quantification of viable DPP4+ primary senescent ECs (Doxorubicin treated) 72 hours after treatment with FIN56 alone or FIN56 + DFP.

[00177] FIG. 18F shows quantification of viable DPP4+ primary senescent ECs (Doxorubicin treated) 72 hours after treatment with FIN56 alone or FIN56 + Fer-1.

[00178] FIG. 18G shows confirmation of GPX4 knockdown by siGPX4 at the mRNA level.

[00179] FIG. 18H shows confirmation of GPX4 knockdown by siGPX4 at the protein level.

[00180] FIG. 18I shows the cytotoxic effect of siGPX4 in senescent IMR90 cells as determined by crystal violet assay. Comparison was made with one-way ANOVA. Error bars represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

[00181] FIG. 19A shows the cytotoxicity of TRX-CBI in S^{DPP4+} and PS^{DPP4+} ECs. For FIG. 19A – 19C, viability was determined by xCELLigence real-time cell analysis (RTCA0 (n = 9 in three donors). Comparison was made with one-way ANOVA. Error bars represent mean ± SEM. **p < 0.01, ***p < 0.001.

[00182] FIG. 19B shows the cytotoxicity of 20 nM of TRX-CBI across time. Cells were treated with 20 nM of TRX-CBI and viability was determined every 15 minutes for four days.

[00183] FIG. 19C shows the cytotoxicity of TRX-CBI in doxorubicin-treated senescent ECs without DPP4-based isolation.

[00184] FIG. 19D shows the cytotoxicity of TRX-CBI in etoposide-treated S^{DPP4+} and PS^{DPP4+} ECs.

[00185] FIG. 19E shows the cytotoxicity of TRX-CBI in S^{DPP4+} and PS^{DPP4-} ECs.

[00186] FIG. 19F shows quantification of viable S^{DPP4+} ECs (doxorubicin treated) 72 hours after treatment with TRX-CBI alone or TRX-CBI + z-vad-fmk.

[00187] FIG. 19G shows a proposed mechanism of action of TRX-CBI cytotoxicity.

Definitions

[00188] Reference in this specification to "one embodiment/aspect" or "an embodiment/aspect" means that a particular feature, structure, or characteristic described in connection with the embodiment/aspect is included in at least one embodiment/aspect of the disclosure. The use of the phrase "in one embodiment/aspect" or "in another embodiment/aspect" in various places in the specification are not necessarily all referring to the same embodiment/aspect, nor are separate or alternative embodiments/aspects mutually exclusive of other embodiments/aspects. Moreover, various features are described which may be exhibited by some embodiments/aspects and not by others. Similarly, various requirements are described which may be requirements for some embodiments/aspects but not other embodiments/aspects. Embodiment and aspect can be in certain instances be used interchangeably.

[00189] The terms used in this specification generally have their ordinary meanings in the art, within the context of the disclosure, and in the specific context where each term is used. Certain terms that are used to describe the disclosure are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner regarding the description of the disclosure. It will be appreciated that the same thing can be said in more than one way.

[00190] Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein. Nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification including

examples of any terms discussed herein is illustrative only and is not intended to further limit the scope and meaning of the disclosure or of any exemplified term. Likewise, the disclosure is not limited to various embodiments given in this specification.

[00191] Without intent to further limit the scope of the disclosure, examples of instruments, apparatus, methods and their related results according to the embodiments of the present disclosure are given below. Note that titles or subtitles may be used in the examples for convenience of a reader, which in no way should limit the scope of the disclosure. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. In the case of conflict, the present document, including definitions, will control.

[00192] The term “aging” refers to progressive physiological changes in an organism that lead to senescence, or a decline of biological functions and of the organism’s ability to adapt to metabolic stress. Aging takes place in a cell, an organ, and/or the total organism with the passage of time. In humans, aging is typically manifested in a variety of physical changes including skin wrinkling, decreased physical strength, reduced muscle mass, loss off/greying hair, atherosclerosis, reduced fertility/libido, shorter reaction times, reduced hearing/vision sensitivity and frailty. The term “slowing aging” or “ameliorating aging” refers to slowing the aging progress, arresting further signs of aging and/or reducing the severity of one or more physical changes associated with aging. Cellular aging is the result of a progressive decline in the proliferative capacity and life span of cells and the effects of continuous exposure to exogenous influences that result in the progressive accumulation of cellular and molecular damage.

[00193] The term “senescence” refers to gradual deterioration of functional characteristics in living organisms. Cellular senescence is often defined as a stress-induced, durable cell cycle arrest of previously replication-competent cells. The effects of senescent cells can be thought of as beneficial or detrimental with regard to host physiology and disease, although in some contexts, senescent cells affect a disease state in a complex manner both promoting and opposing certain conditions.

[00194] The term “senomorph” refers to one of a range of agents that can modulate the phenotypes of senescent cells (SCs) to those of young cells through interfering with senoinflammation/inflammaging, senescence-related signal pathways and SASP, without induction of SC apoptosis.

[00195] The term “senescence-associated disease or disorder” refers to an ailment that is associated with age and can include, for example, atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer’s disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis. Other ailments (including age-related conditions) associated with age or senescence include hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.

[00196] Still other ailments associated with age or senescence include cardiovascular disease (e.g., atherosclerosis, angina, arrhythmia, cardiomyopathy, congestive heart failure, coronary artery disease, carotid artery disease, endocarditis, coronary thrombosis, myocardial infarction, hypertension, aortic aneurysm, cardiac diastolic dysfunction, hypercholesterolemia, hyperlipidemia, mitral valve prolapsed, peripheral vascular disease, cardiac stress resistance, cardiac fibrosis, brain aneurysm, and stroke). A senescence-associated disease or disorder can also be an inflammatory or autoimmune disease or disorder (e.g., osteoarthritis, osteoporosis, oral mucositis, inflammatory bowel disease or kyphosis). A senescence-associated disease or disorder can also be a neurodegenerative disease (e.g., Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, dementia, mild cognitive impairment or motor neuron dysfunction). A senescence-associated disease or disorder can also be a metabolic disease (e.g., diabetes, diabetic ulcer, metabolic syndrome or obesity). A senescence-associated disease or disorder can also be a pulmonary disease (e.g., pulmonary fibrosis, chronic obstructive pulmonary disease, asthma, cystic fibrosis, emphysema, bronchiectasis or age-related loss of pulmonary function). A senescence-associated disease or disorder can also be an eye disease or disorder (e.g., macular degeneration, glaucoma, cataracts, presbyopia or vision loss). A senescence-associated disease or disorder can also be a dermatological disease or disorder (e.g., eczema, psoriasis, hyperpigmentation, nevi, rashes, atopic dermatitis, urticaria,

diseases or disorders related to photosensitivity or photoaging). A senescence-associated disease or disorder can also be renal disease, renal failure, frailty, hearing loss, muscle fatigue, skin conditions, skin wound healing, liver fibrosis, pancreatic fibrosis, oral submucosa fibrosis or sarcopenia.

[00197] The term “secondary senescence” refers to cells that become senescent as a result of exposure to senescence-associated secretory phenotype (SASP) factors produced by primary senescent cells (S). Unlike primary senescent cells that undergo permanent cell cycle arrest upon exposure to the insults (e.g., exposure to DNA damaging agents, telomere loss, mitochondrial dysfunction, or oncogene activation etc.), some cells undergo a senescent state upon prolonged exposure to the SASP factors produced by S. These cells are referred to as secondary SCs (SS). The phenomenon of secondary senescence has not only been demonstrated in cell culture, but also *in vivo* is known to drive the accumulation of SCs and subsequent functional defects with aged mice.

[00198] The term “senescence-associated secretory phenotype factors” or “SASP factors” refers to factors produced by senescent cells. SASP factors can be globally divided into the following categories: soluble signaling factors (interleukins, chemokines, and growth factors), secreted proteases and secreted insoluble proteins/extracellular matrix (ECM) components.

[00199] The term “ferroptosis” refers to a type of programmed cell death dependent on iron and characterized by the accumulation of lipid peroxides. It is genetically and biochemically distinct from other forms of regulated cell death such as apoptosis. Small molecules such as erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, and ML210 are inhibitors of tumor cell growth via induction of ferroptosis. These compounds do not trigger apoptosis and therefore do not cause chromatin margination or poly (ADP-ribose) polymerase (PARP) cleavage. Instead, ferroptosis causes changes in mitochondrial phenotype. Iron is also necessary for small molecule ferroptosis induction; Therefore, these compounds can be inhibited by iron chelators.

[00200] The term “ferroptosis inducer” or “FIN” refers to a substance such as a small

molecule that activates ferroptosis. FINs include, for example, erastin, FIN 56, L-glutamic acid, L-buthionine sulfoximine, ML 210, RSL3, simvastatin, sorafenib and sulfasalazine. Mechanistically, ferroptosis inducers can be divided into two classes: (1) inhibitors of cystine import via system x_c^- (e.g., erastin, SAS and SRF), which subsequently causes depletion of glutathione (GSH), and (2) covalent inhibitors of glutathione peroxidase 4 (GPX4) (e.g., RSL3/5, FINs, FINO2, DPIs, Altretamine and Withaferin A). Because GPX4 reduces lipid hydroperoxides using GSH as a co-substrate, both compound classes ultimately result in loss of GPX4 activity, followed by elevated levels of lipid reactive oxygen species (ROS) and consequent cell death. Other classes of ferroptosis inducers include GLU, iron carrier, BSO, DPI2, Cisplatin, Artemisinin and Nanoparticle inducers.

[00201] The term “erastin” refers to a small molecule capable of initiating ferroptotic cell death. Erastin acts through inhibition of the cystine/glutamate transporter, thus causing decreased intracellular glutathione (GSH) levels. Specifically, erastin binds and activates voltage-dependent anion channels (VDAC) by reversing tubulin's inhibition on VDAC2 and VDAC3, and functionally inhibits the cystine-glutamate antiporter system X_c^- . Cells treated with erastin are deprived of cysteine and are unable to synthesize the antioxidant glutathione. Depletion of glutathione eventually leads to excessive lipid peroxidation and cell death.

[00202] The term “senescence-associated β -galactosidase,” “SA- β -gal” or “SABG” is a hypothetical hydrolase enzyme that catalyzes the hydrolysis of β -galactosides into monosaccharides only in senescent cells. Senescence-associated beta-galactosidase, along with p16Ink4A, is regarded to be a biomarker of cellular senescence.

[00203] The term “senolytic” or “senolytic agent” refers to a therapeutic such as a small molecule that can selectively or preferentially induce death of senescent cells. A senolytic agent may kill senescent cells by inducing (i.e., activating, stimulating or removing inhibition of) an apoptotic pathway that leads to cell death. Senolytic agents may be useful for treatment of a plethora of ailments including diabetes (particularly type 2 diabetes), metabolic syndrome, or obesity; pulmonary diseases, such as chronic

obstructive pulmonary disease or idiopathic pulmonary fibrosis; and inflammatory disorders, such as osteoarthritis. Senolytics are also sought-after for cancer therapy, as chemotherapeutics (e.g., DNA damaging agents) can cause cancer cells to become senescent.

[00204] The term “macrophage” refers to a type of white blood cell that surrounds and kills microorganisms, removes dead cells, and stimulates the action of other immune system cells. Macrophages have important functions in development, tissue homeostasis and immunity. They take various forms (with various names) throughout the body (e.g., histiocytes, Kupffer cells, alveolar macrophages, microglia, etc.). Macrophages also use mechanisms to control erythropoiesis and the handling of iron. For example, red pulp macrophages in the spleen, Kupffer cells in the liver, and central nurse macrophages in the bone marrow ensure a coordinated metabolism of iron to support erythropoiesis. Phagocytosis of senescent red blood cells by macrophages in the spleen and the liver provide a continuous delivery of recycled iron under steady-state conditions and during anemic stress.

[00205] The term “Dipeptidyl peptidase-4” or “DPP4,” also known as cell surface antigen CD26, is an omnipresent transmembrane protease that cleaves NH₂-terminal dipeptides from their abundant substrates. Mass spectrometry analysis has revealed that DPP4 is selectively expressed on the surface of senescent, but not proliferating, human diploid fibroblasts. Further, antibody-dependent cell-mediated cytotoxicity (ADCC) assays has indicated that the cell surface DPP4 preferentially sensitized senescent, but not dividing, fibroblasts to cytotoxicity by natural killer cells.

[00206] The term “high mobility group box 1” or “HMGB1” refers to a nonhistone chromatin-associated protein that has been widely reported to play a pivotal role in the pathogenesis of hematopoietic malignancies. HMGB1 normally exists inside cells but can be secreted into the extracellular environment through passive or active release. Extracellular HMGB1 binds with several different receptors and interactors to mediate the proliferation, differentiation, mobilization, and senescence of hematopoietic stem cells (HSCs).

[00207] The term "biomarker" refers generally to a DNA, RNA, protein, carbohydrate, or glycolipid-based molecular marker, the expression or presence of which in a subject's sample can be detected by standard methods (or methods disclosed herein) and is predictive or prognostic of the effective responsiveness or sensitivity of a mammalian subject with an ailment. Biomarkers may be present in a test sample but absent in a control sample, absent in a test sample but present in a control sample, or the amount or of biomarker can differ between a test sample and a control sample. For example, a protein biomarkers can be present in such a sample, but not in a control sample, or certain biomarkers are seropositive in the sample, but seronegative in a control sample. Also, optionally, expression of such a biomarker may be determined to be higher than that observed for a control sample. The terms "marker" and "biomarker" are used herein interchangeably. Biomarkers for ferroptosis include, for example, DDP4, TFR1, VDAC2/3, Ras, P53 and NOX. Anti-ferroptosis biomarkers include, for example, SLC7A11, HSPB1, NRF2, GSH and GPX4.

[00208] The amount of the biomarker can be measured in a test sample and compared to the "normal control level," utilizing techniques such as reference limits, discrimination limits, or risk defining thresholds to define cutoff points and abnormal values for an ailment. The normal control level means the level of one or more biomarkers or combined biomarker indices typically found in a subject not suffering from the ailment. Such normal control level and cutoff points can vary based on whether a biomarker is used alone or in a formula combining with other biomarkers into an index. Alternatively, the normal control level can be a database of biomarker patterns from previously tested subjects who did not experience the ailment over a clinically relevant time.

[00209] The term "non-coding RNA" or "ncRNA" refers to an RNA molecule that is not translated into a protein. The DNA sequence from which a functional non-coding RNA is transcribed is often called an RNA gene. Abundant and functionally important types of non-coding RNAs include transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), as well as small RNAs such as microRNAs, siRNAs, piRNAs, snoRNAs, snRNAs, exRNAs, scaRNAs, circular RNA and the long ncRNAs such as Xist and

HOTAIR. The number of non-coding RNAs within the human genome is unknown; however, recent transcriptomic and bioinformatic studies suggest that there are thousands of them.

[00210] The term “vorinostat,” “suberoylanilide hydroxamic acid” or “SAHA” refers to a member of a larger class of compounds that inhibit histone deacetylases (HDAC). Histone deacetylase inhibitors (HDI) have a broad spectrum of epigenetic activities.

[00211] The term “emetine” refers to a drug used as both an anti-protozoal and to induce vomiting. It is produced from the ipecac root. It takes its name from its emetic properties.

[00212] The term “natural killer cell” or “NK cell” refers to a type of cytotoxic lymphocyte critical to the innate immune system that belong to the rapidly expanding family of innate lymphoid cells (ILC) and represent 5 – 20% of all circulating lymphocytes in humans. The role of NK cells is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virus-infected cell and other intracellular pathogens acting at around three days after infection and respond to tumor formation. Typically, immune cells detect the major histocompatibility complex (MHC) presented on infected cell surfaces, triggering cytokine release, causing the death of the infected cell by lysis or apoptosis. NK cells are unique, however, as they have the ability to recognize and kill stressed cells in the absence of antibodies and MHC, allowing for a much faster immune reaction. They are referred to as “natural killers” because they do not require activation to kill cells that are missing “self” markers of MHC class 1. This role is especially important because harmful cells that are missing MHC I markers cannot be detected and destroyed by other immune cells such as T lymphocyte cells.

[00213] As used herein, “detecting” or “determining” with respect to a *biomarker* value includes the use of both the instrument required to observe and record a signal corresponding to a *biomarker* value and the material/s required to generate that signal. In various embodiments, the *biomarker* value is detected using any suitable method, including fluorescence, chemiluminescence, surface plasmon resonance, surface

acoustic waves, mass spectrometry, infrared spectroscopy, Raman spectroscopy, atomic force microscopy, scanning tunneling microscopy, electrochemical detection methods, nuclear magnetic resonance, quantum dots, and the like.

[00214] The term “treating” or “treatment” refers to one or more of (1) inhibiting the disease; e.g., inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology); and (2) ameliorating the disease; e.g., ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology) such as decreasing the severity of disease.

[00215] The term “administration” refers to the introduction of an amount of a predetermined substance into a patient by a certain suitable method. The composition disclosed herein may be administered via any of the common routes, as long as it is able to reach a desired tissue, for example, but is not limited to, inhaling, intraperitoneal, intravenous, intramuscular, subcutaneous, intradermal, oral, topical, intranasal, intrapulmonary, or intrarectal administration. However, since peptides are digested upon oral administration, active ingredients of a composition for oral administration should be coated or formulated for protection against degradation in the stomach.

[00216] The term “subject” refers to those who are susceptible to an ailment (e.g., a disease related to senescence) or who are suspected of having or diagnosed with the ailment. However, any subject to be treated with the therapeutic methods described herein is included without limitation.

[00217] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are to be understood as approximations in accordance with common practice in the art. When used herein, the term “about” may connote variation (+) or (-) 1%, 5% or 10% of the stated amount, as appropriate given the context. It is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are

known in the art.

[00218] Many known and useful compounds and the like can be found in Remington's Pharmaceutical Sciences (13th Ed), Mack Publishing Company, Easton, PA—a standard reference for various types of administration. As used herein, the term “formulation(s)” refers to a combination of at least one active ingredient with one or more other ingredient, also commonly referred to as excipients, which may be independently active or inactive. The term “formulation” may or may not refer to a pharmaceutically acceptable composition for administration to humans or animals and may include compositions that are useful intermediates for storage or research purposes.

[00219] Other technical terms used herein have their ordinary meaning in the art that they are used, as exemplified by a variety of technical dictionaries. The particular values and configurations discussed in these non-limiting examples can be varied and are cited merely to illustrate at least one embodiment and are not intended to limit the scope thereof.

DETAILED DESCRIPTION

[00220] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the subject technology as claimed. Additional features and advantages of the subject technology are set forth in the description below, and in part will be apparent from the description, or may be learned by practice of the subject technology. The advantages of the subject technology will be realized and attained by the structure particularly pointed out in the written description and claims hereof.

[00221] Aging is a risk factor for many chronic diseases, disabilities and declining health. The presence of senescent cells in an individual is thought to contribute to aging and aging-related dysfunction. Senescent cells accumulate in tissues and organs of individuals as they age and are found at sites of age-related pathologies. Cells may also become senescent after exposure to an environmental, chemical, or biological insult or as a result of a disease. Cellular senescence can be characterized by (a)

irreversible arrest of proliferation, (b) a pro-inflammatory secretory phenotype and (c) evasion of programmed cell death mechanisms.

[00222] Senescent cells (SCs) can spread the senescent phenotype to other cells by secreting factors referred to as the Senescence Associated Secretory Phenotype (SASP) factors. The cells that become senescent this way are referred to as secondary senescent cells (secondary SCs or SSCs). SSCs have a significant contribution for the accumulation of SCs during the aging process. Efforts made to characterize secondary SCs have been unreliable because conventional studies have relied on analyzing mixed populations of senescent and non-senescent cells without regard to secondary senescence. Recent studies described herein provide evidence that senescence alters cellular iron acquisition and storage and also impedes iron-mediated cell death pathways. Senescent cells accumulate intracellular iron with changes in the levels of iron homeostasis proteins.

[00223] Applicants discovered phenotypic differences between primary senescent cells (SCs) and secondary senescent cells (SSCs) by isolating and enriching SSCs using surface expression of DPP4 (Dipeptidyl Peptidase-4). Using this marker, patient derived primary endothelial cells (ECs) and SSCs were enriched. Applicants observed that DPP4⁺ secondary senescent cells (SS^{DPP4+}) engaged distinct pro-survival pathways compared to DPP4⁺ primary senescent cells (S^{DPP4+}) and were relatively resistant to killing by conventional senolytic drugs. An RNASeq analysis confirmed a distinct molecular signature of SS^{DPP4+}. Interestingly, several genes were identified that were involved in iron homeostasis and ferroptosis in the differentially expressed genes of both SS^{DPP4+} and S^{DPP4+}. These results confirmed the presence of increased accumulation of labile iron in both SS^{DPP4+} and S^{DPP4+}. Further, Applicants demonstrated treatment with Erastin, a pharmacological grade ferroptosis inducer, resulted in significant senolysis of both S^{DPP4+} and SS^{DPP4+} with negligible cytotoxicity to non-senescent (NS) cells. Based on these results, Applicants proposed ferroptosis for use as a senolytic target.

[00224] Accordingly, embodiments include methods of treating senescence-

associated diseases or disorders. Embodiments also include methods of slowing the aging process and/or reducing signs of aging. The methods can include inducing ferroptosis in senescent cells of a subject. Ferroptosis can be induced by, for example, administering a ferroptosis inducer such as erastin, FIN 56, L-glutamic acid, L-buthionine sulfoximine, ML 210, RSL3, simvastatin, sorafenib and/or sulfasalazine.

[00225] In other embodiments, ferroptosis is induced by modulating proteins in senescent cells. The proteins involved in iron homeostasis include iron regulatory proteins (IRPs), IRP1 and IRP2. Other proteins include transferrin receptor 1 (TfR1) [main iron (Fe³⁺) importer], divalent metal transporter 1 (DMT1) (cytosolic iron importer), ferroportin (iron exporter) and ferritin (intracellular iron storage).

[00226] Additional embodiments include methods of identifying and removing cells with high iron content such as senescent cells, cancer cells and aged macrophages. For example, surface proteins expressed on senescent cells can be used to identify and target/eliminate them. The methods described herein can also be used to identify therapeutic targets and pathways for treatment of senescence-associated diseases or disorders.

EXAMPLES

[00227] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of representative embodiments now contemplated. These examples are intended to be a mere subset of all possible contexts in which the components of the formulation may be combined. Thus, these examples should not be construed to limit any of the embodiments described in the present specification, including those pertaining to the type and amounts of components of the formulation and/or methods and uses thereof.

Methods

Cells

[00228] All endothelial cells were purchased from Coriell Institute for medical research and IMR-90 was purchased from the American Type Culture Collection (ATCC).

Human primary endothelial cells were the primary cell type used in this study. IMR-90 and MSC cells were cultured in Dulbecco's modified eagle medium (DMEM 4.5 g/L glucose, without sodium pyruvate - Gibco) supplemented with 10% FBS and penicillin/streptomycin (Gibco). Quiescence was induced by replacing culture media with media containing 0.2% FBS for 24 hours before analysis. All cells were cultured at 37°C and 5% O₂. All cells were mycoplasma free.

Endothelial Cell Maintenance

[00229] Primary human endothelial cells from three apparently healthy individuals (AG09872, AG10774, AG10770) were cultured using the promo cell basal medium MV2 (C-22221) supplemented with Growth Medium MV 2 Supplement Pack (C-39221). CD31 was used as an endothelial cell marker.

Primary Senescence Induction

[00230] Senescence was induced by treating cells with 250µM doxorubicin (DOXO) for 24 hours. After 24 hours, doxorubicin was removed and cells were maintained in complete medium for nine days. At day nine, the complete medium was changed to low serum medium and after 24 hours, multiple senescence markers were determined both on the cells and conditioned medium (CM) collected.

CM preparation and secondary senescence induction

[00231] Primary senescence was induced as described above and CM was generated by culturing cells in appropriate low serum media for 24 hours before harvest. CM collected was spun down to remove cell debris and the supernatant was stored at -80°C. Cells were then trypsinized with TrypLE™ Select Enzyme and counted. All quantitative assays from CM (e.g., ELISA) were normalized to cell number. 24 hours after plating, proliferating cells were treated with 50% CM collected from primary senescent cells and 50% complete medium for seven days. Media was changed at least once and at day 6 medium was replaced with low serum medium. At day seven, CM and cells were collected and multiple senescence markers were determined.

Flow cytometry

[00232] To determine the expression of DPP4, primary and secondary senescence has been induced as above. Cells were resuspended to a concentration of 1×10^6 cells/mL and aliquoted. Cells were fixed with ice cold 100% methanol at -20°C for 20 minutes. Cells were wash two times with 1X PBS and resuspended in FACS buffer (1% BSA in PBS + 0.01% sodium azide). The cells were then incubated with DPP4-PE conjugated antibody (PE Mouse anti-Human CD26 (BD Bioscience Cat 555437 lot 9192757, Clone M-A261 (RUO))) for 1hr at room temperature in the dark. After 1hr incubation, cells were washed three times with FACS buffer and resuspend in 300 μL FACS buffer. Finally, cells pass through a mesh filter of 40 μm pore size to exclude any clumps of cells and expression of DPP4 were determined by flow cytometer (DB Accuri C6). DPP4 immunofluorescence were stained as below (immunofluorescence section) without permeabilization using DPP4/CD26 (D6D8K) Rabbit mAb #67138 (Cell Signaling technology). Data analysis was done using Flowlogic software.

Isolation of senescent cells using DPP4 as a surface marker

[00233] Primary and secondary senescent cells stained with DPP4-PE conjugated antibody on ice for 30 minutes. Cells were washed with a MACS buffer (PBS, 0.5% BSA, and 2 mM EDTA) two times to remove unbound antibodies. Cells were resuspended with 80 μL MACS buffer and incubate with 20 μL of Anti-PE MicroBeads UltraPure (milenyi biotec 130-048-801, lot 5200405519) per 10^7 total cells at 4°C . Cells were washed and resuspended with 500 μL buffer. DPP4 positive cells were then positively selected by separation over MS Columns and MidiMACS™ Separator (milenyi biotec).

Confirmation of senescence in DPP4+ isolated senescent cells

[00234] DPP4 positive cells isolated as above were re-plated with complete medium for 24 hours and multiple senescence markers were determined as described below.

Senescence-associated beta-galactosidase

[00235] SA- β Gal activity was detected as described in the literature (see, e.g., Dimri et

al., Proc Natl Acad Sci U S A. 1995 Sep 26; 92(20): 9363–9367) using a commercial kit (Biovision).

Immunofluorescence

[00236] 10,000 cells per well were plated in 96 well plate and primary and secondary senescence induced as above. Cells were fixed with 4% PFA for 15 minutes at room temperature. After washing the fixative solution, cells were permeabilized with 0.5% triton for 15 minutes, blocked in 5% BSA for 30 minutes and incubated overnight with primary antibody (1:1000 dilution with 5% BSA) at 4°C. Primary antibodies used were gamma H2ax (P Ser139) Mouse Antibody (3F2), NB100-74435 (Novus) and Anti-HMGB1 antibody (ab18256), Rabbit, (Abcam). DPP4 immunofluorescence were performed without permeabilization using DPP4/CD26 (D6D8K) Rabbit mAb #67138 (Cell Signaling technology). Cells were then washed and incubated with fluorescent secondary antibody and Hoechst (Invitrogen H3570) (1: 1000 in 5% BSA) for 20 minutes at room temperature in dark. Secondary antibodies used were Invitrogen Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Catalog # A-11008, lot 1166843) and Invitrogen Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Catalog # A-11030). Images were acquired with Molecular Devices ImageExpress Macro.

Proliferation assay (Click-iT EdU staining)

[00237] Cell proliferation was determined by Click-iT™ EdU Cell Proliferation Kit (ThermoFisher, C10337) following the manufacturer protocol. Briefly, cells were cultured with EdU (10 µM) for 24 hours, fixed in 4% PFA for 10 minutes, washed in PBS, and permeabilized with 0.5% Triton X-100 for 15 minutes. Cells were then washed and incubated in Click-iT® reaction cocktail and DNA was stained with Hoechst 33342 (Invitrogen H3570). Images were acquired by ZEISS Axiovert S 100 microscope at a magnification of 32x.

RT-qPCR Gene Expression

[00238] RNA was extracted using commercially available kits Quick-RNA Miniprep Kit

(Zymo Research, R1055) according to the manufacturer's instructions. cDNA synthesis was performed using Takara PrimeScript™ RT Master Mix (Cat. # RR036A) according to the manufacturer's instructions. Quantitative PCR was performed on a StepOnePlus™ Real-Time PCR System using primers and probes purchased from Applied Biosystems TaqMan Gene Expression assays. Primers and probes used are listed above.

Western blot

[00239] Cells were lysed in RIPA buffer, and protein content determined by BCA assay. 20 µg protein was separated by electrophoresis and transferred to PVDF membranes. Membranes were blocked in 5% milk, incubated overnight with primary antibody (1:1000), washed in TBST, incubated with HRP-conjugated secondary antibody for one hour, and visualized by GeneGnome chemiluminescence imaging system. Primary antibodies obtained from Cell Signalling Technology were Bcl-2 (D55G8) Rabbit mAb (Human Specific) #4223, Bcl-xL Rabbit Antibody #2762, Bcl-w (31H4) Rabbit mAb #2724 and β-Actin Rabbit Antibody #4967. Anti-CDKN2A/p16INK4a Antibody (JC8): sc-56330 were purchased from Santa Cruz Biotechnology.

Enzyme-linked immunosorbent assays (ELISA)

[00240] Primary and secondary senescence were induced as indicated above and cultured in low serum medium for 24 hours. CM were collected, and cell debris were removed by centrifugation at 300G for 10 minutes. Supernatants were transferred to a tube on ice; cells were trypsinized and counted. CM were analysed by ELISA kit (IL-6 and IL-8) as instructed by the manufacturer and normalized to cell number.

Senolytic drugs test

[00241] Senescent and non-senescent cells were treated with a range of doses of senolytic drugs (ABT-199, ABT-263, Quercetin, dasatinib). ABT-199, ABT-263, A-1331852 were purchased from Selleckchem and dasatinib were from LC laboratories. Quercetin was purchased from Sigma-Aldrich. Cell viability was determined by LIVE/DEAD™ Viability/Cytotoxicity Kit, for mammalian cells (Invitrogen, L3224)

following the manufacturer protocol.

RNASeq

[00242] DPP4 positive senescent cells were collected as above and stored at -80°C with RNAZip. RNA was extracted using commercially available kits (Quick-RNA Miniprep Kit (Zymo Research, R1055) according to the manufacturer's instructions. cDNA synthesis was performed using Takara PrimeScript™ RT Master Mix (Cat. # RR036A) according to the manufacturer's instructions.

[00243] After investigating the quality of the raw data, sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were mapped to the reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. BAM files were generated as a result of this step. Unique gene hit counts were calculated by using feature Counts from the Subread package v.1.5.2. Only unique reads that fell within exon regions were counted. After extraction of gene hit counts, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the groups of samples was performed. The Wald test was used to generate p-values and Log2 fold changes. Genes with adjusted p-values < 0.05 and absolute log2 fold changes > 1 were called as differentially expressed genes for each comparison. A PCA analysis was performed using the "plotPCA" function within the DESeq2 R package. The plot shows the samples in a 2D plane spanned by their first two principal components. The top 500 genes, selected by highest row variance, were used to generate the plot.

Detection of intracellular iron by Flow cytometry

[00244] Iron content of cells was determined by FerroFarRed also known as SiRhoNox-1 fluorescent probe based on manufacturers protocol. Briefly, culture medium was removed from flasks and cells rinsed three times with PBS buffer. Cells were then treated with 5 µM of FerroFarRed diluted with serum-free cell culture medium and incubated for one hour at 37°C. After staining, excess probe were washed off with PBS

and cells were collected for flow analysis.

Data representation and statistical analysis

[00245] Statistical analysis was conducted using Graph Pad Prism 9. Statistical analyses of group differences were determined by one-way ANOVA with appropriate correction for multiple comparisons (Bonferroni multiple comparison tests, GraphPad Prism).

Example 1

SCs express DPP4 on their surface

[00246] According to recent studies, endothelial cells (ECs) are the increasingly susceptible to age-associated senescence burden as majority of p16^{high} SCs in mice were found to be ECs. Applicants used primary patient derived ECs (i.e., artery and vein iliac ECs from three young donors aged 20-24) as a model to study secondary senescence. Primary senescence was induced by treating sub-confluent cultures with doxorubicin (300nM) for 24 hours and senescence burden was determined after 7-10 days. To induce secondary senescence, conditioned medium (CM) containing SASP factors was collected from primary SCs. Sub-confluent proliferating ECs were cultured with CM for seven days with media change on every other day. Cells treated with CM collected from quiescent cells were used as a non-senescent (NS) control. The method is depicted in **FIG. 7A**. The top row depicts induction of senescence using doxorubicin (DOXO); the bottom row depicts induction of secondary senescence using cultured media from primary senescent cells (CM).

[00247] The results showed that 90% of doxorubicin treated cells ("S") had SA β Gal activity whereas only 45% of cells treated with CM from senescent cells showed SA β Gal activity. As noted above, these are referred to as secondary senescent cell ("SS"). However, cells treated with vehicle (NS) or conditioned medium (CM) from non-senescent cells (NSCM) had significantly lower SA β Gal activity. These results are depicted graphically in **FIG. 7B** which shows the percentage of cells that expressed SA β Gal.

[00248] Persistent DNA damage caused by treatment with doxorubicin is the main cause of senescence-associated secretory phenotype (SASP) produced by SCs. Immunofluorescence (IF) was performed to detect γ H2AX foci as a proxy of persistent DNA damage. The results demonstrate that 90% of senescent cells ("S") had two or more foci whereas only 40% of secondary senescent cells ("SS") had γ H2AX foci. These results are depicted graphically in **FIG. 7C** which shows the percentage of cells with two or more two foci.

[00249] HMGB1, a histone binding protein is also a component of damage-associated molecular patterns (DAMPs) is secreted upon cellular damage. The loss of HMGB1 from the nucleus and its release from the cells is also considered as one of the hallmarks of senescence. An HMGB1 study showed that nearly all non-senescent (NS) cells or non-senescent cells treated with conditioned medium (NSCM) had HMGB1 localized in the nucleus. About 60% senescent ("S") and only 20% of secondary senescent ("SS") cells had HMGB1 localized to the nucleus and the cytosol. Further, only 5% cells in S or SS group were positive for EdU, indicating loss in proliferative capacity. These results are depicted graphically in **FIG. 7D** which shows the percentage of cells with nuclear versus nuclear/cytosolic HMGB1. Similarly, **FIG. 7E** shows the percentage of EdU positive cells.

[00250] p16 (also known as p16^{INK4a}, cyclin-dependent kinase inhibitor 2A and CDKN2A), is a protein that slows cell division by slowing the progression of the cell cycle from the G1 phase to the S phase, thereby acting as a tumor suppressor. Studies have associated elevated levels of p16 with senescence. Similarly, induction of p21 triggers cell growth arrest associated with senescence and damage response. p21^{Cip1} (also known as p21^{Waf1}) is a cyclin-dependent kinase inhibitor (CKI) that is capable of inhibiting all cyclin/CDK complexes, though is primarily associated with inhibition of CDK2. Quantitative real time PCR also showed a two to eight fold increase in the expression of p16^{INK4a} and p21^{Cip1} (respectively) and a decrease in the expression of LMNB1 in SS^{DPP4+} and S^{DPP4+} cells following enrichment. **FIG. 7F** shows the change in

mRNA expression of p16 Ink4a and p21 Cip1 as determined by RT-qPCR in untreated and doxorubicin-treated ECs. **FIG. 7G** shows mRNA expression of the classical SASP factors *IL-6* and *IL-8* as determined by RT-qPCR. **FIG. 7H** shows mRNA expression of p16 Ink4a, p21 Cip1. *il-6* and *il-8* was determined by RT-qPCR in secondary senescent ECs. Values are presented as mean $\pm$ SEM. Comparison was made with one-way ANOVA.

[00251] DPP4 (Dipeptidyl Peptidase-4), also known as CD26 has been reported as a surface marker of senescent fibroblasts. This was confirmed in an immunofluorescence (IF) study that showed the expression of DPP4 on the surface of senescent fibroblasts (IMR90). These results are shown in images of **FIG. 8A** and **FIG. 8B**. This was also demonstrated in mesenchymal stem cells (MSCs) as shown in **FIG. 8D** and **FIG. 8E**. These results were also confirmed by flow cytometric analysis to determine the surface expression of DPP4 on live SCs. In both IMR90 and MSC cell types over 90% of SCs had DPP4 expression on their surface whereas only 42% and 26% of the non-senescent cells had DPP4 expression on their surface respectively (**FIG. 8C** and **FIG. 8F**). The surface expression of DPP4 was also studied in senescent cells (**FIG. 8G – FIG. 8I**) and secondary senescent cells (**FIG. 8J – FIG. 8L**). Endothelial cells were isolated from the three donors and characterized qualitatively by immunofluorescence (IF) and quantitatively by flow cytometry. On average, 60-80% of senescent cells (SS) and secondary senescent cells (SS) from three donors expressed DPP4 on their surface. Whereas less than 10% of non-senescent cells (NS) had DPP4 expression on their surface. These results are shown graphically in **FIG. 8M**.

Example 2

Isolation of live Senescent Cells using DPP4 as a surface marker

[00252] As only a small percentage of cells exposed to condition media (CM) from primary senescent cells (S) become senescent, Applicants reasoned if secondary senescent cells (SS) could be isolated from the heterogeneous pool of CM treated cells by isolating SCs based on DPP4 expression. To this end, following senescence induction (by doxorubicin or CM treatment) cells were incubated with Anti-DPP4

antibody conjugated with phycoerythrin (PE). Subsequently, cells were incubated with anti-phycoerythrin (anti-PE) Microbeads and passed through magnetic separation column. The column bounded DPP4⁺ cells, presumably SCs, and flow through DPP4⁻ cells were sorted and analyzed for senescence markers. The results confirmed that 80-85% of DPP4⁺ secondary SCs (SS^{DPP4+}) showed SA β Gal activity which was comparable to DPP4⁺ primary SCs (S^{DPP4+}). These results are depicted in **FIG 1A** which shows the percent of SA- β -gal positive cells in DPP4⁺ isolated primary and secondary senescent cells from three donors. **FIG. 9A – FIG. 9D** are images of stained cells. **FIG. 9A** shows SA β Gal staining in DPP4⁺ isolated non-senescent (NS) cells. **FIG. 9B** shows SA β Gal staining in DPP4⁺ isolated non-senescent cells treated with conditioned media (NSCM). **FIG. 9C** shows of SA β Gal staining in isolated DPP4⁺ primary senescent cells (S^{DDP4+}). **FIG. 9D** shows SA β Gal staining in isolated DPP4⁺ secondary senescent cells (SS^{DDP4+}).

[00253] Enrichment of SS was further confirmed by quantitation of γ H2AX IF assay showing that 80-85% of SS^{DPP4+} had two or more foci per nucleus following enrichment. **FIG. 1B** shows the quantification of γ H2AX foci in DPP4⁺ isolated primary and secondary senescent cells. Cells with two or more foci per nucleus were defined as senescent cells. **FIG. 9E – FIG. 9H** are images of stained cells. **FIG. 9E** shows γ H2AX and HMGB1 staining in DPP4⁺ isolated non-senescent (NS) cells. Both primary and secondary senescent cells were isolated based on DPP4 expression and replated. 24 hours after replating cells were stained for SA β Gal, or γ H2AX and HMGB1. **FIG. 9F** shows γ H2AX and HMGB1 staining in DPP4⁺ isolated non-senescent cells treated with conditioned media (NSCM). **FIG. 9G** shows γ H2AX and HMGB1 staining in isolated DPP4⁺ primary senescent cells (S^{DDP4+}). **FIG. 9H** shows γ H2AX and HMGB1 staining in isolated DPP4⁺ secondary senescent cells (SS^{DDP4+}).

[00254] These studies demonstrated that 70% of SS^{DPP4+} lost HMGB1 from the nucleus. **FIG. 1C** shows the percentage of cell in which HMGB1 relocalized. The percentage of cells expressing HMGB1 in the nucleus and nucleus + cytosol was scored. HMGB1 relocalized to the cytosol in 80% of primary and 70% of DPP4⁺

isolated secondary senescent cells. As described above, **FIG. 9E – FIG. 9H** show γ H2AX and HMGB1 staining NS, NSCM, S^{DPP4+} and SS^{DPP4+} cells respectively.

[00255] Quantitative real time PCR also showed a two to eight-fold increase in the expression of p16^{Ink4a} and p21^{Cip1} (respectively) and a decrease in the expression of LMNB1 in SS^{DPP4+} and S^{DPP4+} cells following enrichment. These results are shown in **FIG. 1D – FIG. 1F**. **FIG. 1D** shows mRNA expression of p16 Ink4a. **FIG. 1E** shows mRNA expression of p21 Cip1. **FIG. 1F** shows mRNA expression of LMNB1. Levels of prototypical SASP factors IL-6 and IL-8 were also measured by ELISA in the CM of SS^{DPP4+}. The results demonstrated significantly high secretion of IL-6 and IL-8 in both SS^{DPP4+} and S^{DPP4+} cells as shown in **FIG. 1G**.

[00256] To determine whether DPP4 could be used to isolate senescent cells (SCs) irrespective of senescence induction methods and cell type/origin, primary senescence was induced in endothelial cells by X-irradiation. Cultured media (CM) was collected from X-irradiated cells to induce secondary senescence (SS). Similar to doxorubicin treatment, only 40% of cells treated with the CM collected from irradiated cells (90% senescent) were positive for SA β Gal (**FIG. 9I**) and have two or more γ H2AX foci (**FIG. 9J**).

[00257] Using the same method, DPP4+ SCs were isolated and managed to increase the percentage of secondary SCs to 80%. These results are also shown in **FIG. 9I** and **FIG. 9J**. The study was repeated using fibroblasts. As above, doxorubicin treatment was used to induce primary senescence. Similarly, only 35-40% of cells treated with the CM collected from doxorubicin treated IMR90 cells (90% senescent) were positive for SA β Gal (**FIG. 9K**) and had two or more γ H2AX foci (**FIG. 9L**). In IMR90, isolation of SCs based on DPP4 expression showed 70% secondary senescence (**FIG. 9K** and **FIG. 9L**). These data showed that SS can be isolated and enriched based on surface expression of DPP4 (**FIG. 1J**).

[00258] Further, DPP4⁻ cells were studied to determine whether they express

senescence phenotypes. Applicants studied senescence markers in the DPP4⁻ flowthrough fractions of SS following magnetic sorting. SA β Gal and gH2AX IF assay showed only 20% of DPP4⁻ cells were senescent (compared to 80-85% in DPP4⁺ cells). The results are graphically depicted in **FIG. 9M – FIG. 9N**.

Example 3

SS^{DPP4+} can spread senescence as Tertiary senescence

[00259] Previous investigations regarding the spread of senescence have shown that SASP factors from primary SCs can induce senescence in about half of the cells exposed to the conditioned media (CM). Whereas SASP factors from secondary SCs induced senescence in about 10 – 16% of cells exposed to CM. These studies suggest that the dilution of senescence spreads. However, the present study demonstrated that SS^{DPP4+} secreted comparable levels of SASP factors like IL-6 and IL-8 as S^{DPP4+}. **FIG. 1G** shows IL-6 and IL-8 secretion as measured by ELISA in conditioned media from DPP4⁺ isolated senescent cells in three donors.

[00260] Therefore, Applicants investigated whether SASP factors secreted by SS^{DPP4+} further spread senescence as tertiary senescence. Results showed that, CM from un-enriched SS cells induced senescence phenotype in only 20% of cells as measured by SA β Gal assay and gH2AX foci. Interestingly, CM collected from SS^{DPP4+} induced senescence phenotype in 40% of treated cells (**FIG. 1H** and **FIG. 1I**). These results suggest that SS^{DPP4+} cells are capable of spreading senescence as tertiary senescence. **FIG. 1J** depicts a comparison of previous method of comparing primary and secondary SCs. The top row shows the conventional understanding of induction of secondary and tertiary senescence. The bottom row shows induction using SS^{DPP4+} cells.

Example 4

SS^{DPP4+} are phenotypically different from S^{DPP4+}

[00261] In addition to cell cycle arrest, SCs are also known to resist cell death. To further characterize SS^{DPP4+}, the expression of the anti-apoptotic BCL-2 family proteins

was measured (BCL-2, BCL-xL and BCL-w) which are known to constitutively upregulated in SCs. Western blot analysis in the whole cell lysate showed elevated expression of BCL-2, BCL-xL and BCL-w in S^{DPP4+} . **FIG. 2A** shows relative levels of expression of these proteins with actin as a control. BCL-2 in the whole cell lysate of SS^{DPP4+} was not detected even though comparable levels levels of BCL-xL and BCL-w were observed (compared to S^{DPP4+}).

[00262] As BCL-2 antagonists are often used to selectively kill SCs, the effect of these senolytics were tested on S^{DPP4+} and SS^{DPP4+} . To this, S^{DPP4+} and SS^{DPP4+} were isolated seven days after treatment and replated to 96 well plates followed by senolytic drug treatments. The experimental timeline is depicted in **FIG. 2B**. The results demonstrated that Venetoclax (ABT-199), a specific inhibitor of BCL-2, killed S^{DPP4+} in a dose dependent manner. Approximately 50% were killed at its optimal dose (5mM), while only 25% SS^{DPP4+} were killed at this concentration. **FIG. 2C** shows that ABT-199 has senolytic activity in $DPP4+$ primary but not secondary SCs. However, with increasing concentration, the drug was observed to be toxic to NS cells. Surprisingly, Navitoclax (ABT-263) a broad inhibitor of all BCL-2 family proteins was also not effective on killing SS^{DPP4+} (**FIG. 2D**). At its optimal dose (0.5mM) ABT-263 killed 45% of S^{DPP4+} and only 20% of SS^{DPP4+} . The senolytic effect of Quercetin and its combination with Dasatinib were also studied. These are the most widely tested drugs. Quercetin demonstrated (at optimal dose) that it killed up to 60% of both S^{DPP4+} and SS^{DPP4+} (**FIG. 2E**). However, the combination was toxic to both senescent and NS cells (**FIG. 2F**).

Example 5

Transcriptome profile of S^{DPP4+} and SS^{DPP4+}

[00263] Due to differential expression of BCL-2 family proteins and differential sensitivities of S^{DPP4+} and SS^{DPP4+} to BCL-2 antagonists, frequently used as current senolytics, Applicants further characterized the global gene expression signature of secondary SCs to identify novel senolytic targetable pathways. Applicants isolated $DPP4+$ primary and secondary senescent ECs from three young donors each in

triplicates and performed RNASeq analysis. After stringent filtration ($|\text{LFC}| \geq 1$ and $\text{P}_{\text{adj.}} < 0.05$), 1240 were found genes in $\text{S}^{\text{DPP4+}}$ compared to NS and 1316 genes in $\text{SS}^{\text{DPP4+}}$ compared to NSCM treated cells as differential expressed genes (DEGs). Of 1316 DEGs of $\text{SS}^{\text{DPP4+}}$, 945 (72%) were uniquely expressed in $\text{SS}^{\text{DPP4+}}$ but not in $\text{S}^{\text{DPP4+}}$. **FIG. 3A** shows the transcriptome profile of DPP4+ primary and secondary SCs. **FIG. 10A** is a Venn diagram of DEG's of DPP4+ isolated primary and secondary senescent cells.

[00264] Heatmap analysis of the top 30 and 50 DEG's showed that $\text{SS}^{\text{DPP4+}}$ from three donors were well separated from $\text{S}^{\text{DPP4+}}$ and clustered together. These results are show in **FIG. 3B** and **10B**. Further, 2D principal-component analysis (PCA) showed the variance (PC1) caused by the differences in the tissue of origin ($\text{S}^{\text{DPP4+}}$ and $\text{SS}^{\text{DPP4+}}$ samples from one donor (artery) versus the other two donors (vein)) followed by the variance (PC2) based on mode of senescence induction (i.e., primary vs secondary) (**FIG. 3C**). Comparisons between the $\text{S}^{\text{DPP4+}}$ and $\text{SS}^{\text{DPP4+}}$ at the donor level demonstrated that primary and secondary SCs are separated from each other and from the non-senescent (NS) control (**FIG. 3D** and **FIG. 10C – FIG. 10D**).

[00265] To investigate the biological context of these differences in the gene expression DEGs were categorized based on biological process, molecular function, and cellular component GO term categories. Overall, $\text{S}^{\text{DPP4+}}$ and $\text{SS}^{\text{DPP4+}}$ showed a similar trend in all three categories (**FIG. 10E** and **FIG. 10F**). Further, several senescence associated pathways such as p53 pathway, protein secretion, apoptosis and mTORC1 signaling and proinflammatory pathways such as interferon alpha (IFN- α) and interferon gamma (IFN- γ) were among the top enriched GO terms by upregulated genes in both $\text{S}^{\text{DPP4+}}$ and $\text{SS}^{\text{DPP4+}}$ (**FIG. 3E** and **FIG. 3F**). Further, Applicants validated the RNASeq data by performing RT-qPCR on some of the DEG's identified. RT-qPCR data confirmed that *GDF7*, *WNT5B*, *IGFBP5*, *CST1* were upregulated in both $\text{S}^{\text{DPP4+}}$ and $\text{SS}^{\text{DPP4+}}$ (**FIG. 3G – FIG. 3J**) whereas *IGFBP3*, *PAPPA2*, *EPHA7* and *TNC* were significantly upregulated in $\text{SS}^{\text{DPP4+}}$ compared to $\text{S}^{\text{DPP4+}}$ (**FIG. 3K – FIG. 3N**).

Example 6

SCs have high level of iron accumulation

[00266] RNASeq analysis revealed that the top differentially expressed gene in SS^{DPP4+} SCs were Metallothioneins (*MTs*) isoforms (*MT1A*, *MT1F*, *MT1G*, *MT1H*, *MT1M*) and solute carrier proteins (*SLCs*) related genes (**FIG. 4A**). These genes were also differentially expressed in S^{DPP4+} SCs (**FIG. 4B**). The *MTs* are cysteine rich metal-binding proteins that regulate homeostasis of iron, zinc and copper and known to mitigate heavy metal poisoning and alleviate superoxide stress. The *SLCs* are membrane bound transporters that are known to transport a variety of substances including metal ions. Both *MTs* and *SLCs* genes are known to involve in iron homeostasis and senescent cells are reported to have high iron accumulation (see. e.g., Killilea et al., Ann N Y Acad Sci. 2004 Jun; 1019:365-7). Therefore, Applicants investigated whether primary and secondary senescent ECs accumulate high levels of iron.

[00267] First, RNASeq data revealed differential expression of several iron metabolism related genes in S^{DPP4+} and SS^{DPP4+} (**FIG. 4C**). Quantitative RT-PCR was performed in both S^{DPP4+} and SS^{DPP4+} SCs samples which confirmed elevated levels of *MT1G* (**FIG. 4D**), *SLC7A11* (**FIG. 4E**) and *TGFBR1* (**FIG. 4G**) and downregulation of *FTRC* (**FIG. 4F**) gene expression. Finally, iron content in S^{DPP4+} and SS^{DPP4+} ECs from three donors was analyzed by assaying cells with SiRhoNox-1 prob based flow analysis. In all three donors S^{DPP4+} (**FIG. 4H** and **FIG. 11A**) and SS^{DPP4+} cells (**FIG. 4I** and **FIG. 11B**) had high level of iron compared to their respective quiescence controls. On average, 80% of S^{DPP4+} and SS^{DPP4+} cells and only less than 30% of the quiescence controls were positive for SiRhoNox-1 prob based flow analysis of iron (**FIG. 4J**).

Example 7

Ferroptosis as a novel senolytic approach to target primary and secondary senescent cells

[00268] In addition to iron accumulation, differential expression of several ferroptosis

related genes was observed in S^{DPP4+} and SS^{DPP4+} cells (**FIG. 5A**). This observation was confirmed by RT-qPCR which showed the upregulation of classical ferroptosis suppressors (*NFE2L2*, *CHAC1* and *PTGS2*) in S^{DPP4+} and SS^{DPP4+} cells (**FIG. 5B** and **5C**). High expression of *ACSL4* was confirmed in both S^{DPP4+} and SS^{DPP4+} cells (**FIG. 5D**).

[00269] Recent studies demonstrate that *ACSL4* is required for long chain polyunsaturated fatty acids (PUFA) peroxidation to facilitate ferroptosis. Applicants investigated whether an increase in intracellular labile iron would increase the sensitivity of S^{DPP4+} and SS^{DPP4+} to ferroptosis, an iron dependent nonapoptotic cell death. S^{DPP4+} and SS^{DPP4+} ECs were treated with erastin a well-established ferroptosis inducer. Interestingly, the results demonstrated a dose dependent cytotoxicity of S^{DPP4+} and SS^{DPP4+} ECs (**FIG. 5E**). Treatment with 10 μ M erastin for 72 hours killed 80-90% of both S^{DPP4+} (Doxorubicin treated) and SS^{DPP4+} ECs. Importantly, substantially low cytotoxicity in non-senescent (NS) control cells (about 10%) was observed (**FIG. 5F**). The senolytic effect of erastin was also confirmed in irradiated ECs. Treatment with 10 μ M Erastin again killed 60% of S^{DPP4+} irradiated ECs and 50% of their SS^{DPP4+} with minor effect towards NS cells (**FIG. 5G**). Finally, to determine if the senolytic effect of erastin is independent of cell types of origin, the senolytic activity of erastin in Doxorubicin treated (**FIG. 5H**) and irradiated (**FIG. 5I**) fibroblasts (IMR90) was studied with their derived secondary SCs ($DPP4+$ isolated SCs). 10 μ M erastin killed 80-85% of S^{DPP4+} and SS^{DPP4+} fibroblasts irrespective of the senescence induction methods (Doxorubicin and irradiated). These results showed that S^{DPP4+} and SS^{DPP4+} are highly susceptible to senolysis by erastin treatment.

Example 8

Erastin kills Senescent Cells through ferroptosis

[00270] To determine the mechanism of cell death by erastin, SCs were co-treated with various cell death pathway inhibitors including ferroptosis inhibitor ferrostatin-1 (Fer-1), iron chelator deferoxamine (DFO) and pan-caspase inhibitor z-VAD-fmk to inhibit apoptosis. Images of treated cells over time are shown in **FIG. 6A – 6D**. The

percent cytotoxicity was determined following the treatments to assess cell viability. SCs treated with pan-caspase inhibitor z-VAD-fmk were still sensitive to Erastin, suggesting Erastin is unlikely to kill targets by apoptosis (**FIG. 6A** and **6B**). However, treatment with DFO abrogated the Erastin mediated cytotoxicity of SCs (**FIG. 6A** and **6C**). Furthermore, treatment with Fer-1 also reduced the cytotoxicity of Erastin treatment in SCs (**FIG. 6A** and **6D**). This shows that the increase in intracellular labile iron and ferroptosis are necessary for the senolytic activity of Erastin. Treatment with Erastin also decreased the expression levels of the ferroptosis inhibitor genes *PTGS2* and *CHAC1* (**FIG. 6E** and **FIG. 6F**). Finally, malondialdehyde (MDA) was measured which is a ferroptosis marker. Levels were studied in erastin treated S^{DPP4+} and SS^{DPP4+} SCs. High levels of MDA were observed in S^{DPP4+} compared to NS cells (**FIG. 12A**). Importantly, erastin treatment significantly increased MDA level in S^{DPP4+} whereas its treatment did not change the level of MDA in NS cells (**FIG. 6G**). Further, significantly elevated levels of MDA secretion were observed by S^{DPP4+} treated with erastin but not in NS cells (**FIG. 6H**).

Example 9

RNASeq analysis revealed novel senescence markers

[00271] As detailed in Example 5, Applicants characterized the global gene expression signature of secondary SCs to identify senolytic targetable pathways. Specifically, Applicants isolated DPP4⁺ primary and secondary senescent ECs from three young donors each in triplicates and performed RNASeq analysis.

[00272] The differentially expressed genes from the RNASeq analysis in primary and secondary SCs can be used as senescence markers. The markers can be used to identify primary and/or secondary SCs. In other aspects, these genes can be used as pro-survival genes for primary and secondary senescent cells.

[00273] Table IA lists differentially expressed genes that were upregulated in primary senescent cells as determined by the RNASeq analysis. Downregulated genes are listed in Table IB. Similarly, Table IC lists differentially expressed genes that were

upregulated in secondary senescent cells as determined by the RNASeq analysis. Downregulated genes are listed in Table ID.

Example 10

Noncoding RNA as a senescent cell marker

[00274] In addition to the protein coding RNA analysis, Applicants discovered differentially expressed noncoding RNAs in primary and secondary senescent cells. In aspects, these non-coding RNAs are used as markers of senescence and secondary senescence, as a target to kill senescent cells or to reverse the senescence status or as senomorphs. The differentially expressed noncoding RNAs are listed in Table II (**FIG. 17B**).

[00275] Table IIA lists differentially expressed noncoding RNAs that were upregulated in primary senescent cells. Downregulated noncoding RNAs are listed in Table IIB. Similarly, Table IIC lists differentially expressed noncoding RNAs that were upregulated in secondary senescent cells. Downregulated noncoding RNAs are listed in Table IID.

Example 11

Identification of pro-survival genes of primary and secondary senescent cells

[00276] Applicants have identified pro-survival genes that can play a role on the death resistance mechanism of primary and secondary senescent cells. The RNA sequencing data above was filtered so that it is limited to pro-survival genes. Differential gene analysis was conducted to identify genes that were uniquely upregulated in primary and secondary senescent cells. Based on these studies, Applicants have identified potential pro-survival genes that can be a target for senescent cell removal.

[00277] Table IIIA lists pro-survival genes that were upregulated in primary senescent cells. Downregulated pro-survival genes are listed in Table IIIB. Similarly, Table IIIC lists differentially pro-survival genes that were upregulated in secondary

senescent cells. Downregulated pro-survival genes are listed in Table IIID.

Example 12

Identification of senescent cell surface markers

[00278] In the next study, Applicants identified surface proteins for us as markers for primary and secondary senescence. The RNA sequencing data was filtered to include genes known to code for surface proteins. Next, differential gene analysis was conducted to identify genes which code for surface proteins and are uniquely upregulated in primary and secondary senescent cells. A published list (i.e., Cell Surface Protein Atlas) was used to validate surfaceome proteins (see, e.g., Bausch-Fluck D, Hofmann A, Bock T, Frei AP, Cerciello F, et al., 2015, A Mass Spectrometric-Derived Cell Surface Protein Atlas. PLoS One 10: e0121314). Applicants identified genes that coded for surface proteins and used the DESeq2 package to identify genes that were differentially expressed in primary and secondary senescence. Based on these studies, surface markers were identified for primary and secondary senescent cells which are listed in Table IV (**FIG. 17D**).

[00279] Table IVA lists surface markers that were upregulated in primary senescent cells. Downregulated surface markers are listed in Table IVB. Similarly, Table IVC lists surface markers that were upregulated in secondary senescent cells. Downregulated surface markers are listed in Table IVD.

Example 13

Identification of Secreted Proteins by SCs

[00280] Next, Applicants conducted an RNA sequencing analysis to identify genes coding for secreted proteins that were expressed by primary and secondary senescent cells. A list of secreted proteins was compiled by the Human Protein Atlas to search for genes that coded for secreted proteins. The DESeq2 package was applied on RStudio to run DEG analysis to find genes differentially expressed between primary and secondary senescent cells.

[00281] Table VA lists secreted proteins that were upregulated in primary senescent cells. Downregulated secreted proteins are listed in Table VB. Similarly, Table IVC lists secreted proteins that were upregulated in secondary senescent cells. Downregulated secreted proteins are listed in Table IVD.

Example 14

Identification of Senotherapeutic Drugs based on RNASeq Analysis

[00282] To identify novel senolytics for clearing primary and secondary senescent cells, Applicants used a web-based search engine (i.e., the library of integrated network-based cellular signatures (LINCS) L1000 data set). The data includes more than a million gene expression profiles of chemically perturbed human cell lines. Applicants prioritized thousands of small-molecule signatures that were predicted to reverse the gene expression signature of primary and secondary senescence in three donors.

[00283] Applicants identified 18 hits that targeted either primary or secondary senescence. These hits were categorized into nine classes based on their targets (**FIG. 13**). Interestingly some of the hits such as dasatinib, Geldanamycin and R406 are known senolytics. From the 18 hits five (i.e., QL-X-138, Emetine Dihydrochloride Hydrate (74), R406, vorinostat, TWS119) were predicted to target secondary senescent cells. One particular drug (i.e., emetine dihydrochloride hydrate) was predicted to target both primary and secondary senescent cells.

[00284] Vorinostat is a histone deacetylases (HDAC) inhibitor whereas TWS119 is an inhibitor of GSK-3 β and mTORC1. Emetine is an inhibitor of HIF-1 α , and it has also been shown to downregulate BCL-xL.

Example 15

Testing Activity of Senotherapeutic Drugs

[00285] To determine whether any of these 18 drugs had senolytic activity, Applicants treated DPP4+ isolated primary and secondary senescent cells with five

different doses of each drug for 48 hours. Cell viability was determined using a calcein-AM assay. Sixteen compounds had the same or higher toxicity on non-senescent cells at the concentrations tested or had no effect on senescent cells. Only two drugs reduced viability of primary and secondary senescent cells specifically without significant effect on the viability of non-senescent cells. Based on these observations, these drugs were (i.e., vorinostat and emetine) are considered to have senolytic potential.

[00286] To further validate the primary screening, these two senotherapeutic drugs were tested again using Xceligence machine which allowed for determining the optimal concentration and exposure time. Finally, the senolytic activity of these drugs was determined in multiple donors. The results showed that these two drugs (i.e., vorinostat and emetine) are senolytic towards primary and secondary senescent endothelial cells.

Example 16

Prodrug (TRX-CBI) as a senolytic agent

[00287] In addition to elevated levels of Fe(II), SCs also have high expression of NFE2L2, which activates the cellular antioxidant response to membrane damage during ferroptosis. Applicants also found increased expression of CHAC1 and PTGS2 in both types of SCs, and both these genes are consistently upregulated in (and in the case of CHAC1, required for) ferroptosis. Thus, SCs appear to be primed for ferroptosis, studies suggest that they are recalcitrant to it, suggesting that some other mechanism is responsible for the basal resistance of SCs to ferroptosis.

[00288] FIN56 reduced the viability of SDPP4+ and PSDPP4+ irrespective of the cell type of origin (**FIG. 17**). FIN56 promotes degradation of GPX4 and depletion of the antioxidant CoQ which together compromise the antioxidant defense of the cells. FIN56 may also enhance iron bioavailability by triggering lysosomal membrane permeability. The inability of FIN56 to kill SCs in the presence of iron chelator DFP or ferroptosis inhibitor Fer-1 further confirms the induction of ferroptosis as its senolysis mechanism

(FIG. 18).

[00289] Recently, another ferroptosis inducer, JQ1, which may downregulate both SLC7A11 and GPX4, has also been shown to kill senescent human dermal fibroblasts. Another study showed that RSL3 selectively killed senescent tubular cells. Several strategies to induce ferroptosis are being pursued for cancer therapy that might be repurposed for senotherapeutic purposes. However, ferroptosis inducer drugs such as GPX4 inhibitors may have several undesirable toxicities, as GPX4 is required for the development of normal adult mouse brain and kidney.

[00290] Applicants therefore investigated an alternative ferroptosis-independent strategy to ablate SCs by leveraging the elevated Fe(II) levels. In this study, Applicants tested a known Fe(II)-activated prodrug (TRX-CBI) as a senolytic agent. TRX-CBI is converted to the cytotoxic CBI in a manner dependent on the Fenton reaction promoted by Fe(II). Because SCs display elevated levels of Fe(II), TRXCBI selectively ablates SCs independent of senescence lineage and cell type of origin. While the potent cytotoxicity of its CBI payload may limit the therapeutic index of TRX-CBI as a general senolytic, the TRX prodrug approach is generalizable to diverse classes of agents and might be employed to enhance specificity of current senolytics.

[00291] The results show that prodrugs exploiting SCs' high Fe(II) pool such as TRX CBI could be a safe and effective senolytic approach. Consistent with this, previous in vivo testing with TRX-CBI shows modest uptake in most tissues, with the pericardium, lungs, and liver exhibiting the highest uptake. That study also showed that normal white matter had only half the Fe(II) burden of orthotopic glioma xenografts, and similarly that the level of Fe(II) in U87 MG xenografts as measured by PET was substantially higher than that of normal brain tissue. Moreover, trioxolane-based compounds have been shown to have minimal penetrance across the blood-brain barrier, likely shielding the brain from potential toxic effects. The potential value of a Fe(II)-based senolytic approach was further supported by observations that the Fe(II) probe SiRhoNox-1 detects senescence independent of cell type of origin and mode of senescence induction with high selectivity.

[00292] Recently, it was reported that iron, in its free form or when released from damaged red blood cells, is a potent trigger of cellular senescence in vitro. Iron accumulation is sufficient to initiate senescence, fibrogenesis, and inflammation. In this context, when SCs are killed by senolytics, they can be expected to release high levels of iron, which might cause secondary senescence and initiate fibrosis. It has been reported that elimination of p16^{High} liver sinusoid ECs induces liver and perivascular tissue fibrosis²⁹ and that treatment of a rat model of pulmonary hypertension with ABT263 improved pulmonary hypertension at one week but exacerbated it at three weeks, with loss of pulmonary ECs. However, whether the release of iron from dead SCs contributes to this effect remains to be investigated.

[00293] Finally, whether the release of iron to the circulation when SCs undergo apoptosis during senolytic treatment contributed to the unsuccessful clinical trials of several senolytic drugs remains to be investigated. Our study focused on primary senescent human ECs, an understudied cell population in the senescence field of potentially great consequence. ECs line all blood vessels and function as a critical interface between the circulation and solid organs. They regulate many homeostatic functions like maintaining blood flow fluidity, clotting, and immune responses and inflammation, and they are most directly exposed both to the systemic signaling milieu, which has been found to play a critical role in aging (e.g., in the phenomena of heterochronic parabiosis and therapeutic plasma exchange as well as to the effects of circulating senescent immune cells). Conversely, their free and extracellular vesicle-encapsulated SASP has the most direct access to the circulation, potentially giving senescent ECs the widest range of systemic effects of all SC types of origin. Senescent ECs have also been implicated in aging and several diseases of aging in mice and human studies.

[00294] The data herein demonstrates that many of the most widely used senolytic drugs were especially toxic to human ECs. For example, treatment with ABT-199, quercetin, and the combination of dasatinib and quercetin resulted in 50%, 75%, and 55% cytotoxicity in NSs at higher doses, respectively. It is possible that primary ECs

are more sensitive to these drugs, which may explain the differences between results from other recent studies. Interestingly, there is only 30% loss in viability in NSs and NSCM ECs treated with FIN56 at high doses (**FIG. 17A**) and a 40% loss in viability in IMR-90 fibroblasts (**FIG. 17B**).

[00295] Higher dose TRX-CBI also has up to 60% cytotoxicity toward NSs. Importantly, however, NSs and NSCM cells are far more tolerant to the optimal dose of TRX-CBI even for longer time exposure, with less than a 10% loss in viability in these cells treated with the drug vs. an over 90% loss in viability of S and PSs (**FIG. 19B**). Notably the effects of FIN56 and TRX-CBI seem to be relatively safe to NS and NSCM controls from IMR-90 cells as well.

Methods of Use

[00296] Embodiments include a method of treating an ailment (i.e., a senescence-associated disease or disorder) and/or slowing the aging process or reducing signs of aging. The method can include administering an agent that induces ferroptosis. Embodiments also include therapies for treating a senescence-associated disease or disorder and slowing the aging process. In one embodiment, a method includes administering to a pharmaceutical formulation containing a therapeutic agent that selectively kills senescent cells (i.e., selectively kills senescent cells over non-senescent cells or compared with non-senescent cells). A treatment regimen can include administering a pharmaceutical formulation for a time sufficient and in an amount sufficient to selectively kill senescent cells. The pharmaceutical formulation can include an agent that induces ferroptosis such as erastin.

[00297] The therapeutic method of the present specification can include the step of administering drug product (e.g., vorinostat, emetine or a ferroptosis inducer such as FIN56 and erastin) at a pharmaceutically effective amount. The total daily dose should be determined through appropriate medical judgment by a physician and administered once or several times. The specific therapeutically effective dose level for any particular

patient may vary depending on various factors well known in the medical art, including the kind and degree of the response to be achieved, concrete compositions according to whether other agents are used therewith or not, the patient's age, body weight, health condition, gender, and diet, the time and route of administration, the secretion rate of the composition, the time period of therapy, other drugs used in combination or coincident with the composition disclosed herein, and like factors well known in the medical arts.

[00298] In still another aspect, the present specification provides a use of the pharmaceutical composition including the same in the preparation of drugs for the prevention or treatment of a senescence-associated disease or disorder and/or slowing the aging process/reducing signs of aging.

[00299] In one embodiment, the dose of the composition may be administered daily, semi-weekly, weekly, bi-weekly, or monthly. The period of treatment may be for a week, two weeks, a month, two months, four months, six months, eight months, a year, or longer. The initial dose may be larger than a sustaining dose. In one embodiment, the dose ranges from a weekly dose of at least 0.01 mg/kg, at least 0.25 mg/kg, at least 0.3 mg/kg, at least 0.5 mg/kg, at least 0.75 mg/kg, at least 1 mg/kg, at least 2 mg/kg, at least 3 mg/kg, at least 4 mg/kg, at least 5 mg/kg, at least 6 mg/kg, at least 7 mg/kg, at least 8 mg/kg, at least 9 mg/kg, at least 10 mg/kg, at least 15 mg/kg, at least 20 mg/kg, at least 25 mg/kg, or at least 30 mg/kg. In one embodiment, a weekly dose may be at most 1.5 mg/kg, at most 2 mg/kg, at most 2.5 mg/kg, at most 3 mg/kg, at most 4 mg/kg, at most 5 mg/kg, at most 6 mg/kg, at most 7 mg/kg, at most 8 mg/kg, at most 9 mg/kg, at most 10 mg/kg, at most 15 mg/kg, at most 20 mg/kg, at most 25 mg/kg, or at most 30 mg/kg. In a particular aspect, the weekly dose may range from 5 mg/kg to 20 mg/kg. In an alternative aspect, the weekly dose may range from 10 mg/kg to 15 mg/kg.

[00300] The present specification also provides a pharmaceutical composition for the administration to a subject. The pharmaceutical composition disclosed herein may further include a pharmaceutically acceptable carrier, excipient, or diluent. As used herein, the term "pharmaceutically acceptable" means that the composition is sufficient

to achieve the therapeutic effects without deleterious side effects, and may be readily determined depending on the type of the diseases, the patient's age, body weight, health conditions, gender, and drug sensitivity, administration route, administration mode, administration frequency, duration of treatment, drugs used in combination or coincident with the composition disclosed herein, and other factors known in medicine.

[00301] The pharmaceutical composition including the agent(s) disclosed herein may further include a pharmaceutically acceptable carrier. For oral administration, the carrier may include, but is not limited to, a binder, a lubricant, a disintegrant, an excipient, a solubilizer, a dispersing agent, a stabilizer, a suspending agent, a colorant, and a flavorant. For injectable preparations, the carrier may include a buffering agent, a preserving agent, an analgesic, a solubilizer, an isotonic agent, and a stabilizer. For preparations for topical administration, the carrier may include a base, an excipient, a lubricant, and a preserving agent.

[00302] The disclosed compositions may be formulated into a variety of dosage forms in combination with the aforementioned pharmaceutically acceptable carriers. For example, for oral administration, the pharmaceutical composition may be formulated into tablets, troches, capsules, elixirs, suspensions, syrups or wafers. For injectable preparations, the pharmaceutical composition may be formulated into an ampule as a single dosage form or a multidose container. The pharmaceutical composition may also be formulated into solutions, suspensions, tablets, pills, capsules and long-acting preparations.

[00303] On the other hand, examples of the carrier, the excipient, and the diluent suitable for the pharmaceutical formulations include, without limitation, lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate and mineral oils. In addition, the pharmaceutical formulations may further include fillers, anti-coagulating agents, lubricants, humectants, flavorants, and antiseptics.

[00304] Further, the pharmaceutical composition disclosed herein may have any formulation selected from the group consisting of tablets, pills, powders, granules, capsules, suspensions, liquids for internal use, emulsions, syrups, sterile aqueous solutions, non-aqueous solvents, lyophilized formulations and suppositories.

[00305] The composition may be formulated into a single dosage form suitable for the patient's body, and preferably is formulated into a preparation useful for peptide drugs according to the typical method in the pharmaceutical field so as to be administered by an oral or parenteral route such as through skin, intravenous, intramuscular, intra-arterial, intramedullary, intramedullary, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, intracolonic, topical, sublingual, vaginal, or rectal administration, but is not limited thereto.

[00306] The composition may be used by blending with a variety of pharmaceutically acceptable carriers such as physiological saline or organic solvents. In order to increase the stability or absorptivity, carbohydrates such as glucose, sucrose or dextrans, antioxidants such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers may be used.

[00307] The administration dose and frequency of the pharmaceutical composition disclosed herein are determined by the type of active ingredient, together with various factors such as the disease to be treated, administration route, patient's age, gender, and body weight, and disease severity.

[00308] The total effective dose of the compositions disclosed herein may be administered to a patient in a single dose, or may be administered for a long period of time in multiple doses according to a fractionated treatment protocol. In the pharmaceutical composition disclosed herein, the content of active ingredient may vary depending on the disease severity. Preferably, the total daily dose of the peptide disclosed herein may be approximately 0.0001 μg to 500 mg per 1 kg of body weight of a patient. However, the effective dose of the peptide is determined considering various factors including patient's age, body weight, health conditions, gender, disease severity,

diet, and secretion rate, in addition to administration route and treatment frequency of the pharmaceutical composition. In view of this, those skilled in the art may easily determine an effective dose suitable for the particular use of the pharmaceutical composition disclosed herein. The pharmaceutical composition disclosed herein is not particularly limited to the formulation, and administration route and mode, as long as it shows suitable effects.

[00309] Moreover, the pharmaceutical composition may be administered alone or in combination or coincident with other pharmaceutical formulations showing prophylactic or therapeutic efficacy.

[00310] Given the teachings and guidance provided herein, those skilled in the art will understand that a formulation described herein can be equally applicable to many types of biopharmaceuticals, including those exemplified, as well as others known in the art. Given the teachings and guidance provided herein, those skilled in the art also will understand that the selection of, for example, type(s) or and/or amount(s) of one or more excipients, surfactants and/or optional components can be made based on the chemical and functional compatibility with the biopharmaceutical to be formulated and/or the mode of administration as well as other chemical, functional, physiological and/or medical factors well known in the art. For example, non-reducing sugars exhibit favorable excipient properties when used with polypeptide biopharmaceuticals compared to reducing sugars. Accordingly, exemplary formulations are exemplified further herein with reference to polypeptide biopharmaceuticals. However, the range of applicability, chemical and physical properties, considerations and methodology applied to polypeptide biopharmaceutical can be similarly applicable to biopharmaceuticals other than polypeptide biopharmaceuticals.

[00311] Compositions in accordance with embodiments described herein have desirable properties, such as desirable solubility, viscosity, syringeability and stability. Lyophilates in accordance with embodiments described herein have desirable properties, as well, such as desirable recovery, stability and reconstitution.

[00312] In an embodiment, the pH of the pharmaceutical formulation is at least about

3.5, 3.75, 4, 4.25, 4.5, 4.75, 5, 5.25, 5.5, 5.75, 6, 6.25, 6.5, 6.75, 7, 7.25, 7.5, 7.75, 8, 8.25, 8.5, 8.75, or 9.

[00313] In an embodiment, the pH of the pharmaceutical formulation is from about 3 to about 9, about 4 to about 9, about 5 to about 9, about 6 to about 8, about 6 to about 7, about 6 to about 9, about 5 to about 6, about 5 to about 7, about 5 to about 8, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 7 to about 8, about 7 to about 9, about 7 to about 10.

[00314] Dosing can be single dosage or cumulative (serial dosing), and can be readily determined by one skilled in the art. For example, treatment of a senescence-associated disease or disorder can comprise a one-time administration of an effective dose of a pharmaceutical composition disclosed herein. Alternatively, treatment of a senescence-associated disease or disorder may comprise multiple administrations of an effective dose of a pharmaceutical composition carried out over a range of time periods, such as, e.g., once daily, twice daily, trice daily, once every few days, or once weekly. The timing of administration can vary from individual to individual, depending upon such factors as the severity of an individual's symptoms. For example, an effective dose of a pharmaceutical composition disclosed herein can be administered to an individual once daily for an indefinite period of time, or until the individual no longer requires therapy. A person of ordinary skill in the art will recognize that the condition of the individual can be monitored throughout the course of treatment and that the effective amount of a pharmaceutical composition disclosed herein that is administered can be adjusted accordingly.

[00315] In one embodiment, a therapeutic disclosed herein is capable of reducing the signs/symptoms of a senescence-associated disease or disorder by, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% as compared to a patient not receiving the same treatment. In other aspects of this embodiment, a

therapeutic is capable of reducing the number of signs/symptoms of a senescence-associated disease or disorder in an individual by, e.g., about 10% to about 100%, about 20% to about 100%, about 30% to about 100%, about 40% to about 100%, about 50% to about 100%, about 60% to about 100%, about 70% to about 100%, about 80% to about 100%, about 10% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 10% to about 80%, about 20% to about 80%, about 30% to about 80%, about 40% to about 80%, about 50% to about 80%, or about 60% to about 80%, about 10% to about 70%, about 20% to about 70%, about 30% to about 70%, about 40% to about 70%, or about 50% to about 70% as compared to a patient not receiving the same treatment.

[00316] In one embodiment, a therapeutic disclosed herein is capable of reducing signs/symptoms in an individual suffering from a senescence-associated disease or disorder by, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% as compared to a patient not receiving the same treatment. In other aspects of this embodiment, a therapeutic is capable of reducing signs/symptoms in an individual suffering from a senescence-associated disease or disorder by, e.g., about 10% to about 100%, about 20% to about 100%, about 30% to about 100%, about 40% to about 100%, about 50% to about 100%, about 60% to about 100%, about 70% to about 100%, about 80% to about 100%, about 10% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 10% to about 80%, about 20% to about 80%, about 30% to about 80%, about 40% to about 80%, about 50% to about 80%, or about 60% to about 80%, about 10% to about 70%, about 20% to about 70%, about 30% to about 70%, about 40% to about 70%, or about 50% to about 70% as compared to a patient not receiving the same treatment.

[00317] In a further embodiment, a therapeutic and its derivatives have half-lives of 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours,

12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 1 week, 2 weeks, 3 weeks, 4 weeks, one month, two months, three months, four months or more.

[00318] In an embodiment, the period of administration of a therapeutic is for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or more. In a further embodiment, a period of during which administration is stopped is for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or more.

[00319] In aspects of this embodiment, a therapeutically effective amount of a therapeutic disclosed herein reduces signs/symptoms in an individual suffering from a senescence-associated disease or disorder by, *e.g.*, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 100%. In other aspects of this embodiment, a therapeutically effective amount of a therapeutic disclosed herein reduces signs/symptoms by, *e.g.*, at most 10%, at most 15%, at most 20%, at most 25%, at most 30%, at most 35%, at most 40%, at most 45%, at most 50%, at most 55%, at most 60%, at most 65%, at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, at most 95% or at most 100%. In yet other aspects of this embodiment, a therapeutically effective amount of a therapeutic disclosed herein reduces signs/symptoms by, *e.g.*, about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 70%, about 20% to about 60%,

about 20% to about 50%, about 20% to about 40%, about 30% to about 100%, about 30% to about 90%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, or about 30% to about 50%.

[00320] In other aspects, a therapeutically effective amount of a therapeutic disclosed herein reduces the aging process and/or reduces signs of aging in an individual by, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 100%. In other aspects of this embodiment, a therapeutically effective amount of a therapeutic disclosed herein reduces the aging process and/or reduces signs of aging by, e.g., at most 10%, at most 15%, at most 20%, at most 25%, at most 30%, at most 35%, at most 40%, at most 45%, at most 50%, at most 55%, at most 60%, at most 65%, at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, at most 95% or at most 100%. In yet other aspects of this embodiment, a therapeutically effective amount of a therapeutic disclosed herein reduces the aging process and/or reduces signs of aging by, e.g., about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 70%, about 20% to about 60%, about 20% to about 50%, about 20% to about 40%, about 30% to about 100%, about 30% to about 90%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, or about 30% to about 50%.

[00321] Certain embodiments of the present invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the present invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover,

any combination of the above-described embodiments in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[00322] Groupings of alternative embodiments, elements, or steps of the present invention are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other group members disclosed herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[00323] Unless otherwise indicated, all numbers expressing a characteristic, item, quantity, parameter, property, term, and so forth used in the present specification and claims are to be understood as being modified in all instances by the term “about.” As used herein, the term “about” means that the characteristic, item, quantity, parameter, property, or term so qualified encompasses a range of plus or minus ten percent above and below the value of the stated characteristic, item, quantity, parameter, property, or term. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical indication should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and values setting forth the broad scope of the invention are approximations, the numerical ranges and values set forth in the specific examples are reported as precisely as possible. Any numerical range or value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Recitation of numerical ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate numerical value falling within the range. Unless otherwise indicated herein, each individual value of a numerical range is incorporated into the

present specification as if it were individually recited herein.

[00324] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term “consisting of” excludes any element, step, or ingredient not specified in the claims. The transition term “consisting essentially of” limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the present invention so claimed are inherently or expressly described and enabled herein.

[00325] Groupings of alternative embodiments, elements, or steps of the present invention are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other group members disclosed herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[00326] All patents, patent publications, and other publications referenced and identified in the present specification are individually and expressly incorporated herein by reference in their entirety for the purpose of describing and disclosing, for example, the compositions and methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00327] In closing, it is to be understood that although aspects of the present

specification are highlighted by referring to specific embodiments, one skilled in the art will readily appreciate that these disclosed embodiments are only illustrative of the principles of the subject matter disclosed herein. Therefore, it should be understood that the disclosed subject matter is in no way limited to a particular methodology, protocol, and/or reagent, etc., described herein. As such, various modifications or changes to or alternative configurations of the disclosed subject matter can be made in accordance with the teachings herein without departing from the spirit of the present specification. Lastly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Accordingly, the present invention is not limited to that precisely as shown and described.

Table IA - differentially expressed genes from the RNASeq analysis in primary senescent cells (Upregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Senescent (S); Upregulated (U)
1	ENSG00000225101	7.983215822	2.57E-13	S, U
2	ENSG00000096696	7.832738618	6.94E-11	S, U
3	ENSG00000081052	7.679780146	4.18E-11	S, U
4	ENSG00000163017	6.832619824	1.87E-08	S, U
5	ENSG00000120937	6.767987121	2.45E-11	S, U
6	ENSG00000138316	6.575395988	2.06E-20	S, U
7	ENSG00000111186	6.154495726	2.53E-16	S, U
8	ENSG00000188158	6.092784789	5.66E-10	S, U
9	ENSG00000163393	6.023591918	2.46E-08	S, U
10	ENSG00000136542	5.764861838	3.50E-13	S, U
11	ENSG00000213996	5.553771112	1.07E-10	S, U
12	ENSG00000275226	5.21659741	1.43E-09	S, U
13	ENSG00000108821	5.12781011	1.19E-24	S, U
11	ENSG00000261115	5.122327093	5.74E-11	S, U
15	ENSG00000224294	4.910119164	5.79E-21	S, U
16	ENSG00000086289	4.87307691	4.98E-13	S, U
17	ENSG00000103742	4.70247054	5.38E-12	S, U
18	ENSG00000070159	4.693195489	1.15E-10	S, U
19	ENSG00000130294	4.657741491	0.000117366	S, U
20	ENSG00000165124	4.580456946	8.28E-14	S, U
21	ENSG00000137809	4.485011673	4.31E-07	S, U
22	ENSG00000040731	4.452027608	5.60E-39	S, U
23	ENSG00000038427	4.355977284	9.40E-08	S, U
24	ENSG00000120594	4.32409601	0.008088441	S, U
25	ENSG00000137975	4.133592346	1.58E-07	S, U
26	ENSG00000165617	4.095463734	1.37E-08	S, U
27	ENSG00000223511	4.081036058	1.03E-07	S, U

28	ENSG00000187689	4.0426822	0.027414218	S, U
29	ENSG00000143341	4.032553705	4.12E-07	S, U
30	ENSG00000149090	4.024449428	4.73E-06	S, U
31	ENSG00000197614	4.010749555	0.000721434	S, U
32	ENSG00000180914	3.952851634	4.76E-05	S, U
33	ENSG00000169031	3.949660014	1.63E-05	S, U
34	ENSG00000154736	3.904248383	0.000209297	S, U
35	ENSG00000134259	3.877639387	0.013312237	S, U
36	ENSG00000074181	3.778904281	1.04E-17	S, U
37	ENSG00000006606	3.776306172	2.26E-08	S, U
38	ENSG00000172232	3.737536721	9.20E-07	S, U
39	ENSG00000114270	3.662138562	7.54E-23	S, U
40	ENSG00000135454	3.631964609	0.014847166	S, U
41	ENSG00000116183	3.620588501	1.56E-08	S, U
42	ENSG00000129951	3.60540015	1.86E-12	S, U
43	ENSG00000087085	3.579710323	3.73E-21	S, U
44	ENSG00000162490	3.560234145	2.15E-15	S, U
45	ENSG00000251664	3.556220218	0.032753212	S, U
46	ENSG00000261150	3.556069428	4.24E-05	S, U
47	ENSG00000130176	3.550681202	5.01E-12	S, U
48	ENSG00000139044	3.544324703	1.49E-08	S, U
49	ENSG00000261040	3.507826469	5.56E-07	S, U
50	ENSG00000249669	3.493842917	8.13E-09	S, U
51	ENSG00000060718	3.447442441	0.000549404	S, U
52	ENSG00000146674	3.434314778	3.77E-05	S, U
53	ENSG00000100234	3.433883214	0.003773777	S, U
54	ENSG00000233588	3.43325595	0.000668091	S, U
55	ENSG00000072195	3.408935646	1.91E-08	S, U
56	ENSG00000112902	3.373216675	0.001035756	S, U
57	ENSG00000169432	3.348591979	1.72E-05	S, U
58	ENSG00000122870	3.337838428	0.0179422	S, U

59	ENSG00000126545	3.336651871	2.92E-17	S, U
60	ENSG00000166165	3.330900094	7.05E-28	S, U
61	ENSG00000205795	3.316692933	2.08E-07	S, U
62	ENSG00000123989	3.282104203	1.67E-13	S, U
63	ENSG00000196208	3.2542239	1.97E-09	S, U
64	ENSG00000142623	3.244109427	0.000374852	S, U
65	ENSG00000075275	3.21045412	1.26E-05	S, U
66	ENSG00000133110	3.19500917	3.94E-17	S, U
67	ENSG00000158258	3.183575644	1.47E-13	S, U
68	ENSG00000272398	3.135674805	3.69E-07	S, U
69	ENSG00000169116	3.113139309	3.21E-08	S, U
70	ENSG00000167244	3.112831849	1.73E-05	S, U
71	ENSG00000265107	3.110478032	4.15E-18	S, U
72	ENSG00000172828	3.079177394	1.31E-10	S, U
73	ENSG00000077942	3.075436494	0.006922619	S, U
74	ENSG00000258602	3.073888854	0.04877305	S, U
75	ENSG00000128165	3.048841749	4.95E-07	S, U
76	ENSG00000186310	3.037279095	4.23E-07	S, U
77	ENSG00000177098	3.02599902	3.89E-12	S, U
78	ENSG00000025423	3.000319125	4.46E-07	S, U
79	ENSG00000204941	2.993648157	0.00024537	S, U
80	ENSG00000115457	2.971086575	0.000132049	S, U
81	ENSG00000074211	2.96500983	1.06E-13	S, U
82	ENSG00000049540	2.950902857	2.96E-06	S, U
83	ENSG00000206262	2.931018458	0.04062243	S, U
84	ENSG00000065320	2.926669402	1.75E-13	S, U
85	ENSG00000250120	2.924804888	0.000355368	S, U
86	ENSG00000180921	2.918501083	8.83E-48	S, U
87	ENSG00000176945	2.8755067	7.69E-08	S, U
88	ENSG00000105357	2.871591269	5.80E-20	S, U
89	ENSG00000114923	2.866557114	1.72E-07	S, U

90	ENSG00000123384	2.860380559	1.29E-22	S, U
91	ENSG00000116254	2.855551707	3.21E-08	S, U
92	ENSG00000208028	2.849155089	0.015797954	S, U
93	ENSG00000142185	2.819827719	3.27E-05	S, U
94	ENSG00000155760	2.81745588	0.000216684	S, U
95	ENSG00000279508	2.812825835	6.39E-13	S, U
96	ENSG00000182752	2.80284894	0.014025441	S, U
97	ENSG00000168398	2.791695557	5.33E-05	S, U
98	ENSG00000251493	2.784767752	5.07E-07	S, U
99	ENSG00000215218	2.783492797	0.000604614	S, U
100	ENSG00000067840	2.764300854	7.42E-15	S, U
101	ENSG00000178776	2.76198761	3.88E-05	S, U
102	ENSG00000183833	2.753293792	0.000726863	S, U
103	ENSG00000095587	2.695639688	3.40E-08	S, U
104	ENSG00000182326	2.689756589	1.89E-16	S, U
105	ENSG00000183486	2.67750969	5.78E-08	S, U
106	ENSG00000105650	2.67699834	4.03E-09	S, U
107	ENSG00000160282	2.650648701	0.005089858	S, U
108	ENSG00000140839	2.648636674	8.40E-06	S, U
109	ENSG00000112852	2.64632077	2.17E-22	S, U
110	ENSG00000272674	2.644401211	1.02E-16	S, U
111	ENSG00000163431	2.639012045	2.32E-07	S, U
112	ENSG00000172602	2.635509381	2.84E-06	S, U
113	ENSG00000126709	2.628416127	2.34E-12	S, U
111	ENSG00000155980	2.614396871	0.017439396	S, U
115	ENSG00000251320	2.608052058	0.001118453	S, U
116	ENSG00000153823	2.59602856	0.002170041	S, U
117	ENSG00000145362	2.589303896	0.002593318	S, U
118	ENSG00000139970	2.586170664	0.006434682	S, U
119	ENSG00000163235	2.569508992	2.75E-14	S, U
120	ENSG00000197506	2.55556521	4.29E-05	S, U

121	ENSG00000185352	2.549869231	0.026545591	S, U
122	ENSG00000165868	2.540263181	3.13E-06	S, U
123	ENSG00000120322	2.539502609	1.08E-14	S, U
124	ENSG00000221890	2.534399038	9.30E-28	S, U
125	ENSG00000212724	2.526566394	0.000969099	S, U
126	ENSG00000183186	2.509830711	0.000590774	S, U
127	ENSG00000141338	2.501603858	1.10E-07	S, U
128	ENSG00000105088	2.499068065	1.80E-06	S, U
129	ENSG00000120328	2.494071028	8.09E-05	S, U
130	ENSG00000269720	2.490476177	0.001315627	S, U
131	ENSG00000189423	2.468415324	0.000114697	S, U
132	ENSG00000109846	2.466559494	8.36E-12	S, U
133	ENSG00000259570	2.465350659	0.013516932	S, U
134	ENSG00000233098	2.462672046	0.003787051	S, U
135	ENSG00000189337	2.447553205	1.28E-08	S, U
136	ENSG00000250337	2.434336112	7.21E-16	S, U
137	ENSG00000135299	2.433454579	3.90E-10	S, U
138	ENSG00000261114	2.427102763	0.001450907	S, U
139	ENSG00000154898	2.410637544	0.013364621	S, U
140	ENSG00000067141	2.406454221	0.00060365	S, U
141	ENSG00000169851	2.404990377	0.000494324	S, U
142	ENSG00000185885	2.40354684	5.00E-06	S, U
143	ENSG00000204381	2.398601681	3.27E-05	S, U
144	ENSG00000078098	2.397297273	0.021523755	S, U
145	ENSG00000105696	2.393582624	4.26E-19	S, U
146	ENSG00000102683	2.393030504	1.42E-08	S, U
147	ENSG00000115461	2.388665593	0.00014512	S, U
148	ENSG00000165084	2.370033854	0.019402218	S, U
149	ENSG00000151617	2.369117723	8.44E-06	S, U
150	ENSG00000184160	2.363170668	1.74E-07	S, U
151	ENSG00000113209	2.353711037	0.000977147	S, U

152	ENSG00000243089	2.338032826	0.001132733	S, U
153	ENSG00000181804	2.337113656	5.14E-08	S, U
154	ENSG00000177106	2.331430606	1.42E-37	S, U
155	ENSG00000116032	2.326575495	1.25E-08	S, U
156	ENSG00000167641	2.316340928	5.53E-10	S, U
157	ENSG00000105290	2.307511996	4.61E-56	S, U
158	ENSG00000125637	2.300338816	1.93E-06	S, U
159	ENSG00000255431	2.297485195	7.51E-05	S, U
160	ENSG00000113361	2.294818834	3.05E-05	S, U
161	ENSG00000129946	2.291960371	5.87E-08	S, U
162	ENSG00000182983	2.283814175	1.69E-06	S, U
163	ENSG00000108852	2.279857377	3.19E-05	S, U
164	ENSG00000250889	2.27887522	0.006817821	S, U
165	ENSG00000172379	2.276723979	0.002381343	S, U
166	ENSG00000128342	2.266937336	2.77E-06	S, U
167	ENSG00000187372	2.259752078	3.37E-07	S, U
168	ENSG00000272872	2.258892455	1.82E-06	S, U
169	ENSG00000139508	2.255566363	0.022060401	S, U
170	ENSG00000164683	2.245100546	0.031749168	S, U
171	ENSG00000183484	2.241665177	0.006493404	S, U
172	ENSG00000112139	2.241450851	3.79E-15	S, U
173	ENSG00000235385	2.236717795	3.56E-06	S, U
174	ENSG00000161544	2.236212656	0.000394781	S, U
175	ENSG00000156265	2.235320541	4.60E-05	S, U
176	ENSG00000261270	2.233439461	0.001151404	S, U
177	ENSG00000076864	2.228406029	1.97E-09	S, U
178	ENSG00000187957	2.224156124	8.19E-14	S, U
179	ENSG00000205363	2.218550316	7.86E-10	S, U
180	ENSG00000154864	2.201691085	0.000148299	S, U
181	ENSG00000135519	2.195925765	1.08E-05	S, U
182	ENSG00000176928	2.19592259	0.003523232	S, U

183	ENSG00000267095	2.195163374	1.54E-06	S, U
184	ENSG00000113248	2.191291668	1.80E-11	S, U
185	ENSG00000128655	2.184019326	1.12E-14	S, U
186	ENSG00000139971	2.171499501	5.74E-08	S, U
187	ENSG00000176595	2.168156909	0.046416621	S, U
188	ENSG00000106976	2.164704554	2.17E-11	S, U
189	ENSG00000196358	2.164517992	1.38E-09	S, U
190	ENSG00000134198	2.164308011	0.000198184	S, U
191	ENSG00000115232	2.163408225	0.00121359	S, U
192	ENSG00000171790	2.159081523	0.000102126	S, U
193	ENSG00000236841	2.158399864	0.046904634	S, U
194	ENSG00000227496	2.143866016	0.008303558	S, U
195	ENSG00000134326	2.142408736	0.000164602	S, U
196	ENSG00000188452	2.134374977	7.74E-07	S, U
197	ENSG00000120327	2.130731231	6.46E-08	S, U
198	ENSG00000140450	2.129337923	1.35E-11	S, U
199	ENSG00000197479	2.113947232	0.00067338	S, U
200	ENSG00000106799	2.109225797	6.39E-05	S, U
201	ENSG00000268894	2.107565877	0.000109342	S, U
202	ENSG00000151320	2.107099678	0.000533297	S, U
203	ENSG00000244056	2.104095158	0.027173569	S, U
204	ENSG00000188112	2.100825247	3.84E-08	S, U
205	ENSG00000113389	2.100346777	1.50E-06	S, U
206	ENSG00000092068	2.098012736	2.02E-08	S, U
207	ENSG00000137959	2.091010451	3.87E-08	S, U
208	ENSG00000170390	2.090453825	2.57E-11	S, U
209	ENSG00000254245	2.090403193	0.001044706	S, U
210	ENSG00000152580	2.090289252	0.000422116	S, U
211	ENSG00000095752	2.083501888	5.54E-05	S, U
212	ENSG00000152463	2.079732481	5.23E-10	S, U
213	ENSG00000230838	2.076269123	0.000355514	S, U

214	ENSG00000116701	2.074224926	0.000786396	S, U
215	ENSG00000107147	2.071830763	9.44E-06	S, U
216	ENSG00000134539	2.069847739	1.71E-05	S, U
217	ENSG00000135324	2.067917036	0.024117094	S, U
218	ENSG00000113721	2.067328363	1.83E-06	S, U
219	ENSG00000188613	2.05730901	3.53E-05	S, U
220	ENSG00000151090	2.057041945	0.001009257	S, U
221	ENSG00000176293	2.047474817	2.11E-09	S, U
222	ENSG00000178184	2.046896756	1.84E-51	S, U
223	ENSG00000135916	2.044665027	3.78E-07	S, U
224	ENSG00000145936	2.036269799	0.011993528	S, U
225	ENSG00000134321	2.035453273	5.61E-05	S, U
226	ENSG00000182545	2.02946207	0.013049961	S, U
227	ENSG00000173110	2.026585824	6.94E-07	S, U
228	ENSG00000279838	2.016955848	2.68E-05	S, U
229	ENSG00000088881	2.009833206	5.33E-10	S, U
230	ENSG00000277999	2.001814705	0.005056681	S, U
231	ENSG00000157601	2.000728282	1.89E-05	S, U
232	ENSG00000142173	2.000526748	1.01E-11	S, U
233	ENSG00000133424	2.000020137	5.80E-05	S, U

Table IB - differentially expressed genes from the RNASeq analysis in primary senescent cells (Downregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Senescent (S); Downregulated (D)
1	ENSG00000121211	-2.003283346	5.02E-06	S, D
2	ENSG00000170011	-2.004128208	0.000547744	S, D
3	ENSG00000168309	-2.007050701	0.000464521	S, D
4	ENSG00000113810	-2.011255861	1.90E-32	S, D
5	ENSG00000100479	-2.03564691	4.76E-11	S, D
6	ENSG00000087586	-2.051522585	6.37E-16	S, D
D	ENSG00000187951	-2.054099546	5.53E-44	S, D

8	ENSG00000105851	-2.055550842	1.28E-06	S, D
9	ENSG00000122952	-2.059578062	7.11E-25	S, U
10	ENSG00000127951	-2.059700649	2.14E-05	S, D
11	ENSG00000111788	-2.060705539	1.02E-05	S, D
12	ENSG00000170160	-2.063479396	0.019234315	S, D
13	ENSG00000229156	-2.074060038	0.013979875	S, D
11	ENSG00000256663	-2.077152761	3.56E-09	S, D
15	ENSG00000151725	-2.080104045	2.48E-13	S, D
16	ENSG00000178175	-2.083455245	0.01470074	S, D
17	ENSG00000144063	-2.086428161	0.011983636	S, D
18	ENSG00000101187	-2.086509888	7.21E-06	S, D
19	ENSG00000112029	-2.09026581	1.39E-29	S, D
20	ENSG00000164104	-2.118327575	1.98E-12	S, D
21	ENSG00000153044	-2.136402484	3.21E-15	S, D
22	ENSG00000136982	-2.152661762	6.47E-08	S, D
23	ENSG00000105825	-2.170736544	0.017440887	S, D
24	ENSG00000185432	-2.181078804	0.005292839	S, D
25	ENSG00000231389	-2.213290158	0.035431277	S, D
26	ENSG00000065618	-2.21889102	1.61E-12	S, D
27	ENSG00000135476	-2.237430895	1.93E-18	S, D
28	ENSG00000008118	-2.243432571	0.024640299	S, D
29	ENSG00000161800	-2.257371671	6.81E-46	S, D
30	ENSG00000137310	-2.258767762	6.56E-15	S, D
31	ENSG00000071539	-2.295301276	1.24E-29	S, D
32	ENSG00000164611	-2.298705222	2.41E-13	S, D
33	ENSG00000111247	-2.301031803	3.63E-13	S, D
34	ENSG00000255282	-2.303383451	0.0201527	S, D
35	ENSG00000197632	-2.30519656	0.006524439	S, D
36	ENSG00000197565	-2.313962542	2.81E-08	S, D
37	ENSG00000146678	-2.320926304	3.51E-12	S, D
38	ENSG00000121621	-2.324588758	1.44E-40	S, D

39	ENSG00000127564	-2.336311591	2.37E-05	S, D
40	ENSG00000075702	-2.367302449	5.13E-11	S, D
41	ENSG00000126878	-2.379933873	2.45E-05	S, D
42	ENSG00000146670	-2.381987665	2.29E-08	S, D
43	ENSG00000257167	-2.390147158	6.02E-08	S, D
44	ENSG00000134222	-2.408806731	3.63E-19	S, D
45	ENSG00000164109	-2.424011542	8.20E-24	S, D
46	ENSG00000124440	-2.43283599	0.008941785	S, D
47	ENSG00000093009	-2.438805577	6.66E-08	S, D
48	ENSG00000076382	-2.462211632	2.37E-26	S, D
49	ENSG00000118193	-2.464099308	1.51E-35	S, D
50	ENSG00000140379	-2.468524425	2.25E-09	S, D
51	ENSG00000134057	-2.469239608	1.70E-20	S, D
52	ENSG00000216775	-2.471413102	1.10E-05	S, D
53	ENSG00000139618	-2.474652489	4.04E-22	S, D
54	ENSG00000203995	-2.591347865	0.001050361	S, D
55	ENSG00000198826	-2.602634674	9.60E-123	S, D
56	ENSG00000088325	-2.610437564	2.81E-32	S, D
57	ENSG00000133101	-2.64061379	4.44E-05	S, D
58	ENSG00000144554	-2.684125641	1.04E-42	S, D
59	ENSG00000140451	-2.687105379	0.000536235	S, D
60	ENSG00000156509	-2.690866642	5.13E-06	S, D
61	ENSG00000122966	-2.701435486	2.10E-32	S, D
62	ENSG00000085999	-2.713092547	3.34E-11	S, D
63	ENSG00000167600	-2.716643396	2.20E-05	S, D
64	ENSG00000145386	-2.727605846	2.72E-137	S, D
65	ENSG00000174371	-2.739512801	4.12E-17	S, D
66	ENSG00000138182	-2.743141157	4.95E-58	S, D
67	ENSG00000104147	-2.786565564	1.01E-11	S, D
68	ENSG00000100162	-2.827387893	7.53E-07	S, D
69	ENSG00000068489	-2.865569384	3.11E-101	S, D

70	ENSG00000158402	-2.869154843	6.36E-26	S, D
71	ENSG00000161888	-2.875553289	1.45E-08	S, D
72	ENSG00000113368	-2.886635077	5.80E-24	S, D
73	ENSG00000105011	-2.914754215	1.29E-10	S, D
74	ENSG00000143476	-2.920961166	6.89E-07	S, D
75	ENSG00000189057	-2.958699272	1.65E-09	S, D
76	ENSG0000012223	-2.959258821	3.87E-06	S, D
77	ENSG00000157456	-2.966359586	1.43E-36	S, D
78	ENSG00000112297	-2.98628471	1.49E-05	S, D
79	ENSG00000186479	-3.006459562	0.004123319	S, D
80	ENSG00000134690	-3.026072571	2.38E-31	S, D
81	ENSG00000171320	-3.058406607	2.26E-08	S, D
82	ENSG00000163808	-3.089301996	1.51E-33	S, D
83	ENSG00000138160	-3.099296236	2.43E-85	S, D
84	ENSG00000166851	-3.103789895	7.09E-53	S, D
85	ENSG00000123080	-3.108377163	6.56E-10	S, D
86	ENSG00000166803	-3.116812173	2.71E-07	S, D
87	ENSG00000142731	-3.141974414	2.38E-37	S, D
88	ENSG00000186871	-3.143580637	1.54E-16	S, D
89	ENSG00000051341	-3.149709685	9.40E-18	S, D
90	ENSG00000022556	-3.190027895	0.007128613	S, D
91	ENSG00000265415	-3.227682433	1.82E-30	S, D
92	ENSG00000237649	-3.239234359	1.25E-20	S, D
93	ENSG00000123485	-3.272064038	4.88E-14	S, D
94	ENSG00000007968	-3.27672421	1.48E-10	S, D
95	ENSG00000231007	-3.291773803	8.24E-08	S, D
96	ENSG00000184661	-3.30651502	5.86E-14	S, D
97	ENSG00000170312	-3.317830937	6.18E-10	S, D
98	ENSG00000198901	-3.343017977	1.64E-45	S, D
99	ENSG00000080986	-3.350928959	6.35E-08	S, D
100	ENSG00000129173	-3.35970608	7.19E-22	S, D

101	ENSG00000092853	-3.380526464	4.52E-28	S, D
102	ENSG00000144354	-3.397098459	5.35E-43	S, D
103	ENSG00000171241	-3.429689854	1.22E-21	S, D
104	ENSG00000109805	-3.43886654	1.37E-21	S, D
105	ENSG00000163814	-3.443854276	5.19E-19	S, D
106	ENSG00000101447	-3.468617906	1.35E-36	S, D
107	ENSG00000075218	-3.479920565	7.72E-27	S, D
108	ENSG00000035499	-3.513023729	3.99E-42	S, D
109	ENSG00000167900	-3.567242338	2.41E-13	S, D
110	ENSG00000175063	-3.586912925	1.02E-07	S, D
111	ENSG00000140873	-3.588883858	3.78E-06	S, D
112	ENSG00000178999	-3.609277998	4.15E-10	S, D
113	ENSG00000065328	-3.613994443	1.86E-12	S, D
111	ENSG00000011426	-3.667204305	1.48E-54	S, D
115	ENSG00000198417	-3.688785679	4.14E-06	S, D
116	ENSG00000112742	-3.716799164	4.38E-17	S, D
117	ENSG00000186193	-3.730608445	1.75E-69	S, D
118	ENSG00000230006	-3.73328427	6.90E-06	S, D
119	ENSG00000101188	-3.744472844	2.71E-08	S, D
120	ENSG00000137804	-3.762360248	4.46E-42	S, D
121	ENSG00000109674	-3.775202004	1.70E-13	S, D
122	ENSG00000121152	-3.783490112	2.06E-25	S, D
123	ENSG00000171848	-3.789751219	5.07E-17	S, D
124	ENSG00000072571	-3.799100893	1.54E-30	S, D
125	ENSG00000165480	-3.812480956	1.65E-53	S, D
126	ENSG00000137812	-3.816948315	2.01E-78	S, D
127	ENSG00000117724	-3.828516905	6.67E-71	S, D
128	ENSG00000111206	-3.8286754	7.97E-61	S, D
129	ENSG00000100526	-3.85564143	4.21E-23	S, D
130	ENSG00000101057	-3.875861549	1.20E-18	S, D
131	ENSG00000143228	-3.877197934	1.16E-25	S, D

132	ENSG00000152253	-3.916767139	6.23E-12	S, D
133	ENSG00000024526	-3.93037481	7.44E-37	S, D
134	ENSG00000183856	-3.940208704	1.02E-35	S, D
135	ENSG00000142945	-3.958752486	1.66E-26	S, D
136	ENSG00000156970	-3.964019103	1.28E-26	S, D
137	ENSG00000129810	-3.972981923	7.39E-26	S, D
138	ENSG00000138180	-4.019090264	3.81E-28	S, D
139	ENSG00000115163	-4.021528917	5.28E-15	S, D
140	ENSG00000169607	-4.025178525	3.53E-53	S, D
141	ENSG00000106511	-4.035737622	0.001275396	S, D
142	ENSG00000169679	-4.054512617	4.43E-33	S, D
143	ENSG00000089685	-4.094279662	4.23E-16	S, D
144	ENSG00000179750	-4.119273036	8.58E-17	S, D
145	ENSG00000129195	-4.159113716	1.56E-24	S, D
146	ENSG00000168078	-4.182276804	2.01E-15	S, D
147	ENSG00000154839	-4.212658533	1.77E-29	S, D
148	ENSG00000090889	-4.249062724	8.50E-23	S, D
149	ENSG00000186185	-4.294179991	3.56E-15	S, D
150	ENSG00000135451	-4.340887982	2.27E-21	S, D
151	ENSG00000126787	-4.353161477	2.15E-21	S, D
152	ENSG00000117399	-4.386725435	2.43E-20	S, D
153	ENSG00000131747	-4.393777149	3.43E-27	S, D
154	ENSG00000066279	-4.60309528	1.84E-64	S, D
155	ENSG00000117650	-4.626524476	2.82E-31	S, D
156	ENSG00000112984	-4.73381687	2.12E-47	S, D
157	ENSG00000148773	-4.847761091	2.82E-31	S, D
158	ENSG00000125144	-6.342722431	7.96E-07	S, D

Table IC - differentially expressed genes from the RNASeq analysis in secondary senescent cells (Upregulated in Secondary Senescent Cells)

ID	log2 Fold Change	p-value adjusted	Secondary (SS); Upregulated (U)
----	------------------	------------------	------------------------------------

1	ENSG00000276241	7.239894142	0.000149988	SS, U
2	ENSG00000163393	5.966079901	0.000160714	SS, U
3	ENSG00000111186	5.212924352	7.91E-06	SS, U
4	ENSG00000116183	4.976855148	7.40E-13	SS, U
5	ENSG00000272398	4.701401021	1.97E-07	SS, U
6	ENSG00000188158	4.655082609	0.0116065	SS, U
7	ENSG00000261115	4.520783347	1.06E-08	SS, U
8	ENSG00000277586	4.367201617	0.000824688	SS, U
9	ENSG00000130294	4.357092531	0.0065649	SS, U
10	ENSG00000224294	4.320966733	0.000324888	SS, U
11	ENSG00000070159	4.236641804	0.002039616	SS, U
12	ENSG00000163531	4.137522585	0.00199312	SS, U
13	ENSG00000165124	3.918658268	0.000136543	SS, U
11	ENSG00000223511	3.805706964	0.001808312	SS, U
15	ENSG00000138316	3.656761068	5.89E-08	SS, U
16	ENSG00000163898	3.621949022	6.16E-05	SS, U
17	ENSG00000096696	3.413528355	0.004826994	SS, U
18	ENSG00000154736	3.313919541	0.013814354	SS, U
19	ENSG00000254275	3.290369302	0.000830303	SS, U
20	ENSG00000168280	3.252321227	0.002210493	SS, U
21	ENSG00000204941	3.174640511	0.003726418	SS, U
22	ENSG00000126545	3.124197503	1.37E-17	SS, U
23	ENSG00000115461	3.090533989	0.000229366	SS, U
24	ENSG00000158258	3.088956864	0.004246081	SS, U
25	ENSG00000145362	2.952527731	0.000943023	SS, U
26	ENSG00000251493	2.936470763	0.003150031	SS, U
27	ENSG00000087085	2.882902113	0.003150031	SS, U
28	ENSG00000182175	2.867987127	0.046472379	SS, U
29	ENSG00000100234	2.826451139	0.020832174	SS, U
30	ENSG00000225101	2.649455793	0.029336413	SS, U
31	ENSG00000128165	2.647021347	1.87E-10	SS, U

32	ENSG00000235385	2.626807699	1.75E-08	SS, U
33	ENSG00000074181	2.590426577	0.001886444	SS, U
34	ENSG00000164796	2.536822076	0.038868925	SS, U
35	ENSG00000279484	2.475896909	0.000735182	SS, U
36	ENSG00000115457	2.468292477	0.017105949	SS, U
37	ENSG00000169432	2.453664299	0.001886444	SS, U
38	ENSG00000067840	2.421525904	0.002861097	SS, U
39	ENSG00000067141	2.396585268	0.016003244	SS, U
40	ENSG00000183486	2.335993015	0.000164804	SS, U
41	ENSG00000123989	2.267585574	0.018686104	SS, U
42	ENSG00000136999	2.266308404	0.001776461	SS, U
43	ENSG00000038427	2.217945624	0.01969763	SS, U
44	ENSG00000151617	2.157897673	0.014991904	SS, U
45	ENSG00000166165	2.145045328	0.002654075	SS, U
46	ENSG00000186310	2.143261974	0.018453628	SS, U
47	ENSG00000074211	2.099750757	0.001912486	SS, U
48	ENSG00000180914	2.0865968	0.045734228	SS, U
49	ENSG00000139200	2.083179711	0.013602546	SS, U
50	ENSG00000187957	2.068062279	0.000160714	SS, U
51	ENSG00000221890	2.054223175	0.000398103	SS, U
52	ENSG00000165617	2.048273584	0.045348021	SS, U
53	ENSG00000148798	2.026692744	0.016510685	SS, U
54	ENSG00000126709	2.019714373	7.68E-07	SS, U
55	ENSG00000165868	1.996244607	0.010864654	SS, U
56	ENSG00000072195	1.990120412	0.012592407	SS, U
57	ENSG00000137642	1.983284858	0.039501971	SS, U
58	ENSG00000242242	1.961310321	0.032245402	SS, U
59	ENSG00000272674	1.913634019	0.008217588	SS, U
60	ENSG00000146674	1.899439323	0.049484705	SS, U
61	ENSG00000232855	1.897199617	0.04643321	SS, U
62	ENSG00000105696	1.880851405	0.005155003	SS, U

63	ENSG00000134321	1.858619925	0.010039264	SS, U
64	ENSG00000198910	1.852885568	0.007634351	SS, U
65	ENSG00000185885	1.851482854	0.00031785	SS, U
66	ENSG00000229981	1.848711679	5.87E-08	SS, U
67	ENSG00000182585	1.840917357	0.02079468	SS, U
68	ENSG00000142173	1.824748574	4.71E-08	SS, U
69	ENSG00000108679	1.795694422	0.013359955	SS, U
70	ENSG00000137959	1.781619427	0.000126457	SS, U
71	ENSG00000171224	1.773858329	0.00252997	SS, U
72	ENSG00000206532	1.74689823	0.018909193	SS, U
73	ENSG00000185745	1.735600755	0.000763703	SS, U
74	ENSG00000197506	1.722167543	0.027662881	SS, U
75	ENSG00000269720	1.716085539	0.034890836	SS, U
76	ENSG00000171533	1.715838802	0.002611815	SS, U
77	ENSG00000163347	1.713680625	9.18E-08	SS, U
78	ENSG00000186469	1.702615692	0.002144454	SS, U
79	ENSG00000182326	1.698638091	2.87E-06	SS, U
80	ENSG00000185633	1.688465631	0.002279995	SS, U
81	ENSG00000227517	1.684163034	0.017176334	SS, U
82	ENSG00000132530	1.683990828	0.00504928	SS, U
83	ENSG00000135253	1.678446959	0.013782417	SS, U
84	ENSG00000151090	1.667986015	0.032891873	SS, U
85	ENSG00000123384	1.66096483	0.000114118	SS, U
86	ENSG00000138271	1.657535437	0.008516265	SS, U
87	ENSG00000134326	1.647764886	0.033216222	SS, U
88	ENSG00000152463	1.641059769	1.74E-05	SS, U
89	ENSG00000177106	1.628333984	0.002807137	SS, U
90	ENSG00000198797	1.623495245	0.036456898	SS, U
91	ENSG00000108852	1.622100212	0.042343969	SS, U
92	ENSG00000196517	1.620162766	6.39E-05	SS, U
93	ENSG00000170909	1.611577526	0.001114429	SS, U

94	ENSG00000180921	1.610471446	0.017773389	SS, U
95	ENSG00000135842	1.607021529	0.004047019	SS, U
96	ENSG00000180332	1.601430296	0.008156009	SS, U
97	ENSG00000143341	1.595382887	0.048334561	SS, U
98	ENSG00000238228	1.573472921	1.47E-05	SS, U
99	ENSG00000119917	1.559164705	0.010646332	SS, U
100	ENSG00000135916	1.55338763	0.041207788	SS, U
101	ENSG00000196358	1.550100745	0.00454834	SS, U
102	ENSG00000174672	1.540057088	0.035310915	SS, U
103	ENSG00000133110	1.51395137	9.66E-31	SS, U
104	ENSG00000188112	1.501702041	0.000991752	SS, U
105	ENSG00000171658	1.494403943	0.002651066	SS, U
106	ENSG00000105357	1.482492139	0.020694938	SS, U
107	ENSG00000157601	1.480119078	0.004810197	SS, U
108	ENSG00000100167	1.475072024	0.041437827	SS, U
109	ENSG00000171951	1.462109637	0.000647458	SS, U
110	ENSG00000111335	1.460733225	0.001525956	SS, U
111	ENSG00000128655	1.45028397	0.000735182	SS, U
112	ENSG00000166016	1.444936287	8.56E-06	SS, U
113	ENSG00000186868	1.44470454	0.037615931	SS, U
111	ENSG00000109472	1.433987861	0.045348021	SS, U
115	ENSG00000157680	1.424954071	0.00430321	SS, U
116	ENSG00000258791	1.424321101	0.016422955	SS, U
117	ENSG00000156042	1.412488682	0.000741249	SS, U
118	ENSG00000243415	1.410673179	0.028441685	SS, U
119	ENSG00000135114	1.409840804	0.013782754	SS, U
120	ENSG00000108821	1.408084403	0.000606124	SS, U
121	ENSG00000268225	1.39967392	0.001416747	SS, U
122	ENSG00000089127	1.398093787	0.010646332	SS, U
123	ENSG00000206337	1.382451715	0.003185926	SS, U
124	ENSG00000270011	1.380041436	0.002319116	SS, U

125	ENSG00000143786	1.366787604	0.004847779	SS, U
126	ENSG00000070669	1.363903502	4.75E-10	SS, U
127	ENSG00000250697	1.363614254	0.001208245	SS, U
128	ENSG00000167995	1.358644296	0.00619095	SS, U
129	ENSG00000100321	1.354046078	1.84E-10	SS, U
130	ENSG00000283265	1.352552671	0.045920532	SS, U
131	ENSG00000124721	1.347330007	4.65E-08	SS, U
132	ENSG00000112852	1.345322984	0.007441888	SS, U
133	ENSG00000163431	1.344841918	0.00214727	SS, U
134	ENSG00000237596	1.343633516	0.024700448	SS, U
135	ENSG00000111331	1.336220708	0.00165518	SS, U
136	ENSG00000183570	1.321955312	0.017394137	SS, U
137	ENSG00000107147	1.315414146	0.003011309	SS, U
138	ENSG00000188452	1.315090925	0.043946838	SS, U
139	ENSG00000105613	1.313807819	0.027314965	SS, U
140	ENSG00000130176	1.311934923	0.032621571	SS, U
141	ENSG00000170954	1.307438728	2.35E-05	SS, U
142	ENSG00000140450	1.299026952	0.025522756	SS, U
143	ENSG00000178623	1.298656537	0.006500089	SS, U
144	ENSG00000139364	1.292680139	0.012731879	SS, U
145	ENSG00000187372	1.286815419	0.024224939	SS, U
146	ENSG00000133687	1.281059141	5.63E-10	SS, U
147	ENSG00000138646	1.276823448	0.031370198	SS, U
148	ENSG00000225963	1.273845325	0.038823191	SS, U
149	ENSG00000163888	1.273051813	0.02303783	SS, U
150	ENSG00000171914	1.267388849	0.044937755	SS, U
151	ENSG00000040731	1.264638326	0.01905033	SS, U
152	ENSG00000183671	1.259868083	7.53E-05	SS, U
153	ENSG00000240476	1.251110774	0.011344666	SS, U
154	ENSG00000143369	1.250729643	0.040108135	SS, U
155	ENSG00000178184	1.240841765	0.042891525	SS, U

156	ENSG00000273295	1.240438494	0.018023105	SS, U
157	ENSG00000265972	1.237888387	0.00982143	SS, U
158	ENSG00000240891	1.23024394	0.032398782	SS, U
159	ENSG00000189337	1.227802932	0.019205345	SS, U
160	ENSG00000129946	1.227103363	0.02663838	SS, U
161	ENSG00000130303	1.224769891	0.005037958	SS, U
162	ENSG00000276523	1.220678632	0.023281294	SS, U
163	ENSG00000030419	1.205405929	0.012010975	SS, U
164	ENSG00000135919	1.200828785	0.000607546	SS, U
165	ENSG00000106976	1.187735337	0.030729292	SS, U
166	ENSG00000145777	1.182197745	0.000146465	SS, U
167	ENSG00000168477	1.169655256	0.022112153	SS, U
168	ENSG00000132429	1.163623471	0.002719672	SS, U
169	ENSG00000279700	1.163419357	0.000107231	SS, U
170	ENSG00000261644	1.160868063	0.049173282	SS, U
171	ENSG00000267577	1.160120917	0.010579779	SS, U
172	ENSG00000213244	1.157946252	0.04643321	SS, U
173	ENSG00000231890	1.15681377	0.030773936	SS, U
174	ENSG00000152503	1.142126446	0.045734228	SS, U
175	ENSG00000138642	1.139691645	0.00269636	SS, U
176	ENSG00000163013	1.13537673	0.031343618	SS, U
177	ENSG00000278202	1.13113644	0.01605249	SS, U
178	ENSG00000161040	1.126302321	0.002628522	SS, U
179	ENSG00000281207	1.115562592	0.036225437	SS, U
180	ENSG00000205363	1.107882073	0.044360949	SS, U
181	ENSG00000063660	1.107281327	0.009153923	SS, U
182	ENSG00000160469	1.10437023	0.0096032	SS, U
183	ENSG00000138356	1.099842201	0.033625732	SS, U
184	ENSG00000165949	1.099410553	1.96E-06	SS, U
185	ENSG00000157111	1.098217653	4.39E-05	SS, U
186	ENSG00000175040	1.097365757	0.034166385	SS, U

187	ENSG00000243753	1.087617254	0.017933761	SS, U
188	ENSG00000119514	1.087565125	1.90E-06	SS, U
189	ENSG00000175600	1.082937063	0.003257213	SS, U
190	ENSG00000152154	1.079236848	0.045768448	SS, U
191	ENSG00000187608	1.074964138	6.19E-05	SS, U
192	ENSG00000231672	1.074653942	0.046278866	SS, U
193	ENSG00000048052	1.068331313	0.011215281	SS, U
194	ENSG00000115267	1.057259606	0.001657761	SS, U
195	ENSG00000084710	1.055884039	0.001206107	SS, U
196	ENSG00000162433	1.052688015	0.00018934	SS, U
197	ENSG00000121753	1.04734347	0.001158404	SS, U
198	ENSG00000112139	1.035926598	2.07E-07	SS, U
199	ENSG00000197599	1.034322498	0.042936121	SS, U
200	ENSG00000234745	1.018604308	0.001402934	SS, U
201	ENSG00000214184	1.013837131	0.04152524	SS, U
202	ENSG00000204642	1.012616655	0.03633716	SS, U

Table ID - differentially expressed genes from the RNASeq analysis in secondary senescent cells (Downregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Secondary (SS); Downregulated (D)
1	ENSG00000107821	-1.005101971	0.011551382	SS, D
2	ENSG00000076382	-1.005587899	0.014693005	SS, D
3	ENSG00000071539	-1.009616595	0.009394655	SS, D
4	ENSG00000101003	-1.010575775	0.010039264	SS, D
5	ENSG00000135476	-1.014719025	0.029004865	SS, D
6	ENSG00000109084	-1.021418343	0.000148175	SS, D
7	ENSG00000119969	-1.024093893	0.00964203	SS, D
8	ENSG00000017483	-1.026248463	5.52E-14	SS, D
9	ENSG00000203760	-1.031851778	0.000802042	SS, D
10	ENSG00000137310	-1.03235569	0.018503572	SS, D
11	ENSG00000121621	-1.03797972	0.00583773	SS, D

12	ENSG00000051180	-1.038129886	0.010545128	SS, D
13	ENSG00000088325	-1.040383613	0.042658858	SS, D
11	ENSG00000092470	-1.04330824	0.006407264	SS, D
15	ENSG00000214826	-1.045259204	0.001507003	SS, D
16	ENSG00000164611	-1.04687937	0.00612745	SS, D
17	ENSG00000260549	-1.047337331	0.042763526	SS, D
18	ENSG00000183971	-1.049998798	0.007735123	SS, D
19	ENSG00000130300	-1.069540468	5.95E-05	SS, D
20	ENSG00000139618	-1.075529769	0.042681003	SS, D
21	ENSG00000144554	-1.083561931	0.006993011	SS, D
22	ENSG00000140379	-1.089254218	0.018503572	SS, D
23	ENSG00000176974	-1.097270786	0.002523726	SS, D
24	ENSG00000234779	-1.099662682	0.00049575	SS, D
25	ENSG00000164109	-1.101225431	0.004810197	SS, D
26	ENSG00000138160	-1.101375001	0.010709796	SS, D
27	ENSG00000198826	-1.10532157	0.011551382	SS, D
28	ENSG00000164104	-1.108663434	0.01508202	SS, D
29	ENSG00000130433	-1.115500663	0.026927824	SS, D
30	ENSG00000085840	-1.117269343	0.018795334	SS, D
31	ENSG00000151725	-1.126769124	0.001507003	SS, D
32	ENSG00000165244	-1.129330848	0.005155003	SS, D
33	ENSG00000165152	-1.131971017	0.031084702	SS, D
34	ENSG00000104147	-1.145246929	0.031672722	SS, D
35	ENSG00000107485	-1.146162121	0.000219355	SS, D
36	ENSG00000131153	-1.154852144	0.002628522	SS, D
37	ENSG00000146670	-1.155360442	0.034920021	SS, D
38	ENSG00000145386	-1.168086015	0.003146673	SS, D
39	ENSG00000186603	-1.174771513	0.024618793	SS, D
40	ENSG00000100479	-1.193845257	0.001873445	SS, D
41	ENSG00000117318	-1.198498676	0.000163607	SS, D
42	ENSG00000166851	-1.20197128	0.020170014	SS, D

43	ENSG00000167513	-1.208334371	0.015744804	SS, D
44	ENSG00000134690	-1.212724681	0.029540764	SS, D
45	ENSG00000182492	-1.216062824	0.000381675	SS, D
46	ENSG00000134222	-1.220528333	0.024502596	SS, D
47	ENSG00000075702	-1.221584288	0.04581995	SS, D
48	ENSG00000069482	-1.224554764	0.001026193	SS, D
49	ENSG00000257815	-1.230214221	0.000298261	SS, D
50	ENSG00000128016	-1.254298785	0.020693546	SS, D
51	ENSG00000280123	-1.272042736	0.001095954	SS, D
52	ENSG00000117724	-1.279201658	0.026867387	SS, D
53	ENSG00000035499	-1.280472364	0.009772541	SS, D
54	ENSG00000137812	-1.28060811	0.009445081	SS, D
55	ENSG00000106484	-1.283326913	0.00127854	SS, D
56	ENSG00000184260	-1.295080463	0.026003667	SS, D
57	ENSG00000163808	-1.305728339	0.031084702	SS, D
58	ENSG00000142731	-1.307646414	0.002651066	SS, D
59	ENSG00000273264	-1.318591424	0.008946062	SS, D
60	ENSG00000092853	-1.331211519	0.003237044	SS, D
61	ENSG00000158402	-1.33499196	0.034676069	SS, D
62	ENSG00000171320	-1.336402988	0.035091047	SS, D
63	ENSG00000075218	-1.346699422	0.022052564	SS, D
64	ENSG00000093009	-1.347631865	0.011824125	SS, D
65	ENSG00000105011	-1.348779371	0.03967606	SS, D
66	ENSG00000154027	-1.350412919	0.023316369	SS, D
67	ENSG00000165490	-1.351297056	2.55E-07	SS, D
68	ENSG00000213853	-1.355936736	0.008486816	SS, D
69	ENSG00000186871	-1.356923826	0.009091328	SS, D
70	ENSG00000142945	-1.370627303	0.043394558	SS, D
71	ENSG00000237649	-1.384331913	0.02894621	SS, D
72	ENSG00000143476	-1.392008921	0.031343618	SS, D
73	ENSG00000123080	-1.400342504	0.004598006	SS, D

74	ENSG00000113368	-1.405219427	0.000240213	SS, D
75	ENSG00000136982	-1.411605647	0.000118204	SS, D
76	ENSG00000165480	-1.416022519	0.013766291	SS, D
77	ENSG00000169607	-1.430228627	0.013993577	SS, D
78	ENSG00000143228	-1.432561754	0.032501975	SS, D
79	ENSG00000184661	-1.440193444	0.01412573	SS, D
80	ENSG00000205426	-1.449228784	0.026487509	SS, D
81	ENSG00000257167	-1.451863563	0.017498898	SS, D
82	ENSG00000126878	-1.482762013	0.007360891	SS, D
83	ENSG00000127564	-1.505467857	0.002527472	SS, D
84	ENSG00000179428	-1.505874974	0.03441072	SS, D
85	ENSG00000161888	-1.509772244	0.017773389	SS, D
86	ENSG00000258943	-1.523417692	0.047457819	SS, D
87	ENSG00000140511	-1.523630242	0.009720762	SS, D
88	ENSG00000189057	-1.529840027	0.016121182	SS, D
89	ENSG00000186193	-1.530769032	0.001210351	SS, D
90	ENSG00000073756	-1.541271521	0.00056983	SS, D
91	ENSG00000100162	-1.545337501	0.017105949	SS, D
92	ENSG00000144354	-1.563312746	0.000639738	SS, D
93	ENSG00000109674	-1.566368381	0.018676542	SS, D
94	ENSG00000174371	-1.57721593	0.001519641	SS, D
95	ENSG00000154839	-1.585241912	0.026415087	SS, D
96	ENSG00000114315	-1.592019171	0.022177486	SS, D
97	ENSG00000166803	-1.603800437	0.003626655	SS, D
98	ENSG00000010319	-1.634662112	0.046771483	SS, D
99	ENSG00000129195	-1.653524489	0.0116065	SS, D
100	ENSG00000164683	-1.660256496	0.02435207	SS, D
101	ENSG00000178401	-1.672555063	0.000270896	SS, D
102	ENSG00000129810	-1.690212863	0.001558066	SS, D
103	ENSG00000121211	-1.697928804	0.003381253	SS, D
104	ENSG00000135119	-1.751527916	0.018878811	SS, D

105	ENSG00000065328	-1.754433586	0.000270896	SS, D
106	ENSG00000203995	-1.808942321	0.013766291	SS, D
107	ENSG00000007968	-1.81159346	0.007782156	SS, D
108	ENSG00000179403	-1.821745831	0.037646697	SS, D
109	ENSG00000146678	-1.83638242	0.000129103	SS, D
110	ENSG00000101057	-1.871986949	0.043673532	SS, D
111	ENSG00000174640	-1.882022264	0.043394558	SS, D
112	ENSG00000114812	-2.092031726	0.007443376	SS, D
113	ENSG00000153234	-2.093620422	0.005221022	SS, D
111	ENSG00000205362	-2.104668656	2.55E-05	SS, D
115	ENSG00000160886	-2.189194514	0.002543833	SS, D
116	ENSG00000123358	-2.695086285	0.000297526	SS, D
117	ENSG00000205364	-2.913601454	3.26E-05	SS, D
118	ENSG00000198417	-3.288225828	0.000696395	SS, D
119	ENSG00000125144	-5.631701129	0.000324888	SS, D

Table IIA - differentially expressed noncoding RNAs (lncRNA) in primary senescent cells (Upregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	External Gene
1	ENSG00000276241	5.2605896	0.02580792	AC243829.2
2	ENSG00000275226	5.15074016	5.47E-09	LINC00547
3	ENSG00000236039	4.91975396	5.27E-07	AC019117.2
4	ENSG00000224294	4.87012157	1.28E-06	PINCR
5	ENSG00000276462	4.73216528	0.00674662	BX255923.2
6	ENSG00000249835	4.70412892	0.00637973	VCAN-AS1
7	ENSG00000099869	4.4095324	0.00024075	IGF2-AS
8	ENSG00000253658	4.37648665	0.03725906	LINC01592
9	ENSG00000226954	4.3234543	0.00628006	AL023802.1
10	ENSG00000224691	4.27077984	0.01933848	AL133553.1
11	ENSG00000248587	4.19969305	0.02657068	GDNF-AS1
12	ENSG00000226067	4.13693888	0.04338084	LINC00623

13	ENSG00000229227	4.11875405	0.01540157	AL356056.1
11	ENSG00000253503	3.9939446	0.00463341	AC060765.1
15	ENSG00000223511	3.95408486	0.00061823	AL683807.1
16	ENSG00000255082	3.80135413	0.00655074	GRM5-AS1
17	ENSG00000261584	3.69388892	0.0407812	AL513548.1
18	ENSG00000232679	3.55120508	0.03770416	LINC01705
19	ENSG00000249669	3.42474614	1.37E-09	CARMN
20	ENSG00000248362	3.27956186	0.00209984	AC011352.1
21	ENSG00000265282	3.20904627	0.03846356	AC005828.4
22	ENSG00000227619	3.08513585	0.03891031	AL391056.1
23	ENSG00000218416	2.75261014	0.00655074	AC110619.1
24	ENSG00000254275	2.73338446	0.0019756	LINC00824
25	ENSG00000244564	2.68922208	0.04472823	PRICKLE2-DT
26	ENSG00000224549	2.68678685	0.00282272	AC017067.1
27	ENSG00000262772	2.65691508	0.01032415	LINC01977
28	ENSG00000212939	2.57544618	0.01952271	Z97192.1
29	ENSG00000235947	2.55270346	0.00339359	EGOT
30	ENSG00000267251	2.54845953	0.00067032	AC139100.1
31	ENSG00000228318	2.54688788	0.02828196	AP001610.2
32	ENSG00000251320	2.5258769	0.04813994	AC011352.3
33	ENSG00000236494	2.44002039	0.02424555	AC008080.4
34	ENSG00000228063	2.42748292	0.00463341	LYPLAL1-DT
35	ENSG00000267605	2.42207458	0.01009486	AC016590.1
36	ENSG00000233098	2.38405764	0.00856038	CCDC144NL-AS1
37	ENSG00000250337	2.35986083	1.95E-06	PURPL
38	ENSG00000258569	2.28757496	0.00982058	AC007376.2
39	ENSG00000261114	2.28174179	0.00193137	AC012181.1
40	ENSG00000243089	2.2346048	0.01516686	AC107029.1

41	ENSG00000231187	2.22034296	0.00079106	AL356056.2
42	ENSG00000272872	2.19967959	0.0002109	AP000525.1
43	ENSG00000228208	2.18753338	0.03725906	LINC02869
44	ENSG00000274654	2.18666847	0.00573338	AC022467.1
45	ENSG00000235385	2.17196334	0.00108682	LINC02154
46	ENSG00000226674	2.1517009	0.03882014	TEX41
47	ENSG00000223764	2.15098898	0.0067499	LINC02593
48	ENSG00000267095	2.13196512	0.00067032	AC025048.1
49	ENSG00000261270	2.08880305	0.00232549	AC012181.2
50	ENSG00000235033	2.0796022	0.00941489	DAAM2-AS1
51	ENSG00000254966	2.06280432	0.01516686	AC103974.1
52	ENSG00000268894	2.04306694	0.00108682	PLCE1-AS1
53	ENSG00000230838	1.97450423	0.00282272	LINC01614
54	ENSG00000277999	1.91313724	0.00697879	AC009093.6
55	ENSG00000281207	1.89812267	0.00069759	SLFNL1-AS1
56	ENSG00000255400	1.88189515	0.01144506	AC124276.2
57	ENSG00000231971	1.86703756	0.02338523	CT69
58	ENSG00000229373	1.81548631	0.00200507	LINC00452
59	ENSG00000276649	1.81141679	0.01735808	AL117335.1
60	ENSG00000261586	1.77753273	0.03738595	AC068987.3
61	ENSG00000225886	1.76621545	0.00061137	AL445490.1
62	ENSG00000234840	1.67771998	0.00039954	LINC01239
63	ENSG00000177234	1.63633149	0.00063749	LINC01561
64	ENSG00000225963	1.61227563	0.03365734	AC009950.1
65	ENSG00000270164	1.59977405	0.03891031	LINC01480
66	ENSG00000271897	1.59867704	0.04815322	AL357518.1
67	ENSG00000237596	1.5933812	0.03297438	AL138828.1
68	ENSG00000276851	1.57503474	0.03048933	AC002401.4

69	ENSG00000226835	1.51383315	0.03365734	ARHGAP29-AS1
70	ENSG00000259840	1.50121573	0.03882014	Z97653.1
71	ENSG00000232759	1.46807899	0.00628006	AC002480.1
72	ENSG00000259081	1.44526586	0.04338084	AF111169.3
73	ENSG00000278202	1.4334675	0.02211042	AC243919.2
74	ENSG00000276523	1.4132337	0.01735808	AC025287.3
75	ENSG00000238184	1.38917134	0.0199123	CD81-AS1
76	ENSG00000250320	1.34556776	0.0142169	EDIL3-DT
77	ENSG00000206195	1.34419121	0.01873268	DUXAP8
78	ENSG00000270157	1.32343403	0.00142679	AC004918.5
79	ENSG00000228495	1.29955799	0.00435629	LINC01013
80	ENSG00000257556	1.25936562	0.01636746	LINC02298
81	ENSG00000260193	1.20274933	0.03916684	LINC02846
82	ENSG00000261488	1.17203103	0.016287	TBILA
83	ENSG00000260578	1.13384163	0.00032374	AC110597.1
84	ENSG00000260285	1.1192697	0.0229427	AL133367.1
85	ENSG00000203392	1.101733	0.00637973	AC105020.1
86	ENSG00000232977	1.07140872	0.01018612	LINC00327
87	ENSG00000261888	1.05513682	0.01498397	AC144831.1
88	ENSG00000188004	1.03047038	0.03048933	SNHG28

Table IIB - differentially expressed noncoding RNAs (lncRNA) in primary senescent cells (Downregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	External Gene
1	ENSG00000260401	-1.1004028	0.00039954	AP002761.4
2	ENSG00000253661	-1.1065922	0.00034909	ZFH4-AS1
3	ENSG00000250899	-1.1099201	1.54E-05	AC125807.2
4	ENSG00000273891	-1.1526948	0.01154228	AL731566.2
5	ENSG00000271826	-1.1956628	0.04268456	PLS3-AS1

6	ENSG00000255062	-1.2120957	0.00637973	AP001318.2
7	ENSG00000281344	-1.2259465	0.01296271	HELLPAR
8	ENSG00000272599	-1.2538212	1.85E-07	AC016394.3
9	ENSG00000226330	-1.4151139	0.0298511	AL606489.1
10	ENSG00000237352	-1.5376532	0.00554939	LINC01358
11	ENSG00000234779	-1.5593966	0.0210313	BNC2-AS1
12	ENSG00000174171	-1.5985398	0.02133172	AC020659.1
13	ENSG00000224063	-1.6789321	0.03557586	AC007319.1
11	ENSG00000230400	-1.7324859	0.00934191	LINC01747
15	ENSG00000234694	-1.7819859	0.03891031	AL139289.1
16	ENSG00000232445	-1.822495	0.01933848	EMSLR
17	ENSG00000267774	-1.993749	0.0002109	AC010776.2
18	ENSG00000187951	-2.1082932	4.06E-10	AC091057.1
19	ENSG00000267698	-2.2299771	0.04268456	AC002116.2
20	ENSG00000229771	-2.3023166	0.00168815	AL035665.1
21	ENSG00000257167	-2.4290891	1.28E-06	TMPO-AS1
22	ENSG00000229558	-2.5530547	0.04338084	SACS-AS1
23	ENSG00000243193	-2.7707884	0.00053253	PRKAR2B-AS1
24	ENSG00000243384	-2.9359642	0.00349067	AC119424.1
25	ENSG00000265415	-3.2901605	8.58E-12	AC099850.4
26	ENSG00000249574	-3.5078551	0.01261044	AC226118.1
27	ENSG00000267886	-3.7377289	0.04244616	AC074135.1

Table IIC - differentially expressed noncoding RNAs (lncRNA) in secondary senescent cells (Upregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	External Gene
1	ENSG00000276241	7.0913239	0.00146305	AC243829.2
2	ENSG00000224294	4.17571534	0.00034885	PINCR
3	ENSG00000253503	3.83278258	0.03131111	AC060765.1

4	ENSG00000223511	3.65155376	0.00990348	AL683807.1
5	ENSG00000254275	3.18807275	0.0003334	LINC00824
6	ENSG00000275226	3.08195812	0.03131111	LINC00547
7	ENSG00000235385	2.60391246	8.08E-05	LINC02154
8	ENSG00000250337	2.3707921	7.94E-06	PURPL
9	ENSG00000229981	1.85111465	3.24E-06	LINC01435
10	ENSG00000206337	1.3510122	0.03131111	HCP5
11	ENSG00000260578	0.963093	0.02043229	AC110597.1
12	ENSG00000223745	0.68960739	0.00099708	CCDC18-AS1
13	ENSG00000282851	0.65772874	0.02790495	BISPR

Table IID - differentially expressed noncoding RNAs (lncRNA) in secondary senescent cells (Downregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	External Gene
1	ENSG00000266053	-0.897779	0.03006747	NDUFV2-AS1
2	ENSG00000257815	-1.2460004	0.04242414	PRANCR
3	ENSG00000257167	-1.4294043	0.04922138	TMPO-AS1

Table IIIA - pro-survival genes in primary senescent cells (Upregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000134259	3.877639387	0.013312237	NGF
2	ENSG00000162490	3.560234145	2.15E-15	DRAXIN
3	ENSG00000112902	3.373216675	0.001035756	SEMA5A
4	ENSG00000123384	2.860380559	1.29E-22	LRP1
5	ENSG00000168398	2.791695557	5.33E-05	BDKRB2
6	ENSG00000163235	2.569508992	2.75E-14	TGFA
7	ENSG00000109846	2.466559494	8.36E-12	CRYAB
8	ENSG00000067141	2.406454221	0.00060365	NEO1
9	ENSG00000151617	2.369117723	8.44E-06	EDNRA
10	ENSG00000172379	2.276723979	0.002381343	ARNT2
11	ENSG00000188452	2.134374977	7.74E-07	CERKL

12	ENSG00000106799	2.109225797	6.39E-05	TGFBR1
13	ENSG00000113721	2.067328363	1.83E-06	PDGFRB
11	ENSG00000118971	1.971394852	0.006832418	CCND2
15	ENSG00000242419	1.956022246	0.007466077	PCDHGC4
16	ENSG00000137642	1.870161071	0.008140799	SORL1
17	ENSG00000119508	1.826454732	0.03136161	NR4A3
18	ENSG00000167772	1.816962283	7.62E-11	ANGPTL4
19	ENSG00000157168	1.800853098	4.20E-07	NRG1
20	ENSG00000197405	1.756019919	0.000168664	C5AR1
21	ENSG00000176046	1.717976117	4.54E-05	NUPR1
22	ENSG00000171951	1.699927831	0.001118453	SCG2
23	ENSG00000240764	1.660505826	0.007303082	PCDHGC5
24	ENSG00000130203	1.635282131	1.63E-05	APOE
25	ENSG00000139211	1.584688742	0.000455987	AMIGO2
26	ENSG00000203780	1.454962199	9.33E-06	FANK1
27	ENSG00000162552	1.434229443	0.000549017	WNT4
28	ENSG00000104290	1.433737963	0.008894543	FZD3
29	ENSG00000188763	1.422864048	1.17E-11	FZD9
30	ENSG00000132821	1.384627876	2.17E-10	VSTM2L
31	ENSG00000128602	1.335864828	3.76E-05	SMO
32	ENSG00000119917	1.325150932	7.32E-09	IFIT3
33	ENSG00000115414	1.322742844	0.000112547	FN1
34	ENSG00000154188	1.280714864	0.021719029	ANGPT1
35	ENSG00000176884	1.271078539	0.020967633	GRIN1
36	ENSG00000183087	1.199969666	8.97E-17	GAS6
37	ENSG00000260428	1.190468906	0.00199352	SCX
38	ENSG00000148053	1.190215694	0.000225868	NTRK2
39	ENSG00000137331	1.182842927	5.54E-10	IER3
40	ENSG00000070669	1.148382367	0.000639801	ASNS
41	ENSG00000138448	1.14673493	0.013705887	ITGAV
42	ENSG00000176907	1.110375342	0.008525635	TCIM

43	ENSG00000110492	1.071227592	0.00304491	MDK
44	ENSG00000146469	1.036154799	0.047676084	VIP
45	ENSG00000112715	1.033827789	0.000437286	VEGFA
46	ENSG00000172057	1.029687459	4.51E-15	ORMDL3
47	ENSG00000151651	1.011429079	0.003211125	ADAM8

Table IIIB - pro-survival genes in primary
senescent cells (Downregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000188906	-1.006980406	0.005572478	LRRK2
2	ENSG00000135111	-1.027609132	2.21E-16	TBX3
3	ENSG00000078081	-1.059979931	0.014578164	LAMP3
4	ENSG00000141480	-1.200411711	0.001063237	ARRB2
5	ENSG00000176208	-1.345264482	5.48E-09	ATAD5
6	ENSG00000123473	-1.392764662	1.34E-19	STIL
7	ENSG00000165490	-1.688803795	1.03E-15	DDIAS
8	ENSG00000012048	-1.717951496	1.03E-20	BRCA1
9	ENSG00000176194	-1.763019331	0.032903051	CIDEA
10	ENSG00000196584	-1.827232338	4.40E-08	XRCC2
11	ENSG00000119969	-1.844371557	1.37E-10	HELLS
12	ENSG00000185862	-1.897920549	6.61E-07	EVI2B
13	ENSG00000197299	-1.980645306	4.73E-13	BLM
11	ENSG00000087586	-2.051522585	6.37E-16	AURKA
15	ENSG00000105851	-2.055550842	1.28E-06	PIK3CG
16	ENSG00000164104	-2.118327575	1.98E-12	HMGB2
17	ENSG00000197632	-2.30519656	0.006524439	SERPINB2
18	ENSG00000164109	-2.424011542	8.20E-24	MAD2L1
19	ENSG00000118193	-2.464099308	1.51E-35	KIF14
20	ENSG00000166851	-3.103789895	7.09E-53	PLK1
21	ENSG00000170312	-3.317830937	6.18E-10	CDK1
22	ENSG00000178999	-3.609277998	4.15E-10	AURKB
23	ENSG00000101188	-3.744472844	2.71E-08	NTSR1

24	ENSG00000089685	-4.094279662	4.23E-16	BIRC5
----	-----------------	--------------	----------	-------

Table IIIC - pro-survival genes in secondary senescent cells (Upregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000277586	4.36720162	0.00082469	NEFL
2	ENSG00000067141	2.39658527	0.01600324	NEO1
3	ENSG00000151617	2.15789767	0.0149919	EDNRA
4	ENSG00000137642	1.98328486	0.03950197	SORL1
5	ENSG00000123384	1.66096483	0.00011412	LRP1
6	ENSG00000119917	1.55916471	0.01064633	IFIT3
7	ENSG00000171951	1.46210964	0.00064746	SCG2
8	ENSG00000070669	1.3639035	4.75E-10	ASNS
9	ENSG00000188452	1.31509093	0.04394684	CERKL
10	ENSG00000145777	1.18219775	0.00014647	TSLP

Table IIID - pro-survival genes in secondary senescent cells (Downregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000119969	-1.0240939	0.00964203	HELLS
2	ENSG00000164109	-1.1012254	0.0048102	MAD2L1
3	ENSG00000164104	-1.1086634	0.01508202	HMGB2
4	ENSG00000107485	-1.1461621	0.00021936	GATA3
5	ENSG00000166851	-1.2019713	0.02017001	PLK1
6	ENSG00000165490	-1.3512971	2.55E-07	DDIAS
7	ENSG00000073756	-1.5412715	0.00056983	PTGS2
8	ENSG00000153234	-2.0936204	0.00522102	NR4A2
9	ENSG00000123358	-2.6950863	0.00029753	NR4A1

Table IVA – surface markers of primary senescent cells (Upregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
--	----	------------------	------------------	-----------

1	ENSG00000137975	4.133592346	1.58E-07	CLCA2
2	ENSG00000169851	2.404990377	0.000494324	PCDH7
3	ENSG00000078098	2.397297273	0.021523755	FAP
4	ENSG00000196517	1.788693009	0.000354045	SLC6A9
5	ENSG00000204642	1.601836943	0.002667841	HLA-F
6	ENSG00000106624	1.480892506	0.041498109	AEBP1
7	ENSG00000139364	1.04021924	6.75E-05	TMEM132B
8	ENSG00000026103	0.417176381	0.024200813	FAS

Table IVB – surface markers of primary
senescent cells (Downregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000130702	-0.473108908	0.023589562	LAMA5
2	ENSG00000154639	-0.492665842	0.045736735	CXADR
3	ENSG00000069702	-0.524075965	0.013078473	TGFBR3
4	ENSG00000153956	-0.542194728	0.034912619	CACNA2D1
5	ENSG00000049130	-0.578856035	0.016850483	KITLG
6	ENSG00000164342	-0.591882693	0.010578173	TLR3
7	ENSG00000134531	-0.689072361	1.12E-05	EMP1
8	ENSG00000005108	-0.716327009	0.023476844	THSD7A
9	ENSG00000120156	-0.751775793	0.005224809	TEK
10	ENSG00000116678	-0.760134355	0.021589047	LEPR
11	ENSG00000099219	-0.839072319	0.000192445	ERMP1
12	ENSG00000162407	-0.849170316	0.024068231	PLPP3
13	ENSG00000130164	-0.850093855	0.019319873	LDLR
11	ENSG00000169760	-0.925801692	3.00E-05	NLGN1
15	ENSG00000149573	-1.023706917	0.00016777	MPZL2
16	ENSG00000112414	-1.152884123	0.000102381	ADGRG6
17	ENSG00000122861	-1.169435578	0.000782224	PLAU
18	ENSG00000184005	-1.347309455	1.89E-09	ST6GALNAC3
19	ENSG00000205336	-1.376856426	1.10E-07	ADGRG1
20	ENSG00000171246	-1.439351735	0.01811733	NPTX1

21	ENSG00000196549	-1.545890911	5.92E-07	MME
22	ENSG00000159164	-1.686783522	0.00368493	SV2A
23	ENSG00000140678	-1.757191321	9.33E-10	ITGAX
24	ENSG00000213853	-1.795043783	7.96E-07	EMP2
25	ENSG00000197635	-1.799943552	0.001414487	DPP4
26	ENSG00000168077	-1.949862955	4.87E-11	SCARA3
27	ENSG00000127951	-2.059700649	2.14E-05	FGL2
28	ENSG00000101187	-2.086509888	7.21E-06	SLCO4A1
29	ENSG00000105825	-2.170736544	0.017440887	TFPI2
30	ENSG00000197632	-2.30519656	0.006524439	SERPINB2
31	ENSG00000012223	-2.959258821	3.87E-06	LTF
32	ENSG00000163814	-3.443854276	5.19E-19	CDCP1
33	ENSG00000101188	-3.744472844	2.71E-08	NTSR1

Table IVC – surface markers of secondary senescent cells (Upregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000108679	1.795694422	0.013359955	LGALS3BP
2	ENSG00000196517	1.620162766	6.39E-05	SLC6A9
3	ENSG00000170909	1.611577526	0.001114429	OSCAR
4	ENSG00000139364	1.292680139	0.012731879	TMEM132B
5	ENSG00000204642	1.012616655	0.03633716	HLA-F
6	ENSG00000179242	0.838114047	0.048391135	CDH4
7	ENSG00000149256	0.833064793	0.037379359	TENM4
8	ENSG00000138623	0.801416799	3.69E-08	SEMA7A
9	ENSG00000026103	0.735567586	0.011052744	FAS

Table IVD – surface markers of secondary senescent cells (Downregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000213853	-1.355936736	0.008486816	EMP2
2	ENSG00000174640	-1.882022264	0.043394558	SLCO2A1

Table VA – secreted proteins from primary

senescent cells (Upregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000100234	3.433883214	0.003773777	TIMP3
2	ENSG00000128165	3.048841749	4.95E-07	ADM2
3	ENSG00000204941	2.993648157	0.00024537	PSG5
4	ENSG00000078098	2.397297273	0.021523755	FAP
5	ENSG00000115461	2.388665593	0.00014512	IGFBP5
6	ENSG00000171951	1.699927831	0.001118453	SCG2
7	ENSG00000159674	1.586844207	0.049351126	SPON2
8	ENSG00000106624	1.480892506	0.041498109	AEBP1
9	ENSG00000198732	1.251641923	0.021723585	SMOC1
10	ENSG00000143369	1.04019613	0.00644673	ECM1

Table VB – secreted proteins from primary
senescent cells (Downregulated in Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000105835	-0.421334898	0.049610241	NAMPT
2	ENSG00000130702	-0.473108908	0.023589562	LAMA5
3	ENSG00000159167	-1.092355101	0.041265039	STC1
4	ENSG00000184232	-1.097546253	0.000373084	OAF
5	ENSG00000153993	-1.125690732	0.010301297	SEMA3D
6	ENSG00000122861	-1.169435578	0.000782224	PLAU
7	ENSG00000010438	-1.174647107	0.001398667	PRSS3
8	ENSG00000171246	-1.439351735	0.01811733	NPTX1
9	ENSG00000183287	-1.587529677	8.94E-05	CCBE1
10	ENSG00000171388	-1.706892868	0.000357562	APLN
11	ENSG00000173083	-1.816429813	7.03E-36	HPSE
12	ENSG00000127951	-2.059700649	2.14E-05	FGL2
13	ENSG00000105825	-2.170736544	0.017440887	TFPI2
14	ENSG00000197565	-2.313962542	2.81E-08	COL4A6
15	ENSG00000146678	-2.320926304	3.51E-12	IGFBP1
16	ENSG00000012223	-2.959258821	3.87E-06	LTF

Table VC – secreted proteins from secondary
senescent cells (Upregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000204941	3.174640511	0.003726418	PSG5
2	ENSG00000115461	3.090533989	0.000229366	IGFBP5
3	ENSG00000100234	2.826451139	0.020832174	TIMP3
4	ENSG00000128165	2.647021347	1.87E-10	ADM2
5	ENSG00000136999	2.266308404	0.001776461	NOV
6	ENSG00000108679	1.795694422	0.013359955	LGALS3BP
7	ENSG00000198797	1.623495245	0.036456898	BRINP2
8	ENSG00000171951	1.462109637	0.000647458	SCG2
9	ENSG00000143369	1.250729643	0.040108135	ECM1
10	ENSG00000115008	0.85772707	0.038051777	IL1A
11	ENSG00000198732	0.850114817	0.049624959	SMOC1

Table VD – secreted proteins from secondary
senescent cells (Downregulated in Secondary Senescent Cells)

	ID	log2 Fold Change	p-value adjusted	Gene name
1	ENSG00000146678	-1.83638242	0.000129103	IGFBP1

CLAIMS

What is claimed is:

1. A method of treating an ailment, the method comprising administering a therapeutic amount of a senolytic agent to a subject.
2. The method of claim 1, wherein the senolytic agent selectively targets senescent cells.
3. The method of claim 1, wherein the senolytic agent targets secondary senescent cells.
4. The method of claim 1, wherein the senolytic agent targets primary senescent cells and secondary senescent cells.
5. The method of claim 1, wherein the senolytic agent is a small molecule.
6. The method of claim 5, wherein the small molecule is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.
7. The method of claim 1, wherein the senolytic agent induces ferroptosis in senescent cells.
8. The method of claim 1, wherein the ailment is a senescence-associated disease or disorder.
9. The method of claim 8, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD)

and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.

10. The method of claim 8, further comprising a step of identifying the senescence-associated disease or disorder based on the presence of one or more biomarkers in a sample from the subject.

11. The method of claim 10, wherein the one or more biomarkers comprise dipeptidyl peptidase-4 (DPP4).

12. A method of slowing the aging process or reducing signs of aging, the method comprising administering a therapeutic amount of a senolytic agent to a subject.

13. The method of claim 12, wherein the senolytic agent selectively targets senescent cells.

14. The method of claim 12, wherein the senolytic agent targets secondary senescent cells.

15. The method of claim 12, wherein the senolytic agent targets primary senescent cells and secondary senescent cells.

16. The method of claim 12, wherein the senolytic agent is a small molecule.

17. The method of claim 16, wherein the small molecule is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.

18. The method of claim 12, wherein the senolytic agent induces ferroptosis in senescent cells.

19. A method of selectively killing senescent cells, the method comprising administering a therapeutic amount of a senolytic agent to a subject.
20. The method of claim 19, wherein the senolytic agent selectively targets senescent cells.
21. The method of claim 19, wherein the senolytic agent targets secondary senescent cells.
22. The method of claim 19, wherein the senolytic agent targets primary senescent cells and secondary senescent cells.
23. The method of claim 19, wherein the senolytic agent is a small molecule.
24. The method of claim 23, wherein the small molecule is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.
25. The method of claim 19, wherein the senolytic agent induces ferroptosis in senescent cells.
26. The method of claim 19, wherein the senescent cells cause a senescence-associated disease or disorder.
27. The method of claim 19, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.

28. The method of claim 19, further comprising a step of identifying the senescence-associated disease or disorder based on the presence of one or more biomarkers in a sample from the subject.
29. The method of claim 28, wherein the one or more biomarkers comprise dipeptidyl peptidase-4 (DPP4).
30. A method of treating a senescence-associated disease or disorder in a subject, the method comprising steps of:
- a) identifying the senescence-associated disease or disorder based on altered expression levels of at least one biomarker from the subject,
 - b) treating the subject by administering a therapeutic amount of a senolytic agent.
31. The method of claim 30, wherein the at least one biomarker comprises dipeptidyl peptidase-4 (DPP4).
32. The method of claim 30, wherein the senolytic agent selectively targets senescent cells.
33. The method of claim 30, wherein the senolytic agent targets secondary senescent cells.
34. The method of claim 30, wherein the senolytic agent targets primary senescent cells and secondary senescent cells.
35. The method of claim 30, wherein the senolytic agent is a small molecule.
36. The method of claim 35, wherein the small molecule is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.

37. The method of claim 30, wherein the senolytic agent induces ferroptosis in senescent cells.
38. The method of claim 30, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.
39. A method of treating a senescence-associated disease or disorder in a subject, the method comprising inducing ferroptosis in senescent cells of the subject.
40. The method of claim 39, wherein ferroptosis is induced by administering a small molecule medicament.
41. The method of claim 40, wherein the small molecule medicament is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.
42. The method of claim 39, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.
43. A formulation for treating a senescence-associated disease or disorder, wherein the formulation includes an agent that selectively kills senescent cells by inducing ferroptosis.

44. The formulation of claim 43, wherein the agent is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.
45. The formulation of claim 43, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.
46. A method of eliminating senescent cells from a subject, the method comprising a step of identifying senescent cells by one or more biomarkers in a sample from the subject.
47. The method of claim 46, wherein the one or more biomarkers comprise dipeptidyl peptidase-4 (DPP4).
48. A method of slowing the aging process and/or reducing signs of aging in a subject, the method comprising administering a therapeutic amount of a senolytic agent to a subject.
49. The method of claim 48, wherein the senolytic agent selectively targets senescent cells.
50. The method of claim 48, wherein the senolytic agent targets secondary senescent cells.
51. The method of claim 48, wherein the senolytic agent targets primary senescent cells and secondary senescent cells.
52. The method of claim 48, wherein the senolytic agent is a small molecule.

53. The method of claim 52, wherein the small molecule is one or more of erastin, sulfasalazine, sorafenib, (1S, 3R)-RSL3, ML162, ML210, L-glutamic acid, L-buthionine sulfoximine, ML 210, simvastatin, sorafenib and sulfasalazine.
54. The method of claim 52, wherein the small molecule induces ferroptosis in senescent cells.
55. A method treating a senescence-associated disease or disorder in a subject, the method comprising a step of modulating a pathway that induces senescence,
wherein the pathway is selected from p53 pathway, a protein secretion pathway, apoptosis, mTORC1 signaling pathway and a proinflammatory pathway.
56. The method of claim 55, wherein the proinflammatory pathway is selected from interferon alpha (IFN- α) and interferon gamma (IFN- γ).
57. The method of claim 55, wherein the modulation reduces numbers of senescent cells in the subject.
58. The method of claim 55, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.
59. A method of slowing the aging process or reducing signs of aging in a subject, the method comprising a step of modulating a pathway that induces senescence,
wherein the pathway is selected from p53 pathway, a protein secretion pathway, apoptosis, mTORC1 signaling pathway and a proinflammatory pathway.

60. The method of claim 59, wherein the proinflammatory pathway is selected from interferon alpha (IFN- α) and interferon gamma (IFN- γ).
61. The method of claim 59, wherein the modulation reduces numbers of senescent cells in the subject.
62. A method selectively removing or killing senescent cells from a subject, the method comprising a step of modulating protein expression or protein activity in senescent cells.
63. The method of claim 62, wherein the protein is selected from GDF7, WNT5B, IGFBP5 and CST1.
64. The method of claim 62, wherein the protein is selected from IGFBP3, PAPP2, EPHA7 and TNC.
65. The method of claim 62, wherein the protein is a Metallothionein.
66. The method of claim 62, wherein the protein is a Metallothionein is one or more of MT1A, MT1F, MT1G, MT1H and MT1M.
67. The method of claim 62, wherein the protein is a solute carrier protein.
68. The method of claim 62, wherein the senescent cells cause a senescence-associated disease or disorder.
69. The method of claim 68, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.

70. The method of claim 62, wherein the senescent cells cause aging and/or signs of aging.

71. The method of claim 62, wherein the step of modulating protein expression induces ferroptosis in senescent cells.

72. A method of identifying a senescence-associated disease or disorder based on the presence of one or more biomarkers in a sample from the subject,
wherein the one or more biomarkers are selected from the genes of Table IA – ID.

73. A method of slowing the aging process or reducing signs of aging, the method comprising modulating activity of one or more genes in a subject,
wherein the one or more genes are selected from the genes of Table IA – ID.

74. A method of identifying a senescence-associated disease or disorder based on the presence of one or more biomarkers in a sample from the subject,
wherein the one or more biomarkers are non-coding RNA genes.

75. The method of claim 74, wherein the non-coding RNA genes are selected from the genes of Table IIA – IID.

76. A method of slowing the aging process or reducing signs of aging, the method comprising modulating activity of one or more non-coding RNA genes in a subject,
wherein the one or more non-coding RNA genes are selected from the genes of Table IIA – IID.

77. A method of identifying a senescence-associated disease or disorder based on the presence of one or more biomarkers in a sample from the subject,
wherein the one or more biomarkers are selected from the surface proteins of Table IVA – IVD.

78. A method of identifying senescence in cells based on the presence of one or more surface proteins,

wherein the one or more surface proteins are selected from those identified in Table IVA – IVD.

79. The method of claim 78, wherein the senescence is primary senescence.

80. The method of claim 78, wherein the senescence is secondary senescence.

81. A method treating a senescence-associated disease or disorder in a subject, the method comprising administering a therapeutic amount of a senolytic agent to the subject, wherein the senolytic agent is selected from vorinostat (SAHA) and Ementine.

82. The method of claim 81, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.

83. A method treating a senescence-associated disease or disorder in a subject, the method comprising a step of modulating activity of one or more genes in the subject, wherein the one or more genes are selected from those identified in Table III.

84. The method of claim 83, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.

85. A method of slowing the aging process or reducing signs of aging, the method comprising modulating activity of one or more genes in a subject, wherein the one or more genes are selected from those identified in Table IIIA – IIID.
86. A method of killing senescent cells, the method comprising modulating activity of one or more genes identified in Table I – Table V.
87. A method of treating a senescence-associated disease or disorder, the method comprising modulating activity of one or more genes identified in Table I – Table V.
88. The method of claim 87, wherein the senescence-associated disease or disorder is one or more of atherosclerosis, osteoarthritis, osteoporosis, hypertension, arthritis, cataracts, cancer, Alzheimer's disease, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, hair graying, sarcopenia, adiposity, neurogenesis, fibrosis and glaucoma.
89. A method of slowing the aging process or reducing signs of aging, the method comprising modulating activity of one or more genes identified in Table I – Table V.

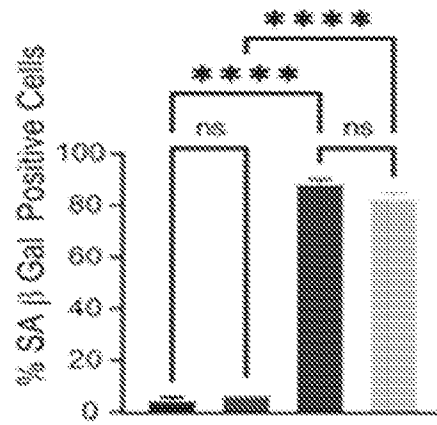


FIG. 1A

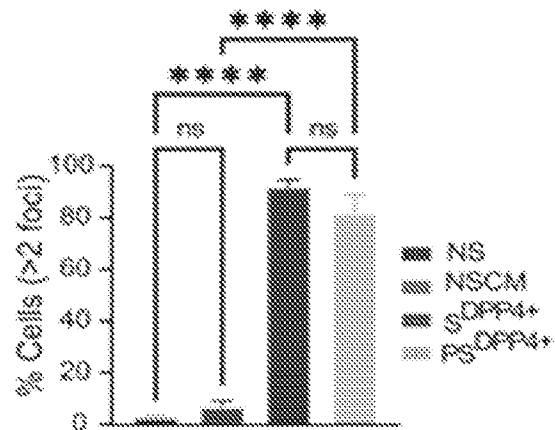


FIG. 1B

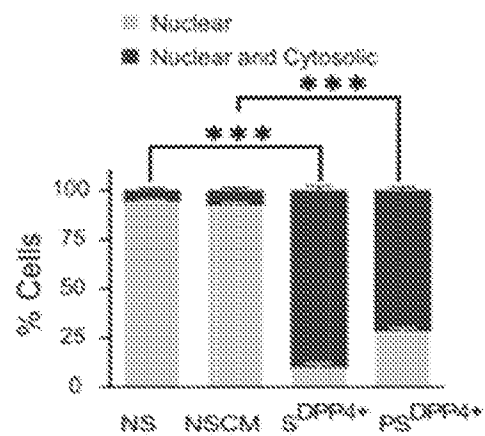


FIG. 1C

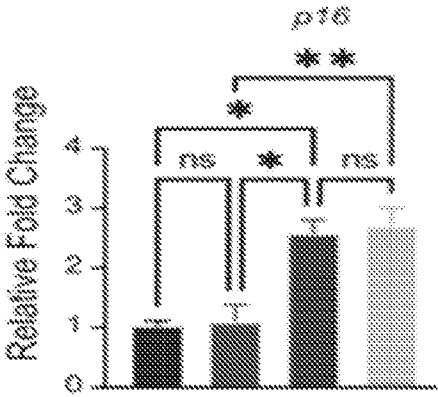


FIG. 1D

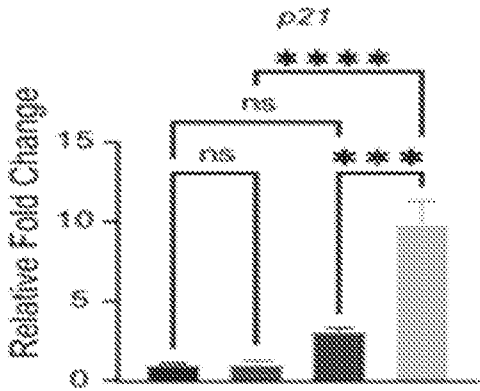


FIG. 1E

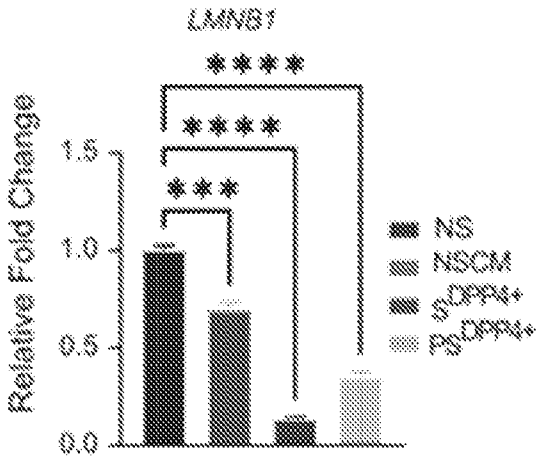


FIG. 1F

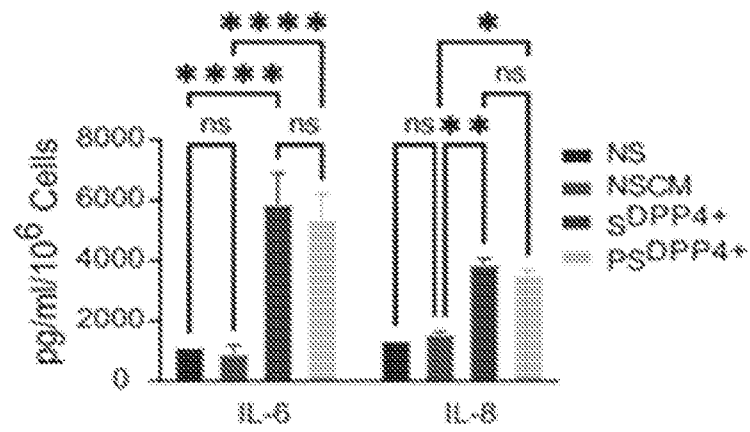


FIG. 1G

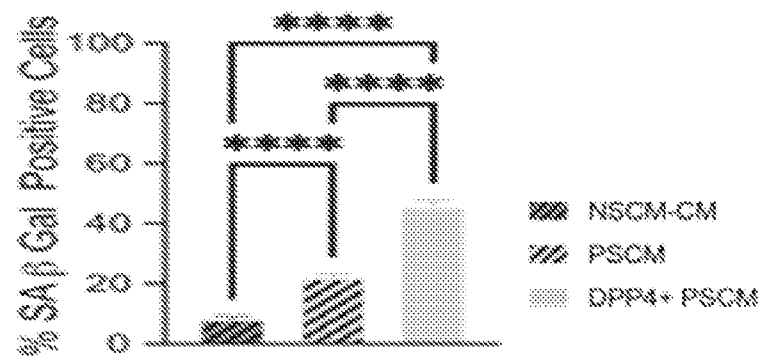


FIG. 1H

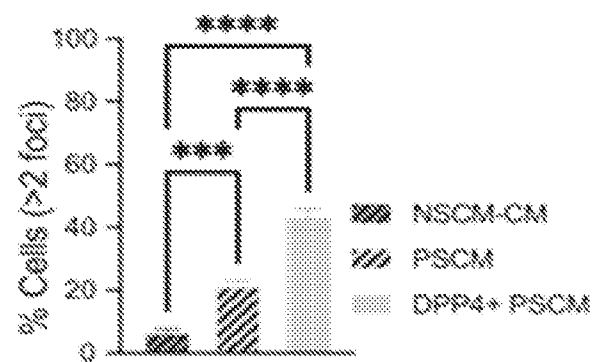


FIG. 1I

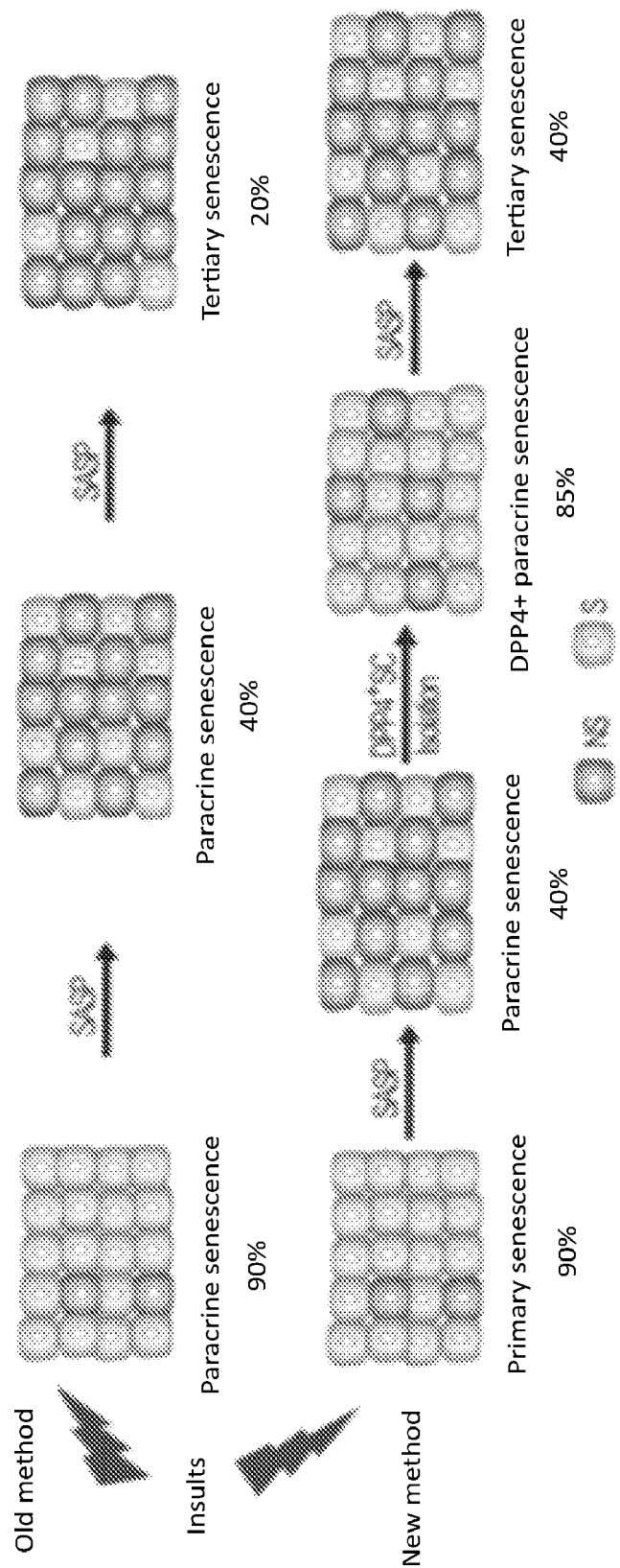


FIG. 1J

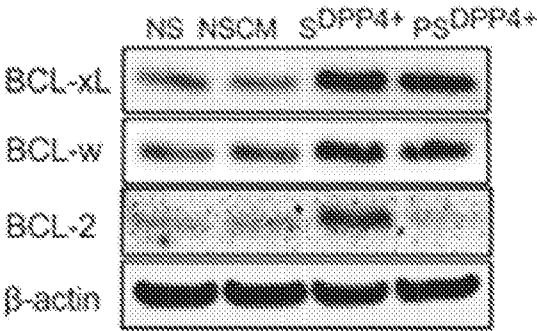


FIG. 2A



FIG. 2B

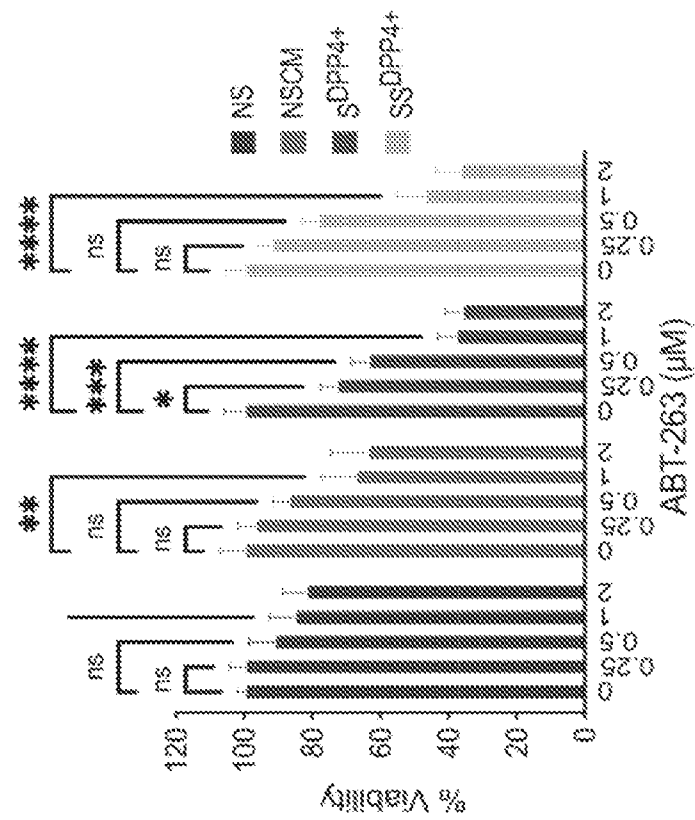


FIG. 2D

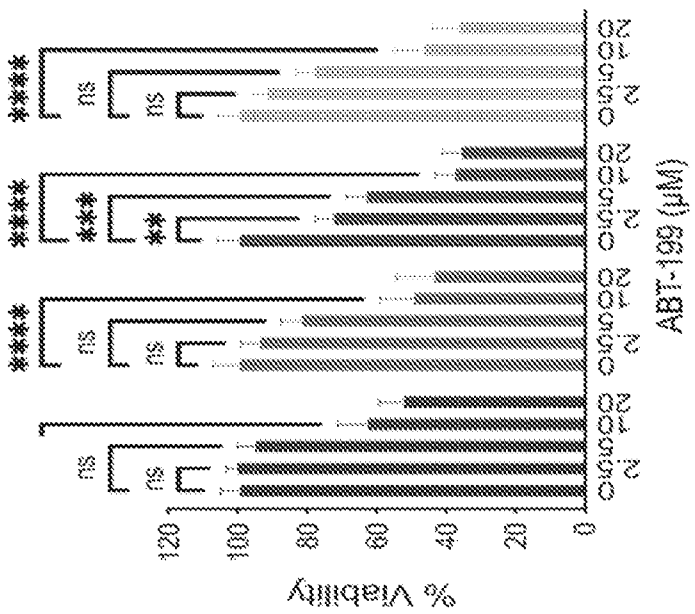


FIG. 2C

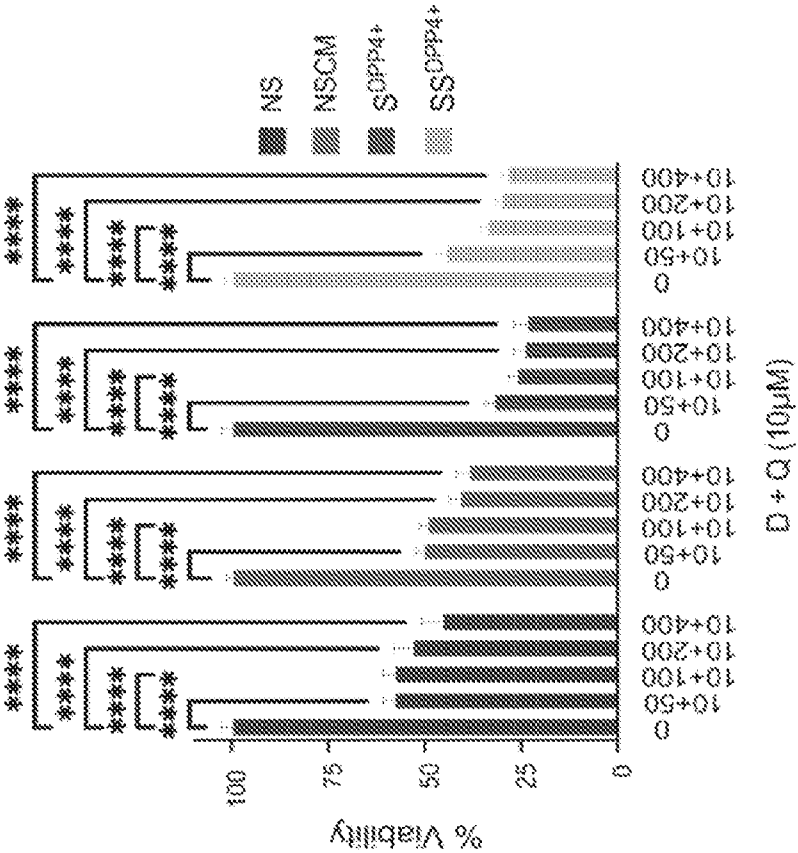


FIG. 2F

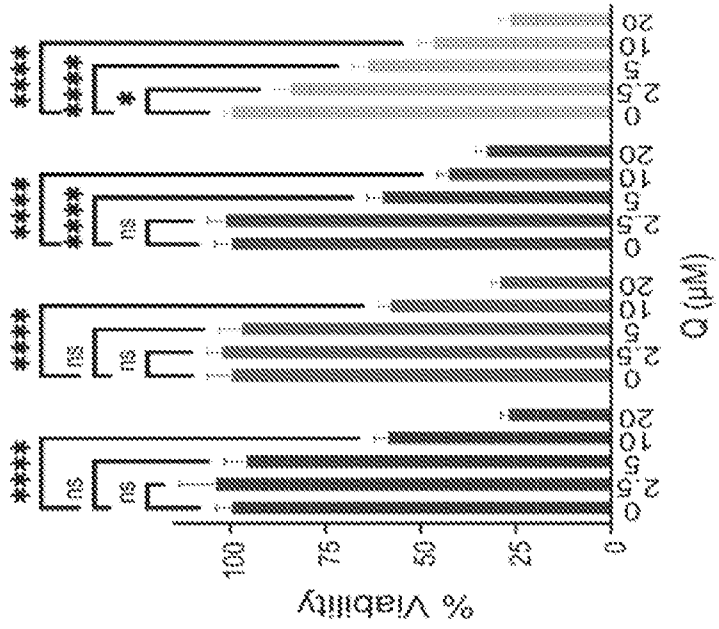
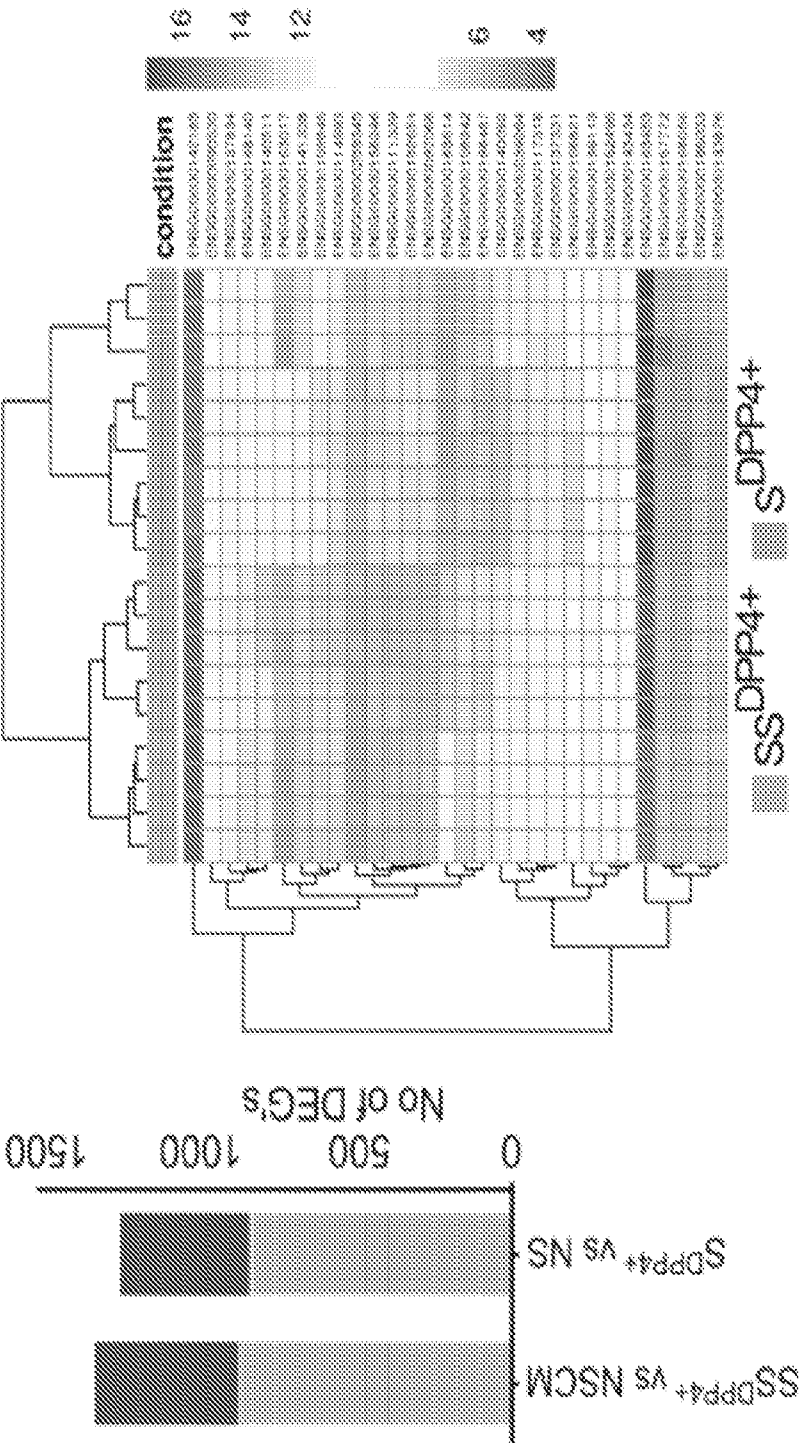


FIG. 2E



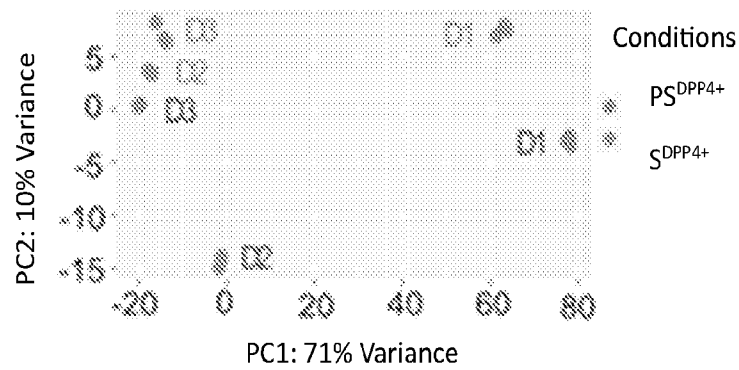


FIG. 3C

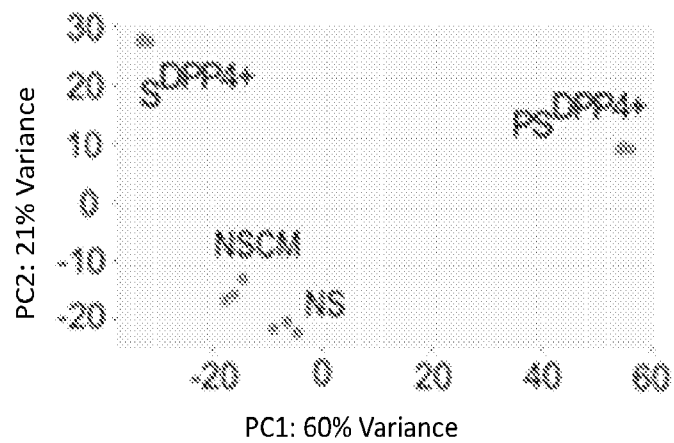


FIG. 3D

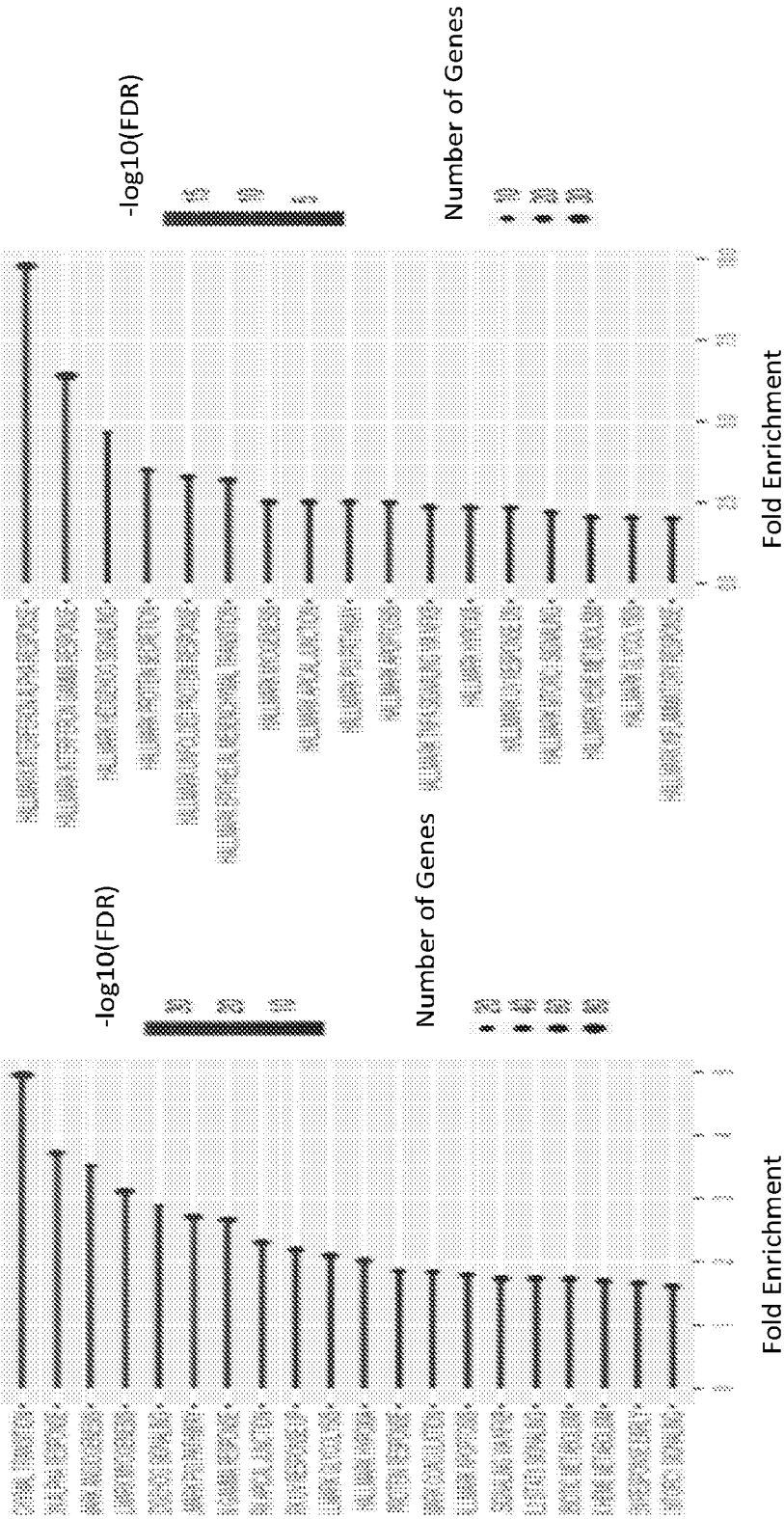


FIG. 3F

FIG. 3E

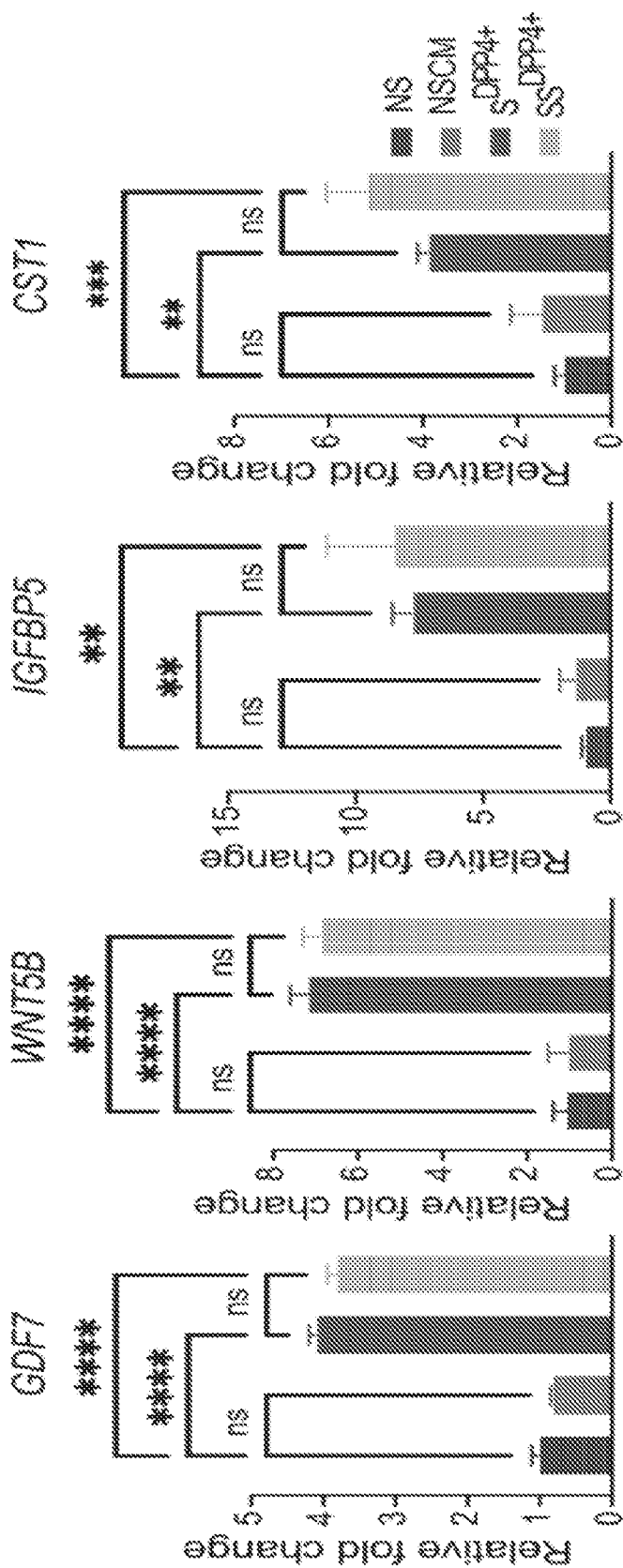


FIG. 3J

FIG. 3I

FIG. 3H

FIG. 3G

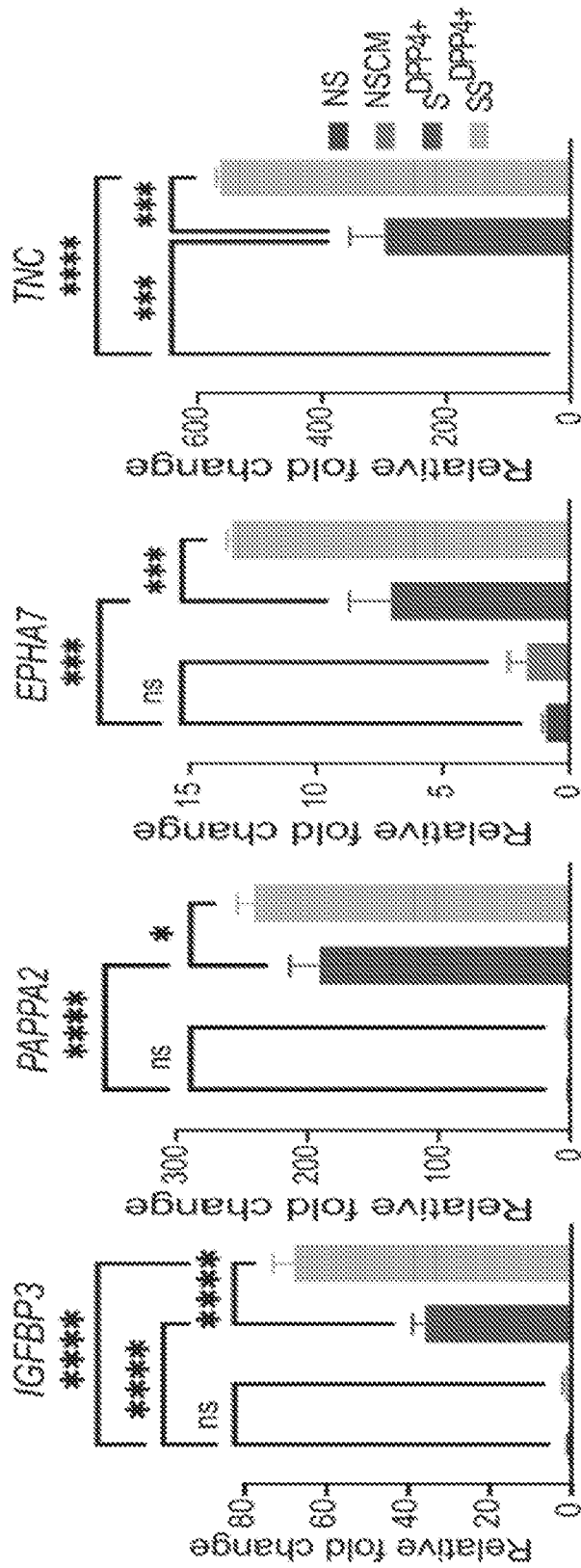


FIG. 3K

FIG. 3L

FIG. 3M

FIG. 3N

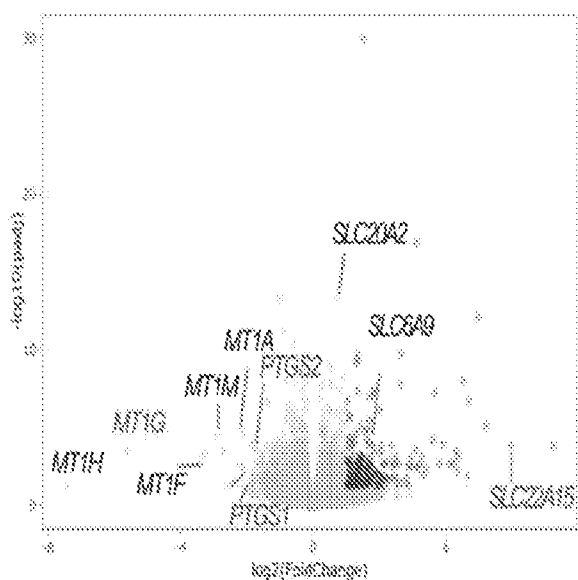


FIG. 4A

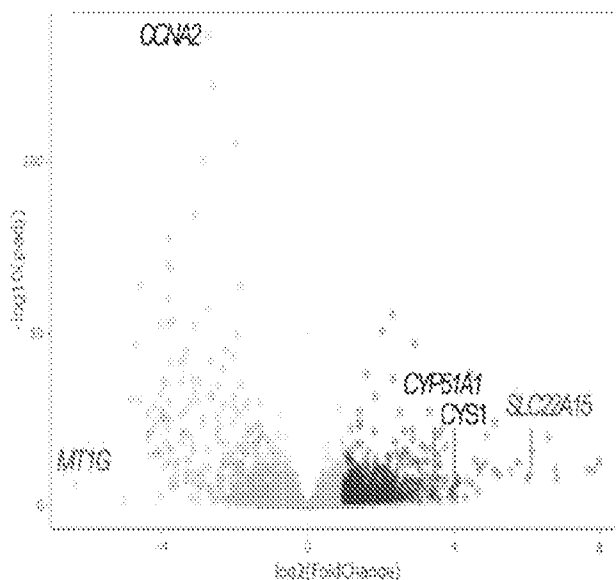


FIG. 4B

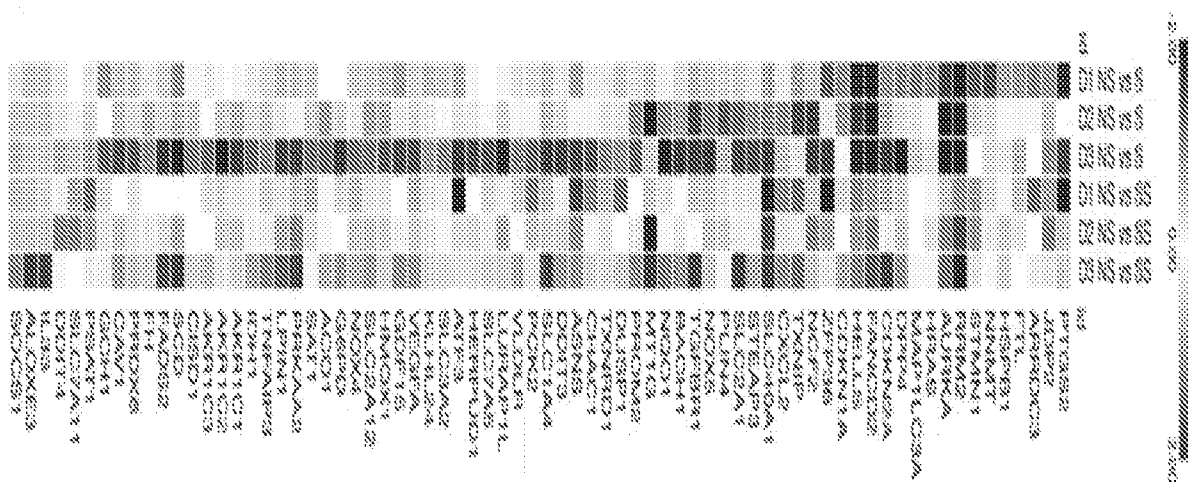


FIG. 4C

FIG. 4D

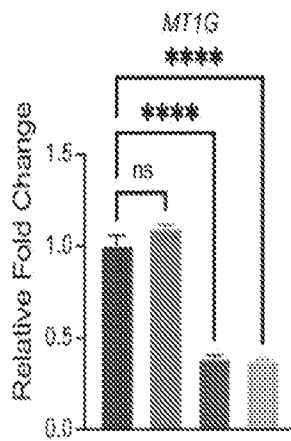


FIG. 4E

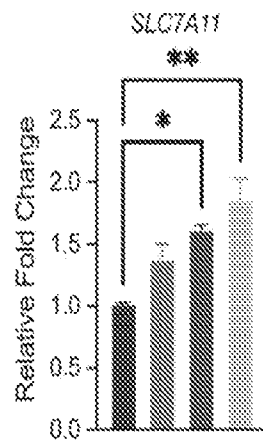


FIG. 4F

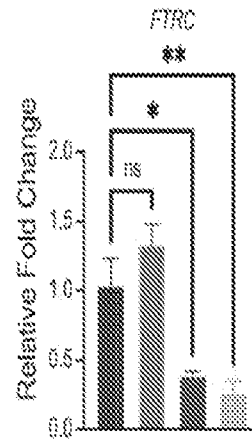
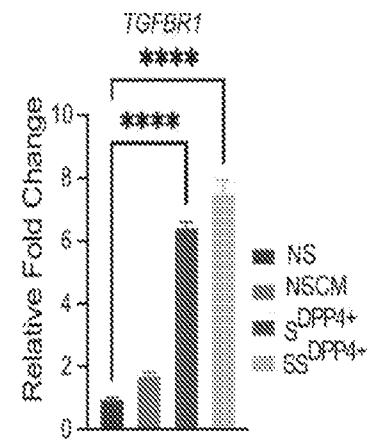


FIG. 4G



Flow cytometry histogram showing Relative Cell Number (Y-axis, 0 to 100%) versus Fluorescence Intensity (X-axis, logarithmic scale from 0 to 10⁷). The histogram compares two cell populations: NS (solid line) and ODF4+ (hatched line). The NS population shows a peak at approximately 10³ fluorescence intensity, while the ODF4+ population shows a peak at approximately 10⁵ fluorescence intensity.

FIG. 4H

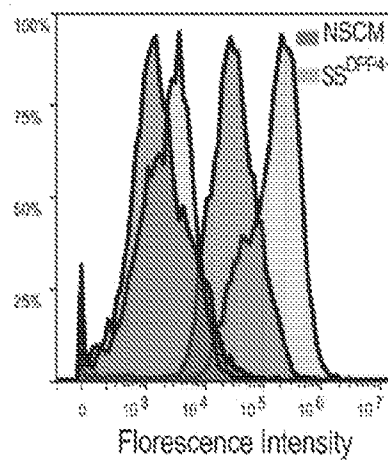


FIG. 4I

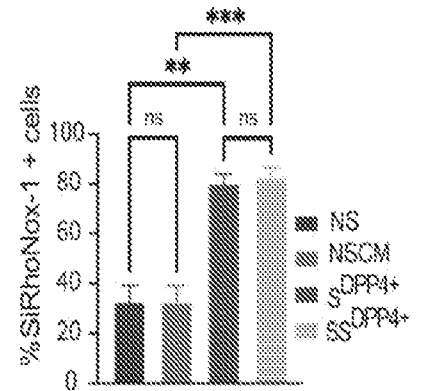


FIG. 4J

FIG. 5A

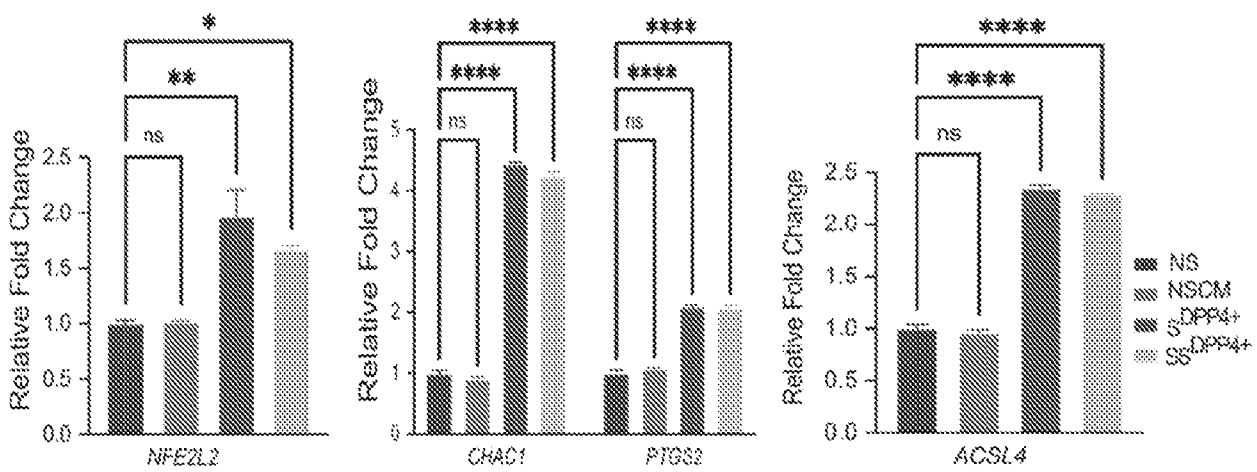
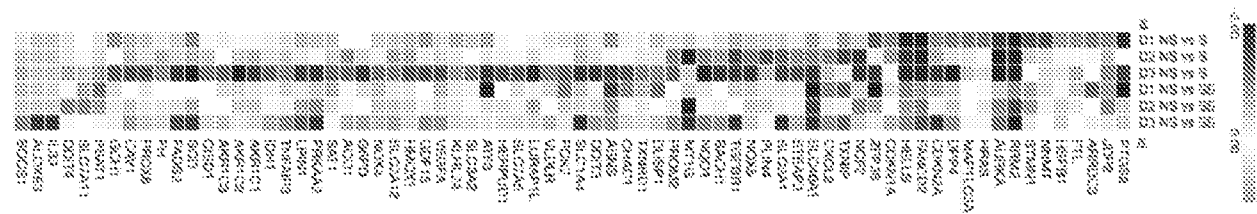


FIG. 5B

FIG. 5C

FIG. 5BD

FIG. 5E

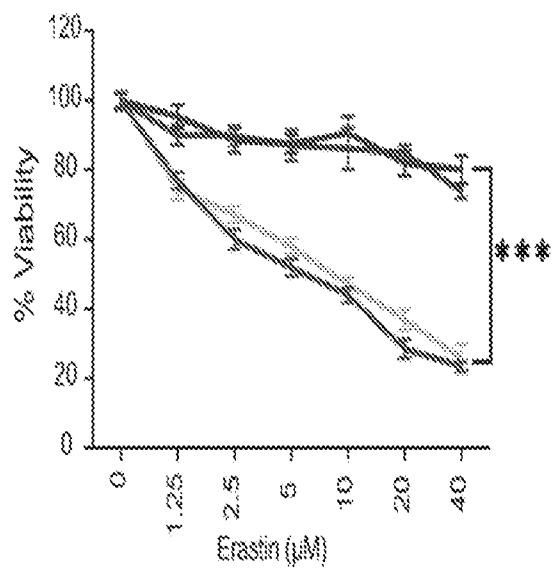


FIG. 5F

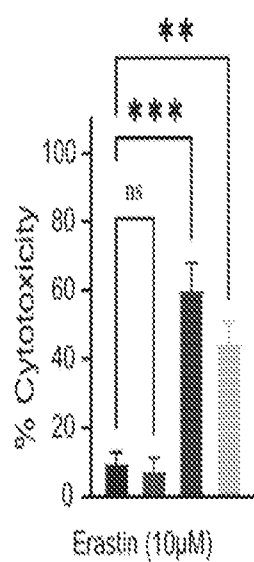
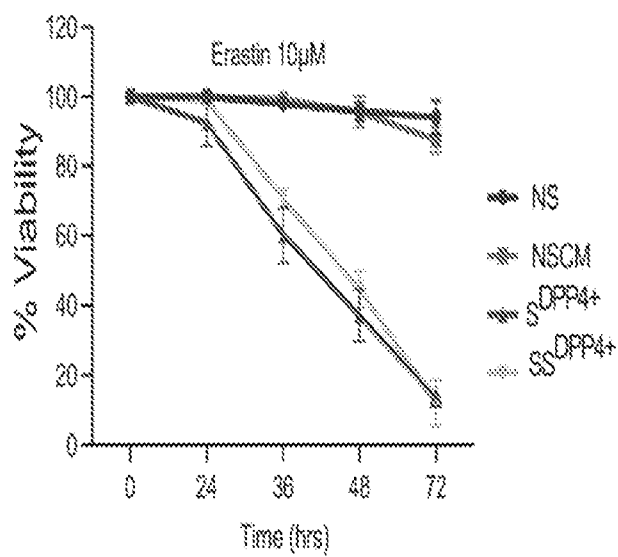


FIG. 5G

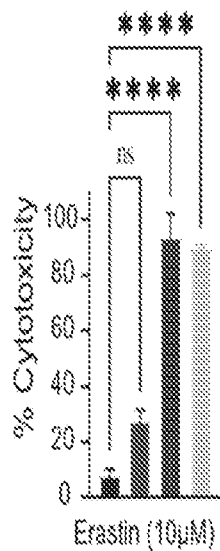


FIG. 5H

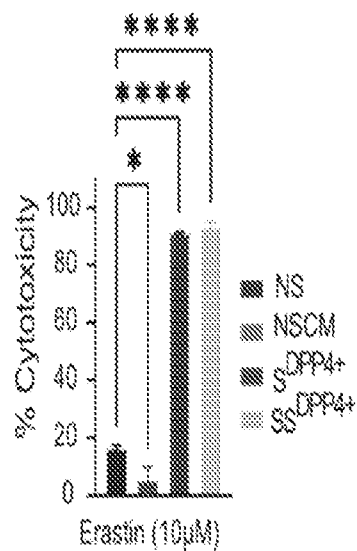


FIG. 5I

FIG. 6A

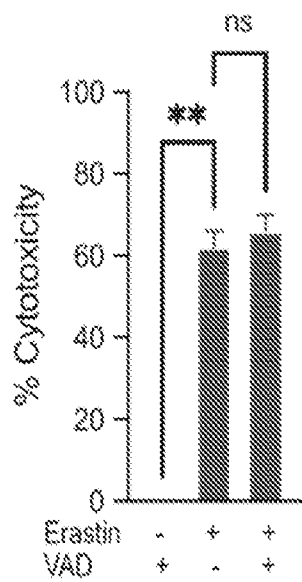
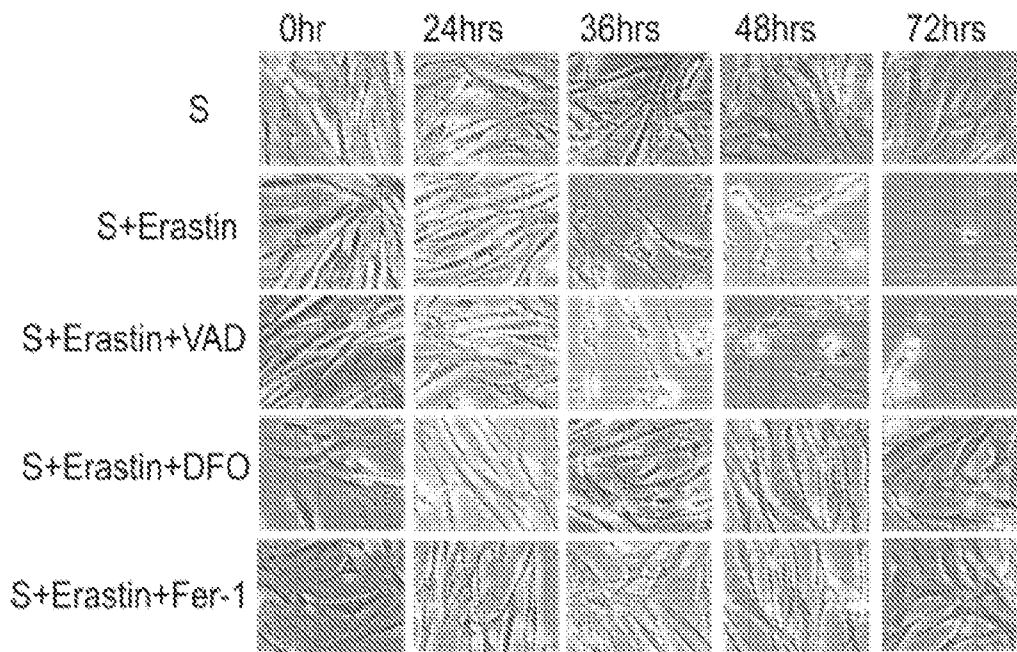


FIG. 6B

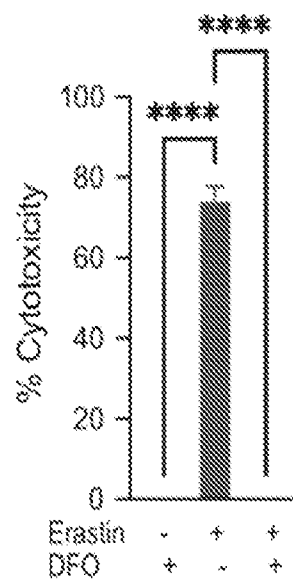


FIG. 6C

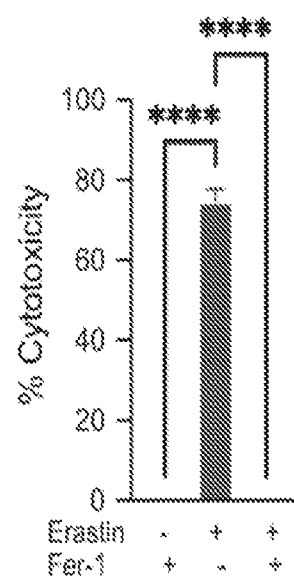


FIG. 6D

FIG. 6E

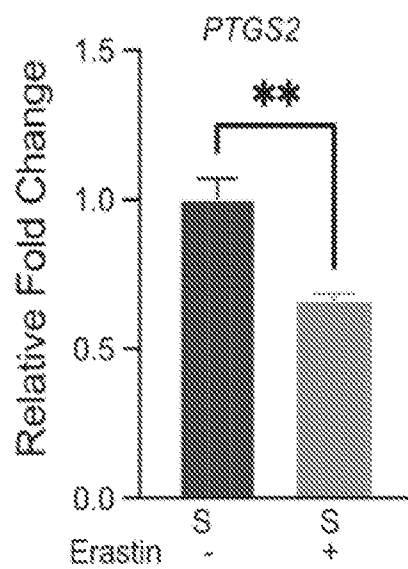


FIG. 6F

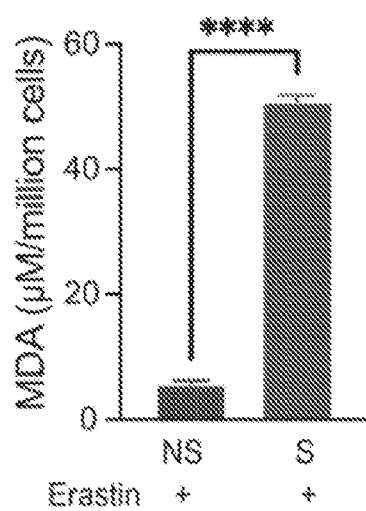
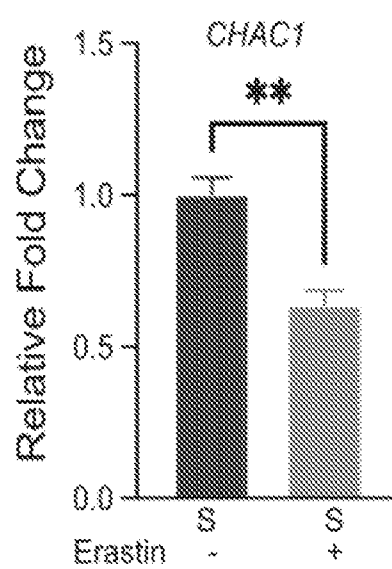


FIG. 6G

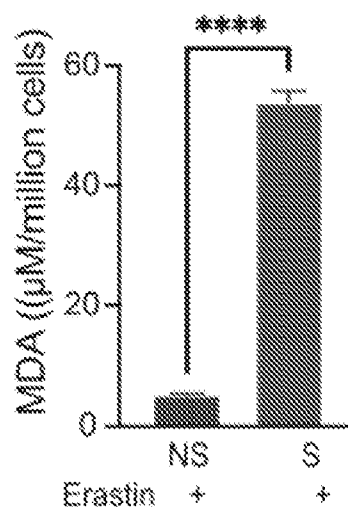


FIG. 6G

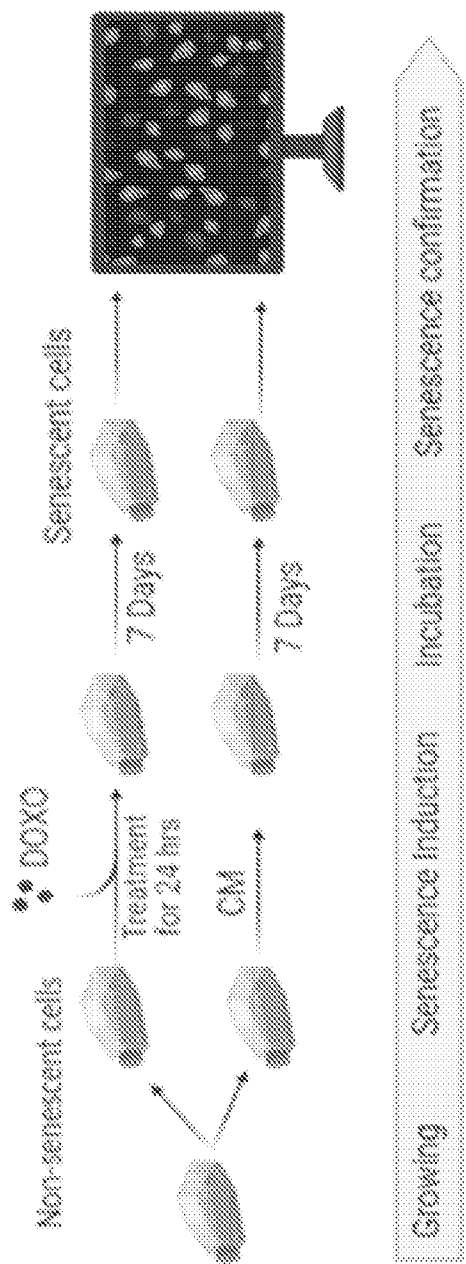


FIG. 7A

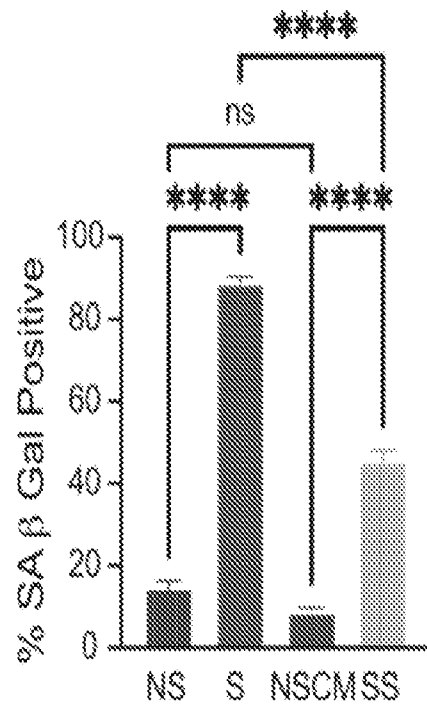


FIG. 7B

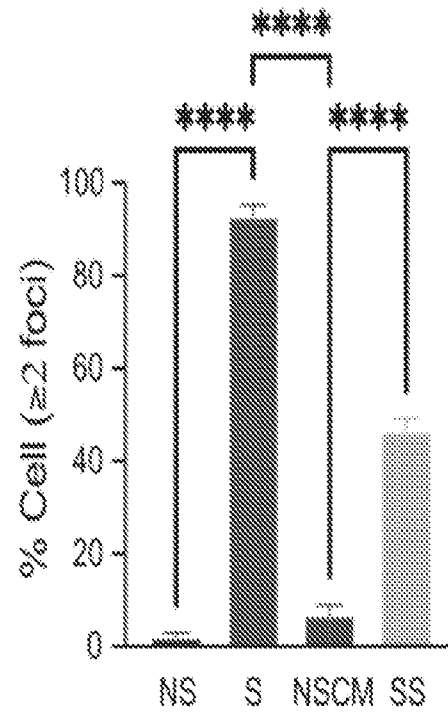


FIG. 7C

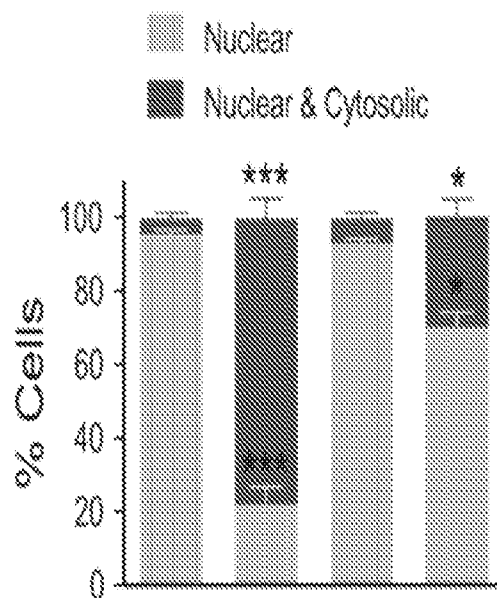


FIG. 7D

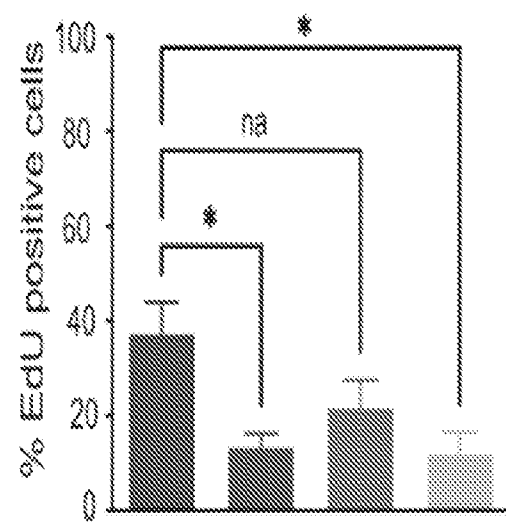


FIG. 7E

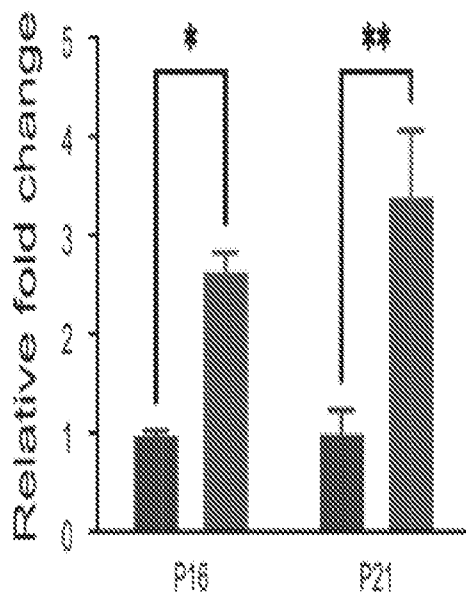


FIG. 7F

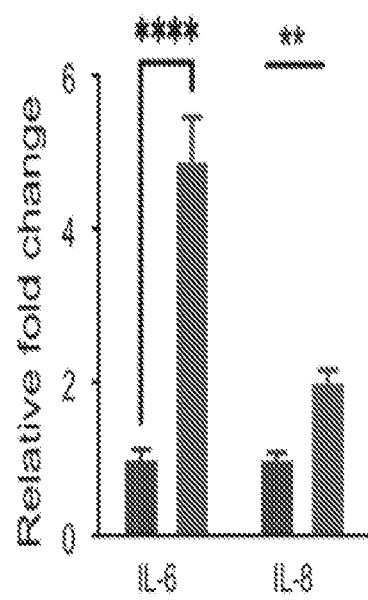


FIG. 7G

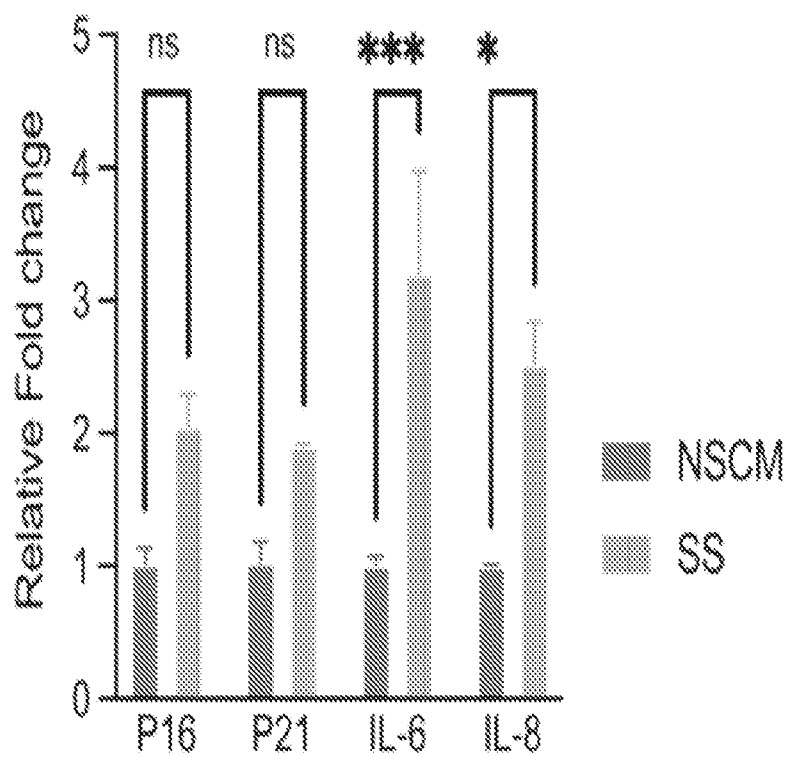


FIG. 7H

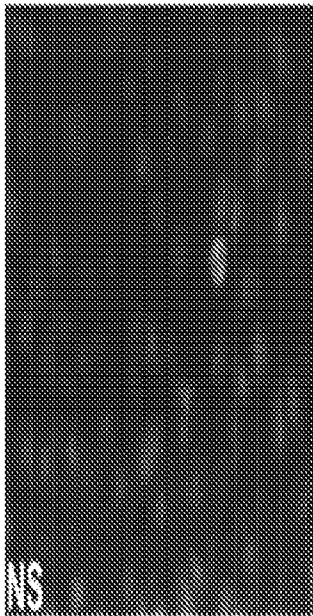


FIG. 8G

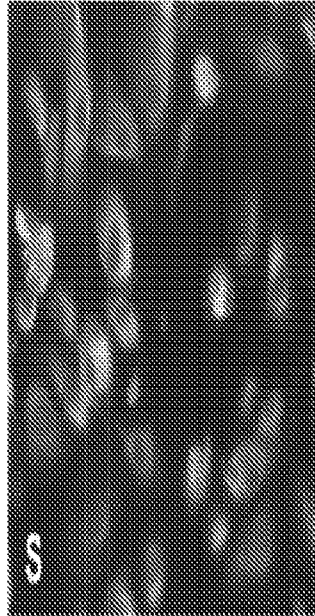


FIG. 8H

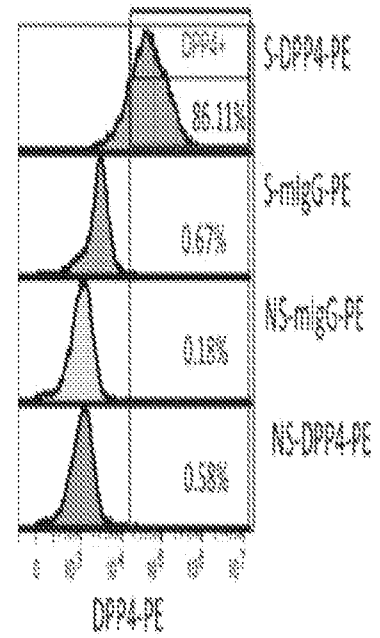


FIG. 8I

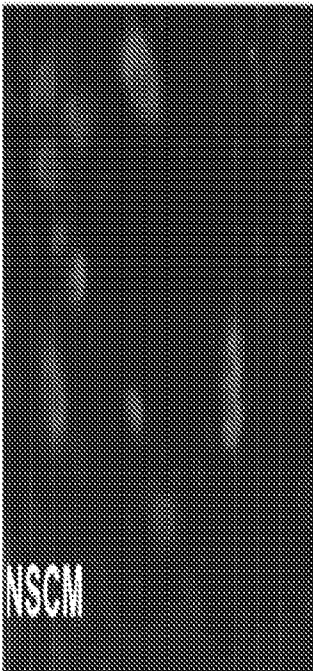


FIG. 8J

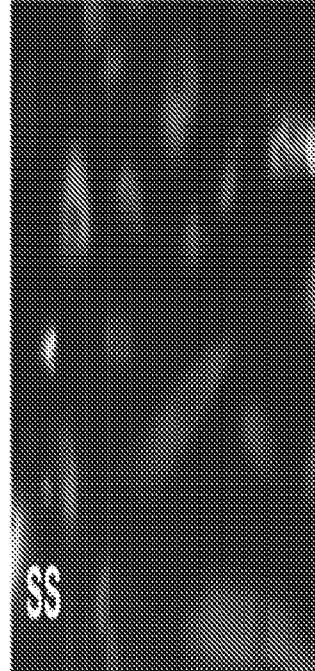


FIG. 8K

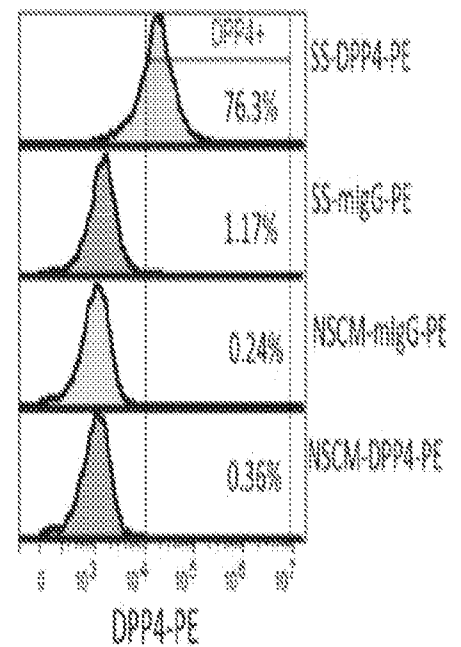


FIG. 8L

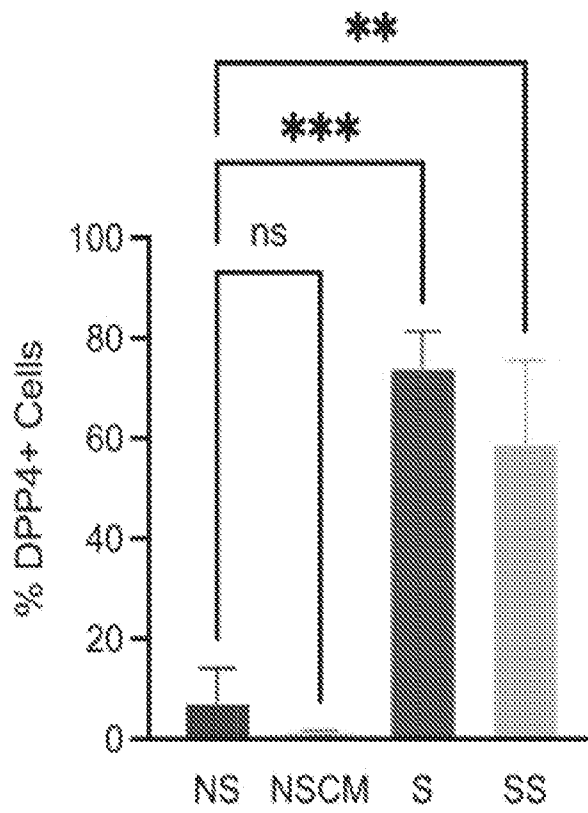


FIG. 8M

FIG. 8

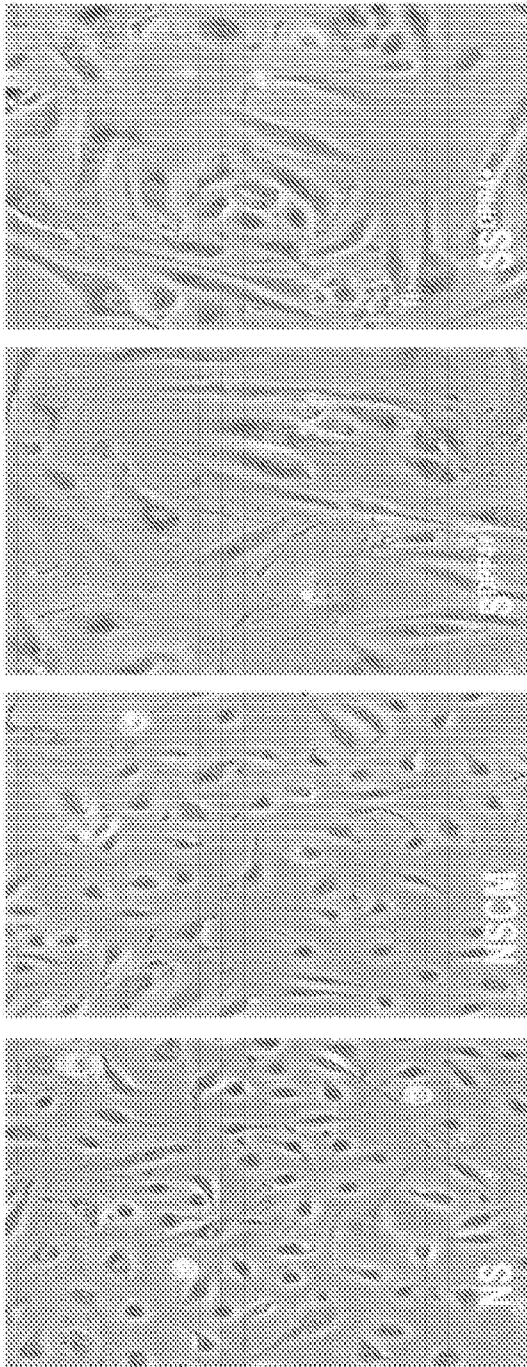


FIG. 9A

FIG. 9B

FIG. 9C

FIG. 9D

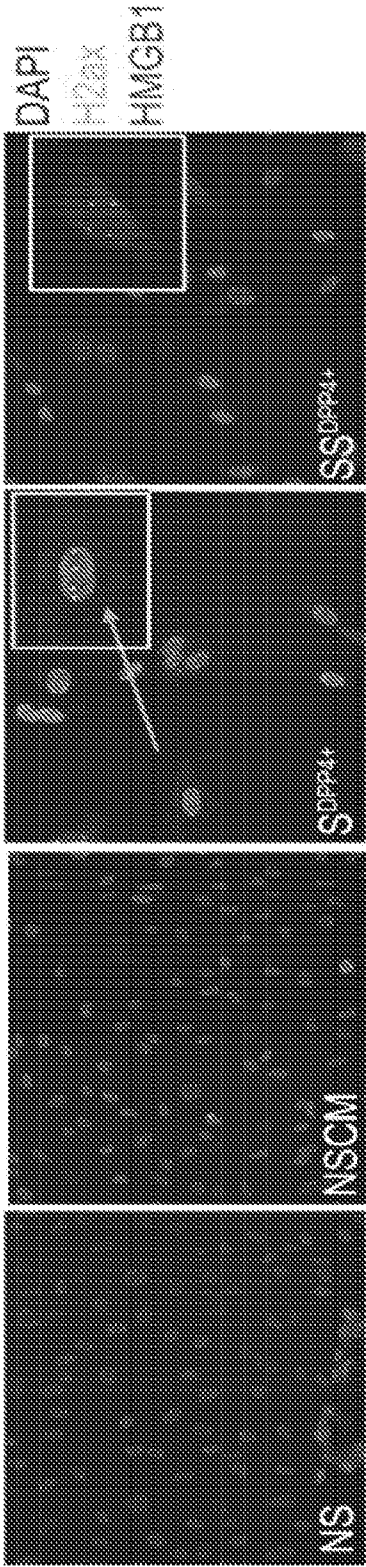


FIG. 9E

FIG. 9F

FIG. 9G

FIG. 9H

FIG. 9I

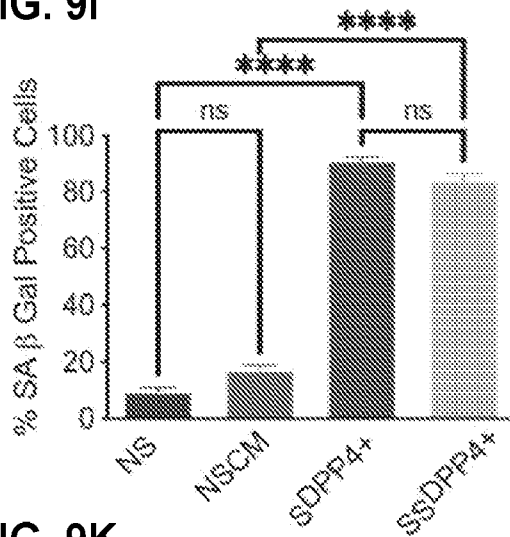


FIG. 9J

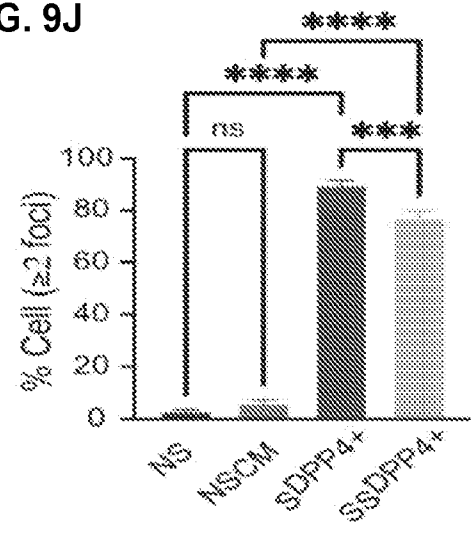


FIG. 9K

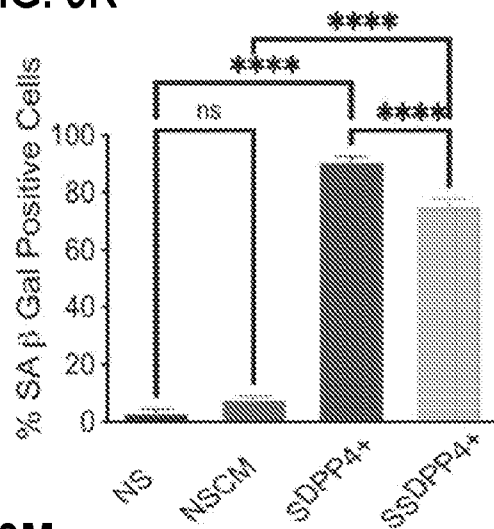


FIG. 9L

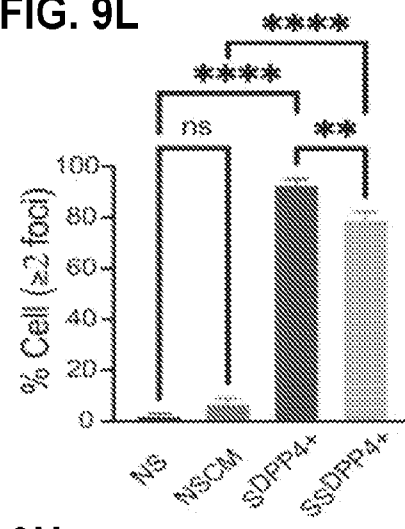


FIG. 9M

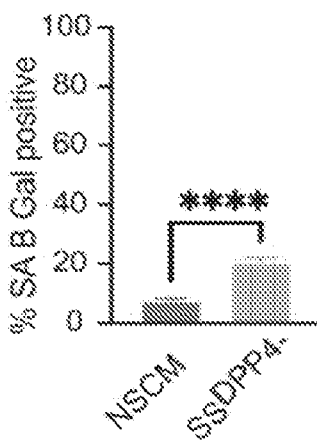
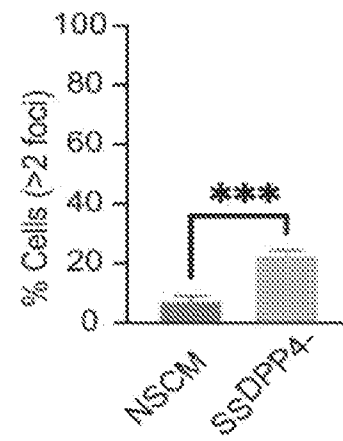


FIG. 9N



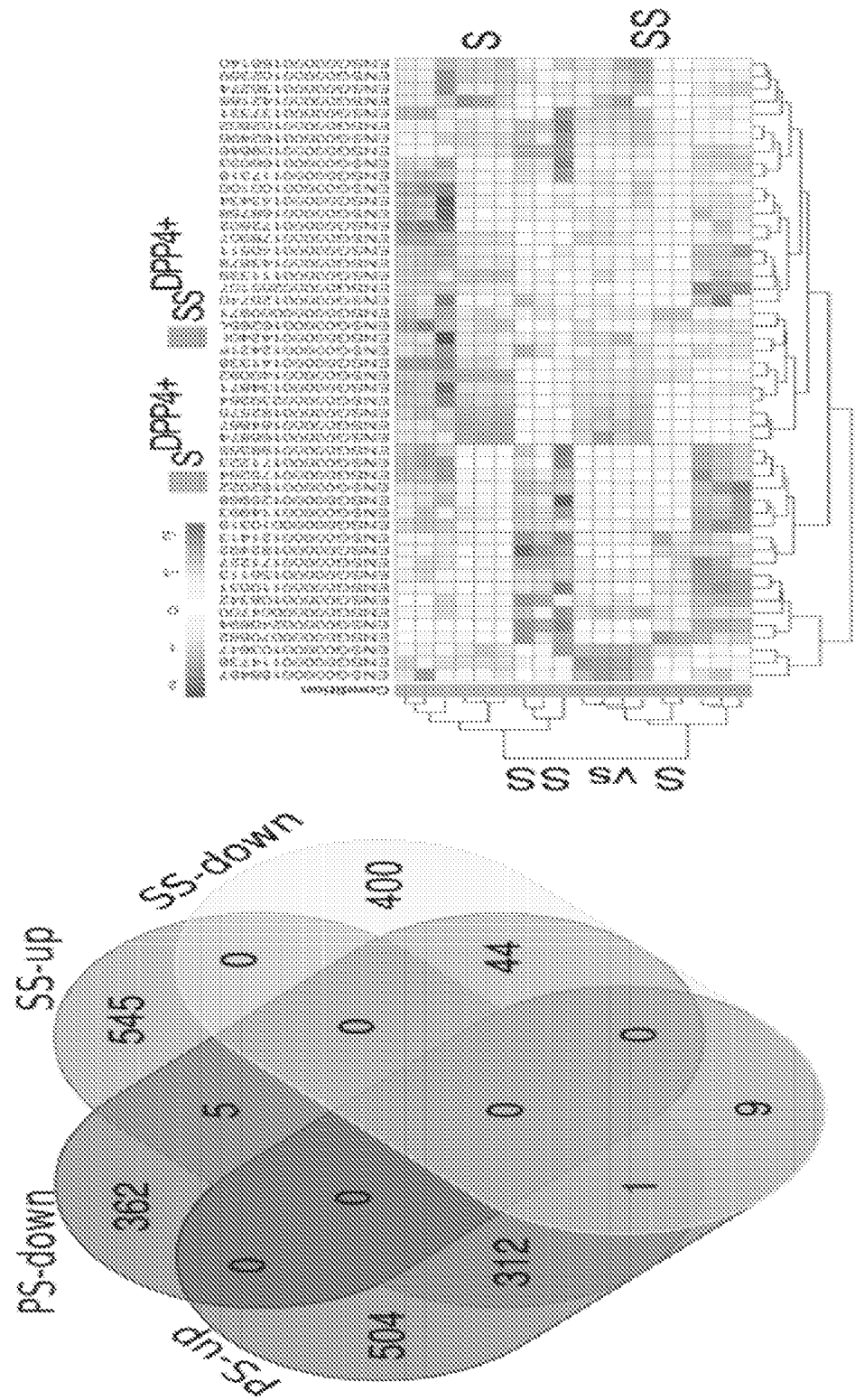


FIG. 10B

FIG. 10A

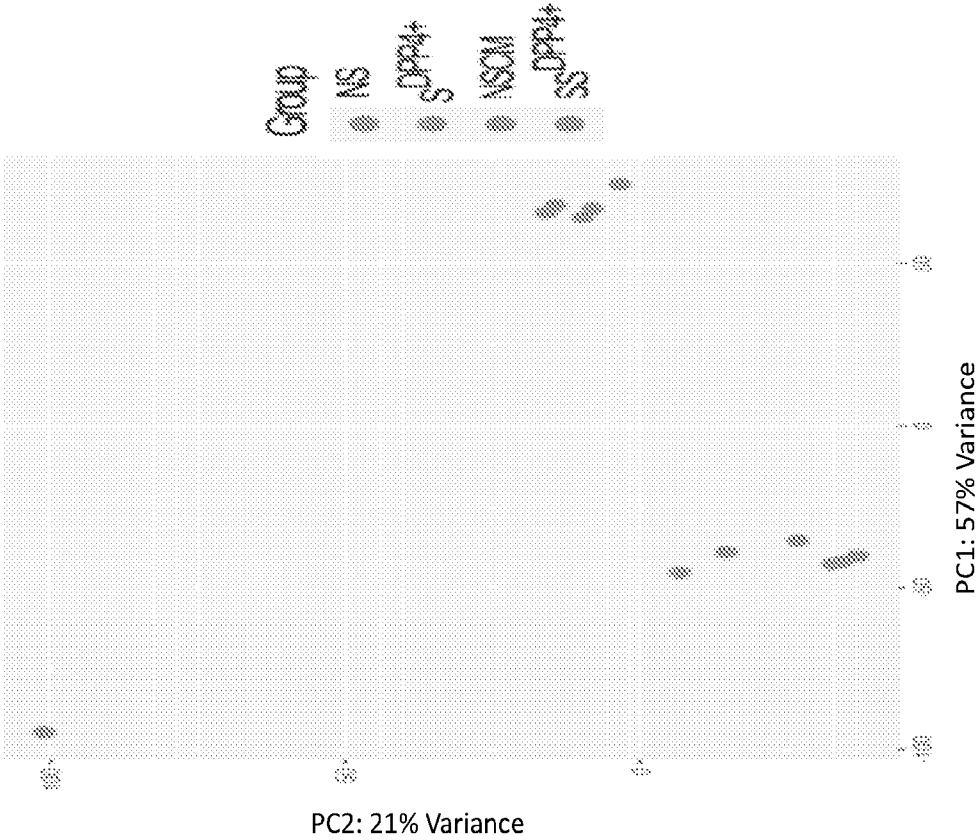


FIG. 10D

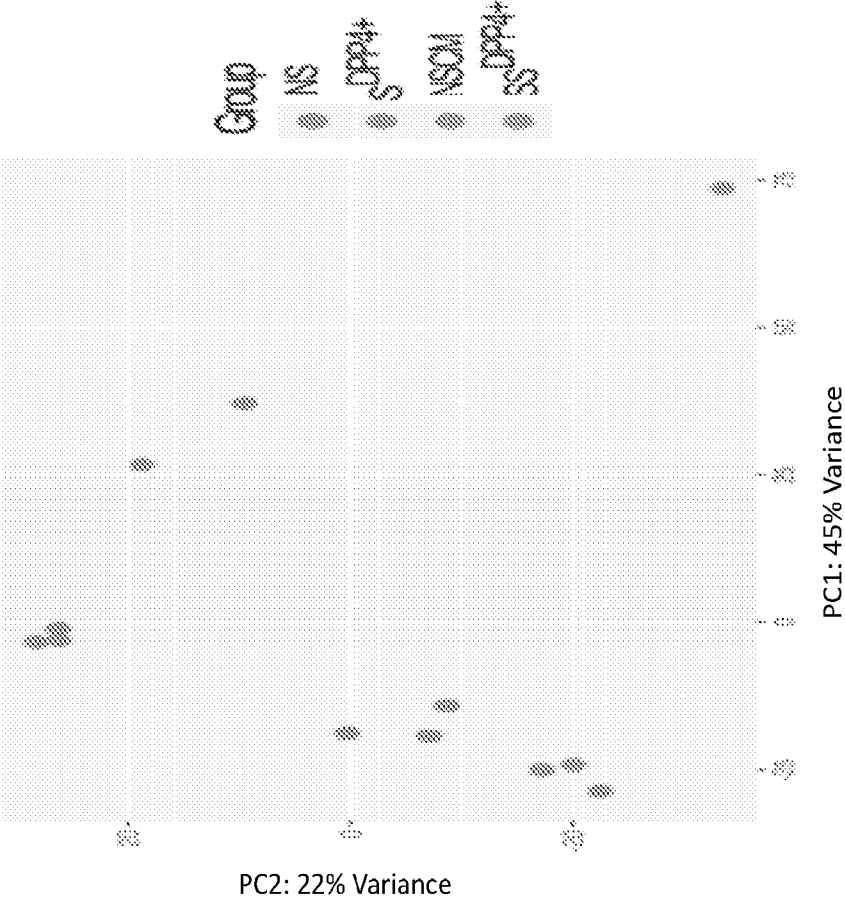
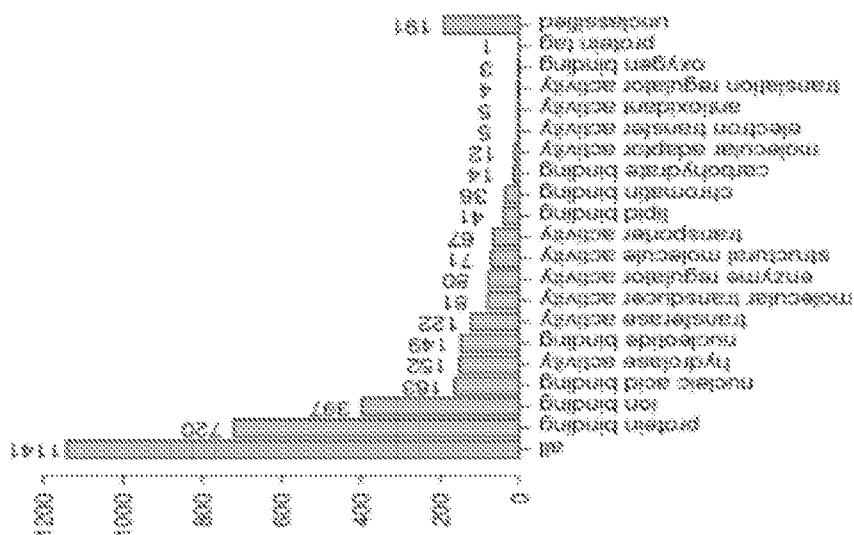
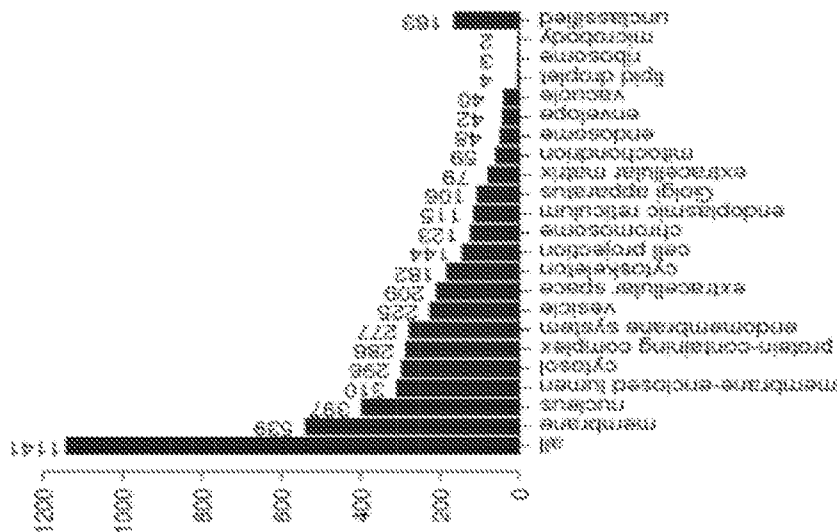


FIG. 10C

Bar Chart of Molecular Function Categories



Bar Chart of Cellular Component Categories



Bar Chart of Biological Process Categories

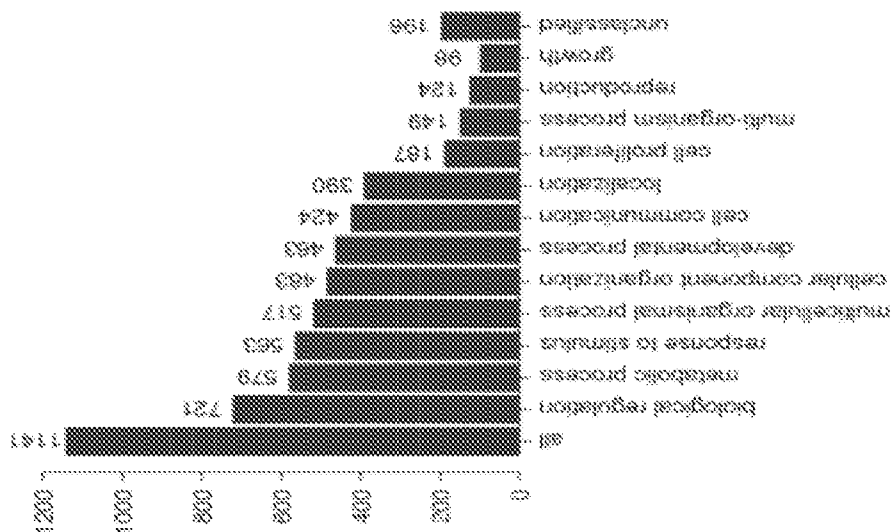
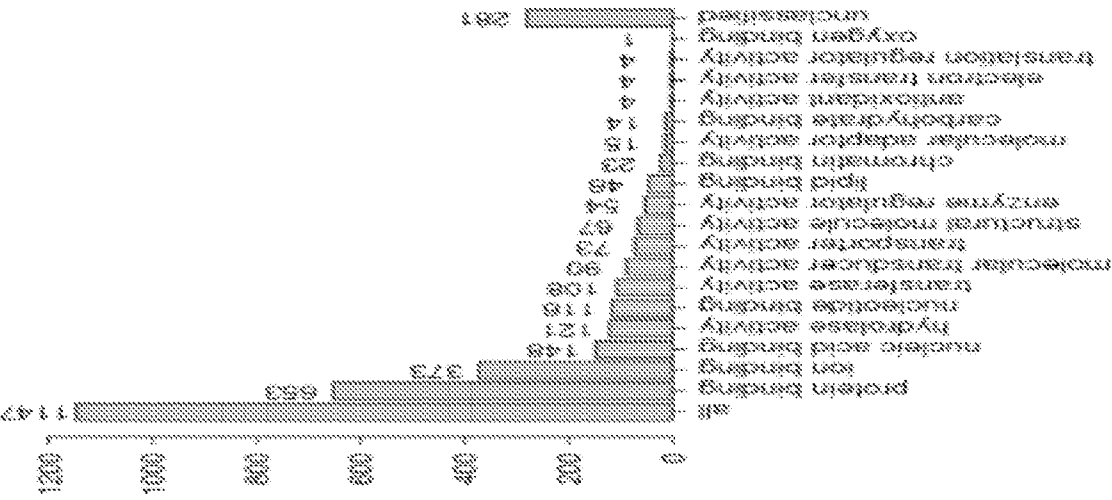


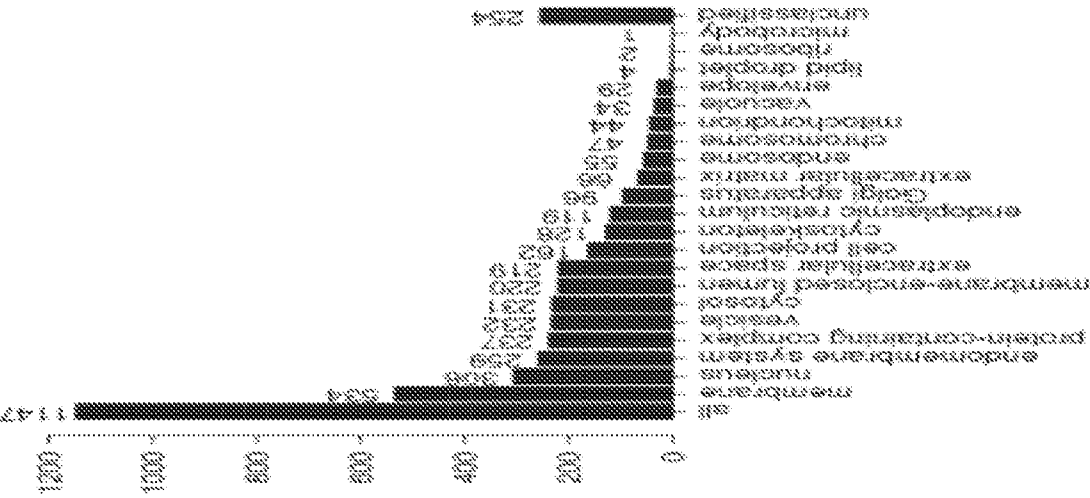
FIG. 10E

FIG. 10F

Bar Chart of Molecular Function Categories



Bar Chart of Cellular Component Categories



Bar Chart of Biological Process Categories

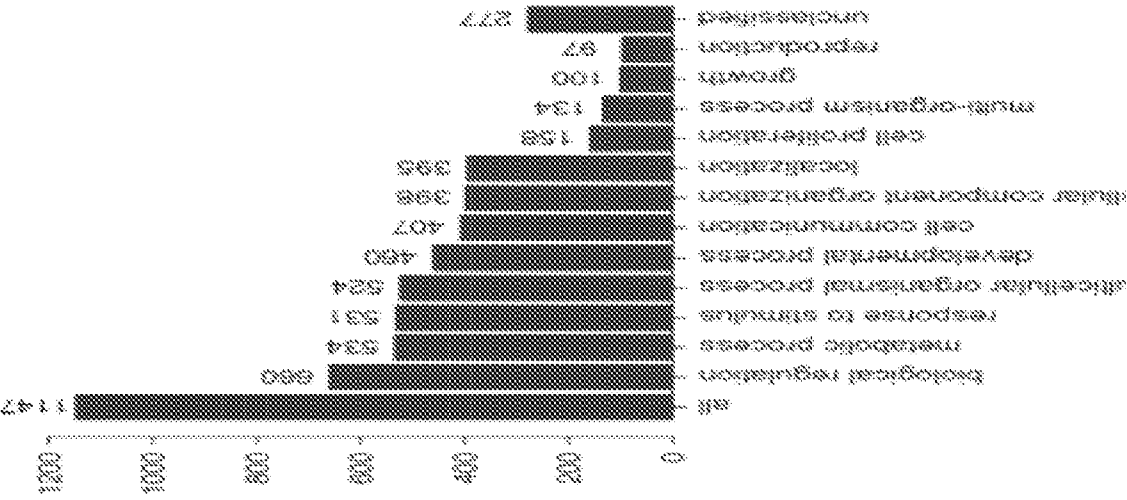


FIG. 11A

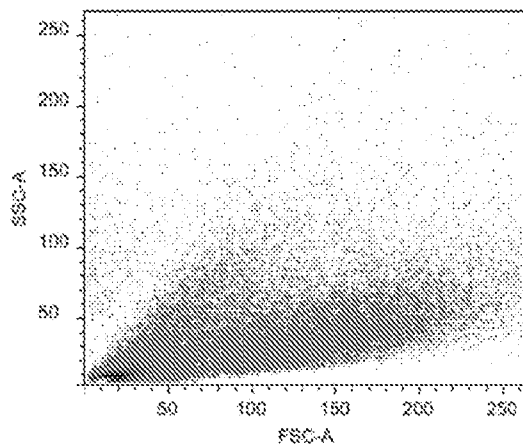


FIG. 11B

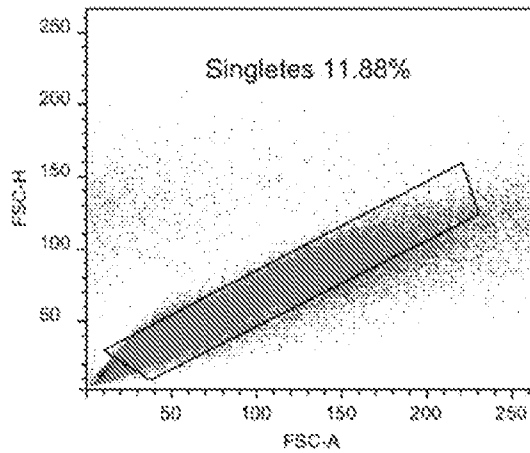


FIG. 11C

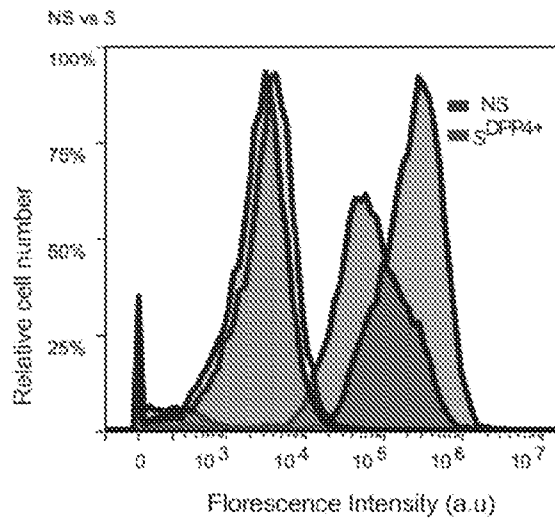


FIG. 11D

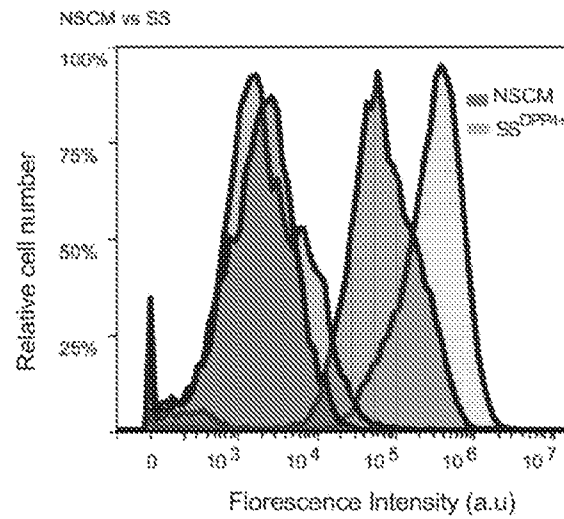


FIG. 11E

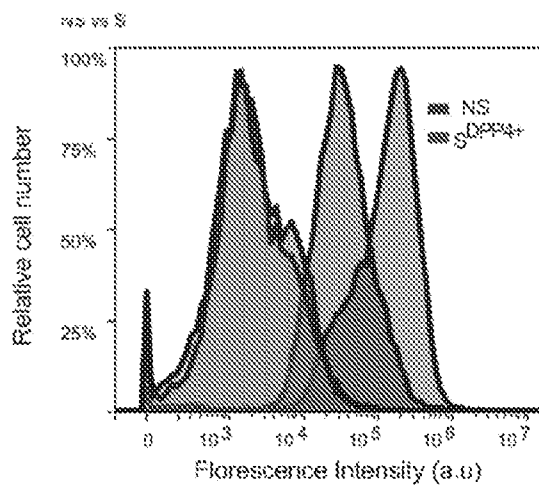
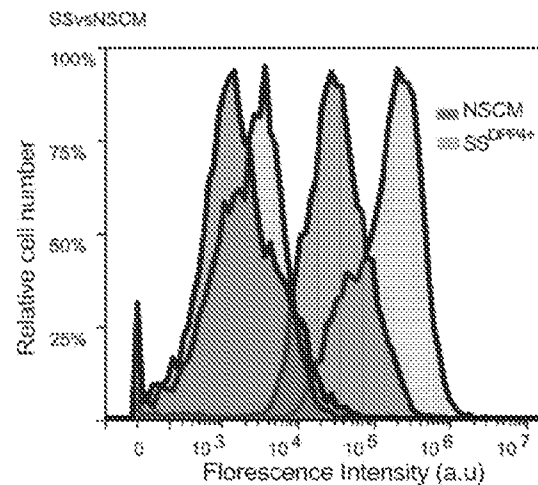


FIG. 11F



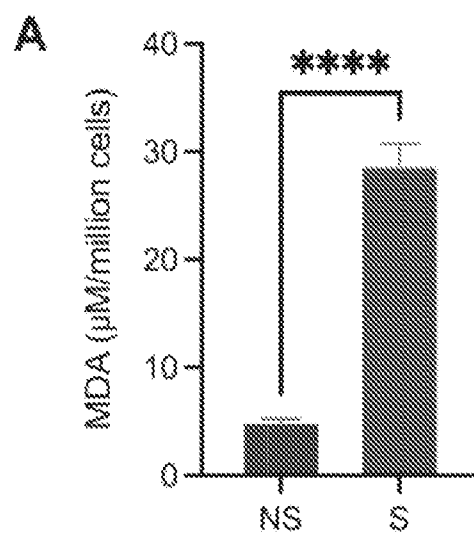


FIG. 12A

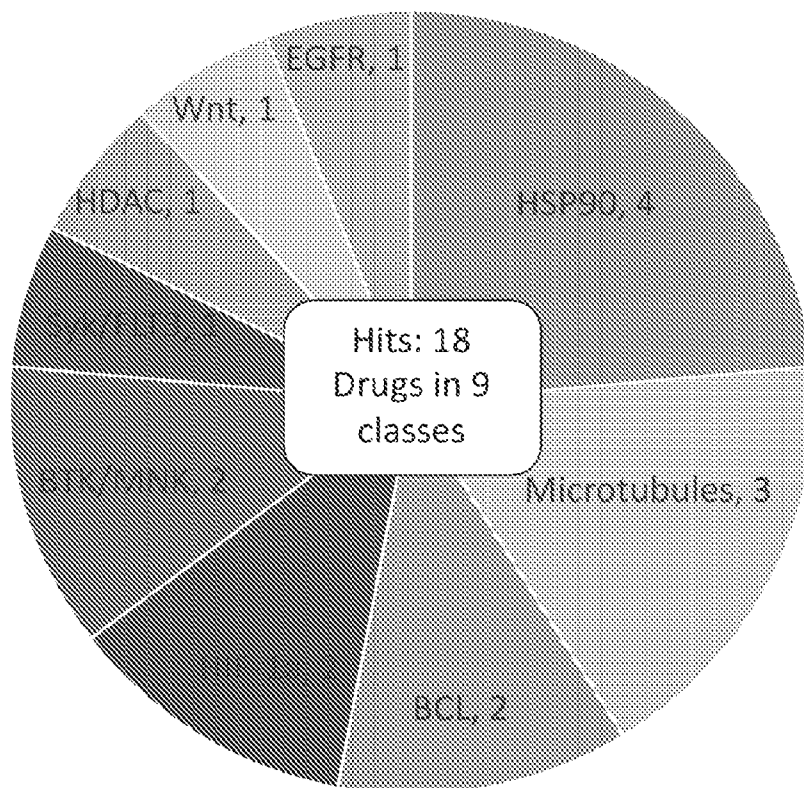


FIG. 13

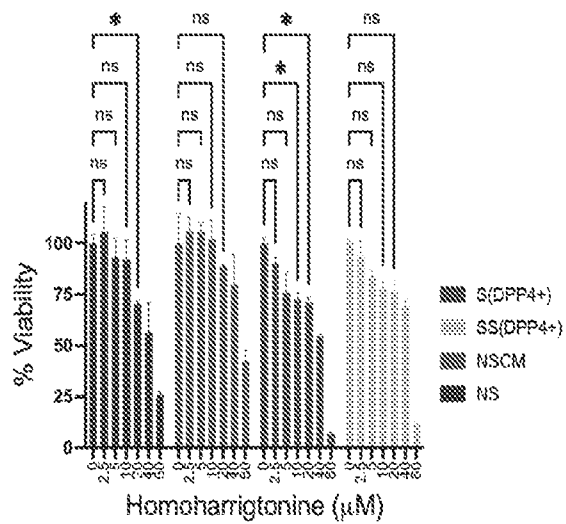


FIG. 14A

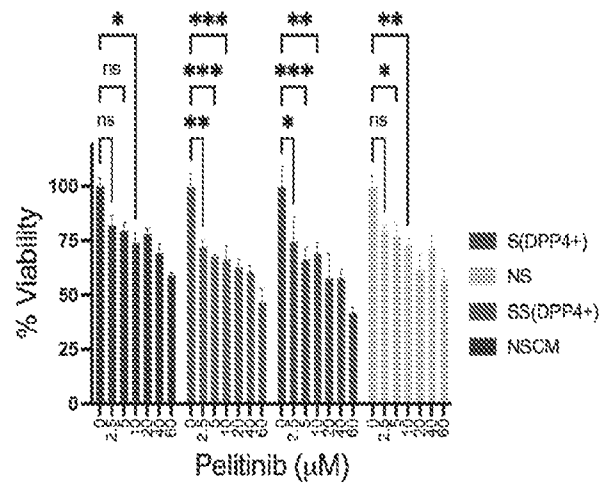


FIG. 14B

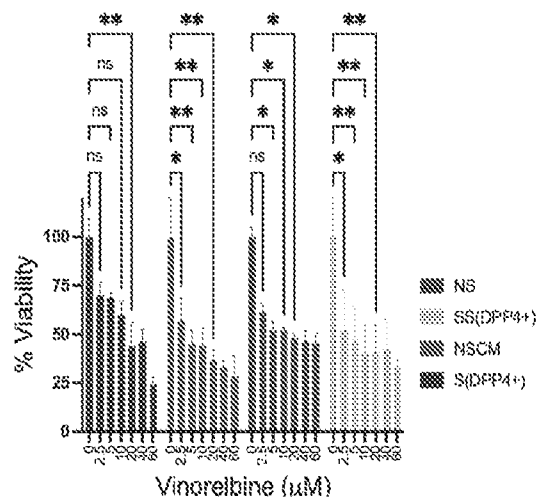


FIG. 14C

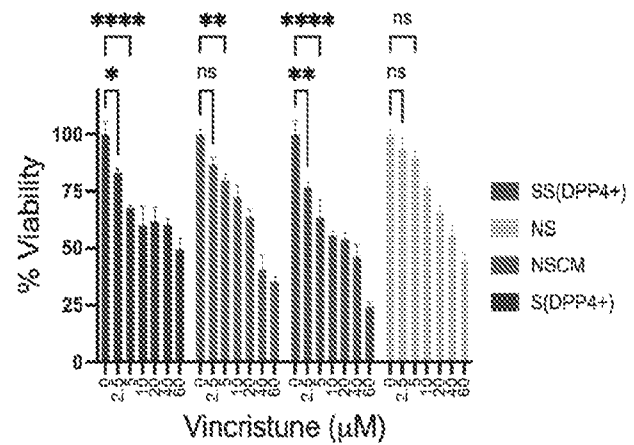


FIG. 14D

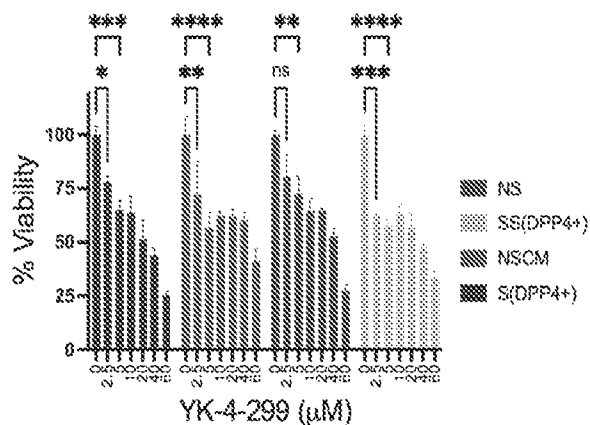


FIG. 15A

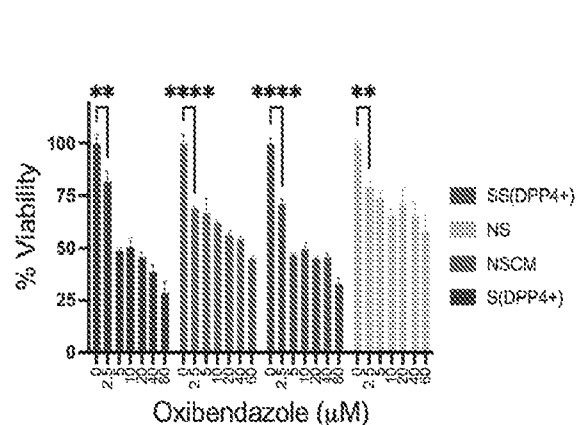


FIG. 15B

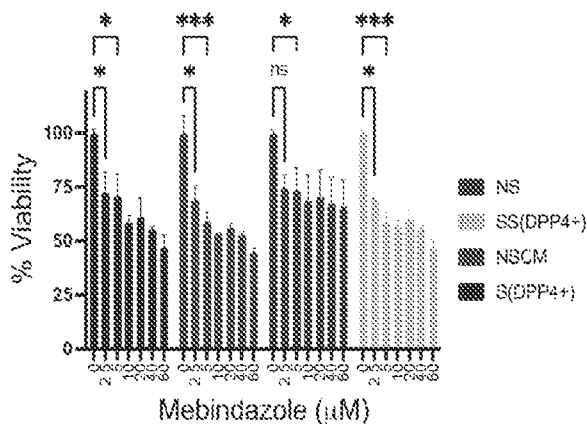


FIG. 15C

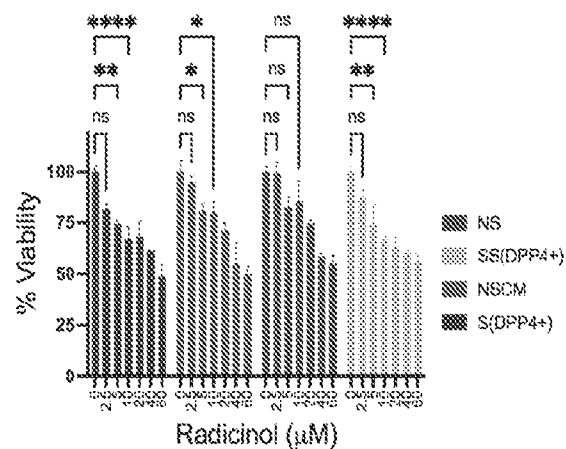


FIG. 15D

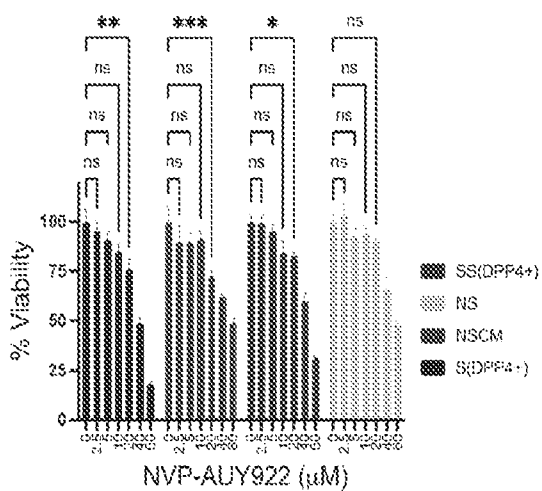


FIG. 15E

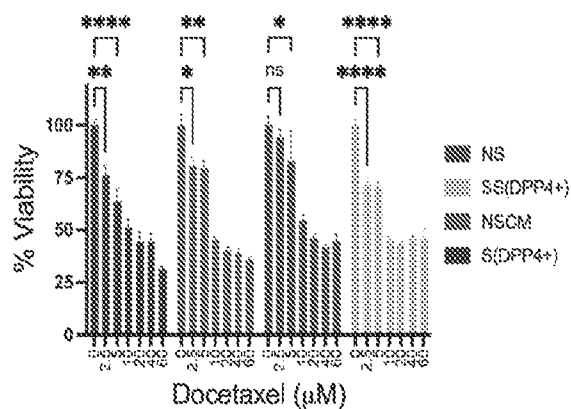


FIG. 15F

Senolytics optimization using Xcellegence machine -
Vorinostat

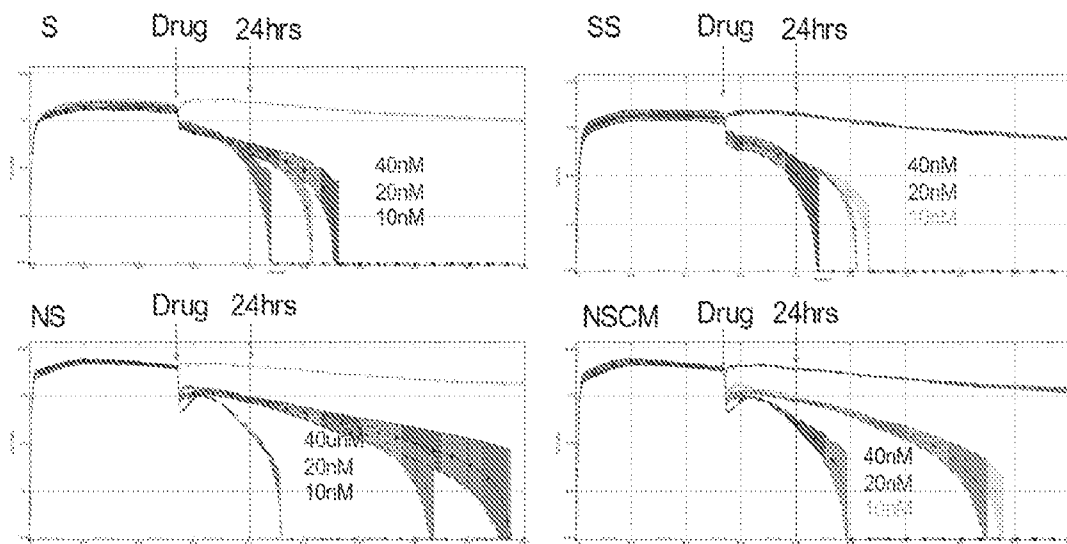


FIG. 16A

Senolytics optimization using Xcellegence machine -
Emetine

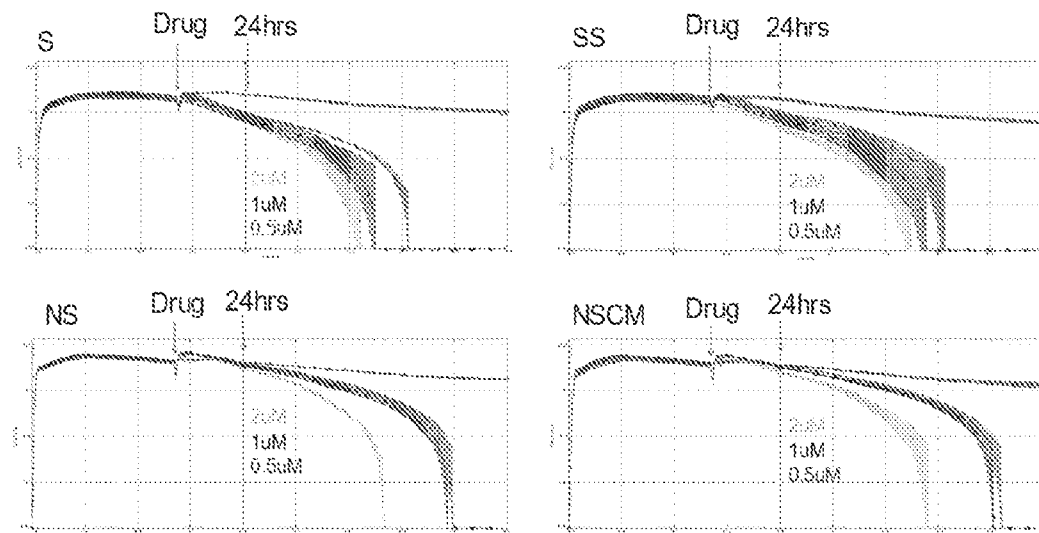


FIG. 16B

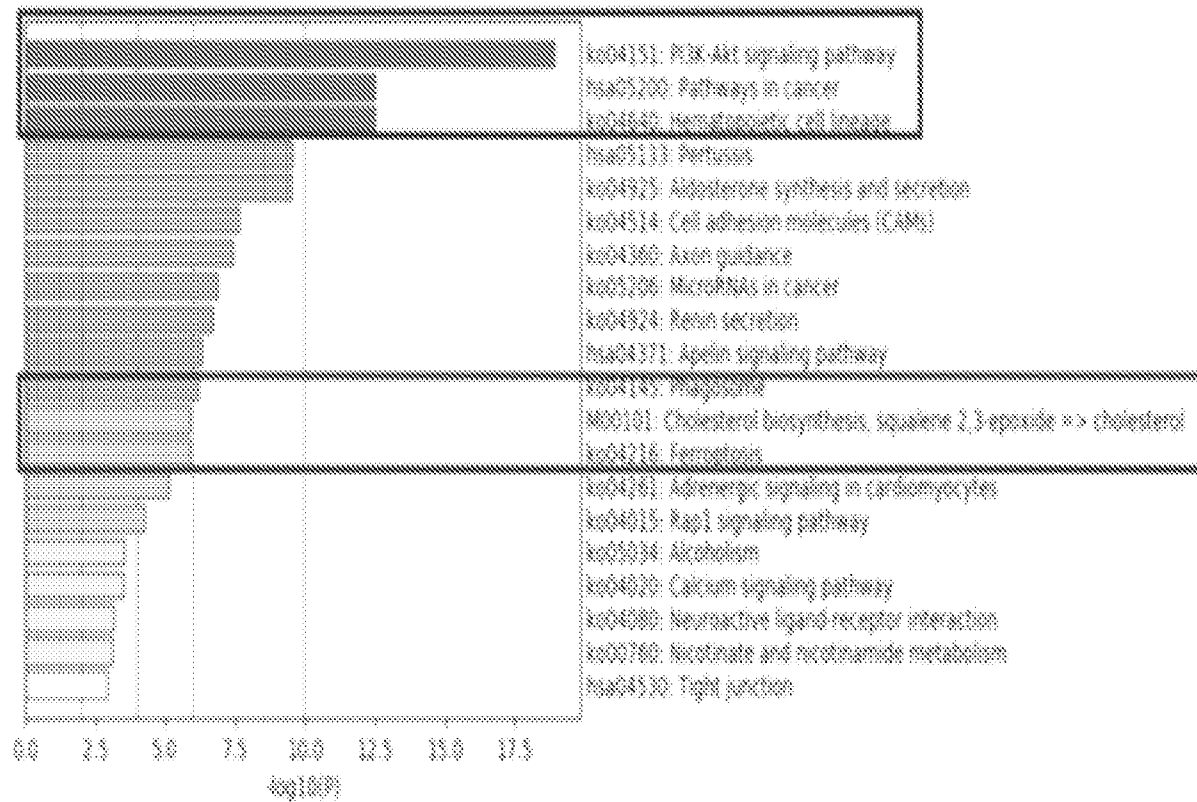


FIG. 17A

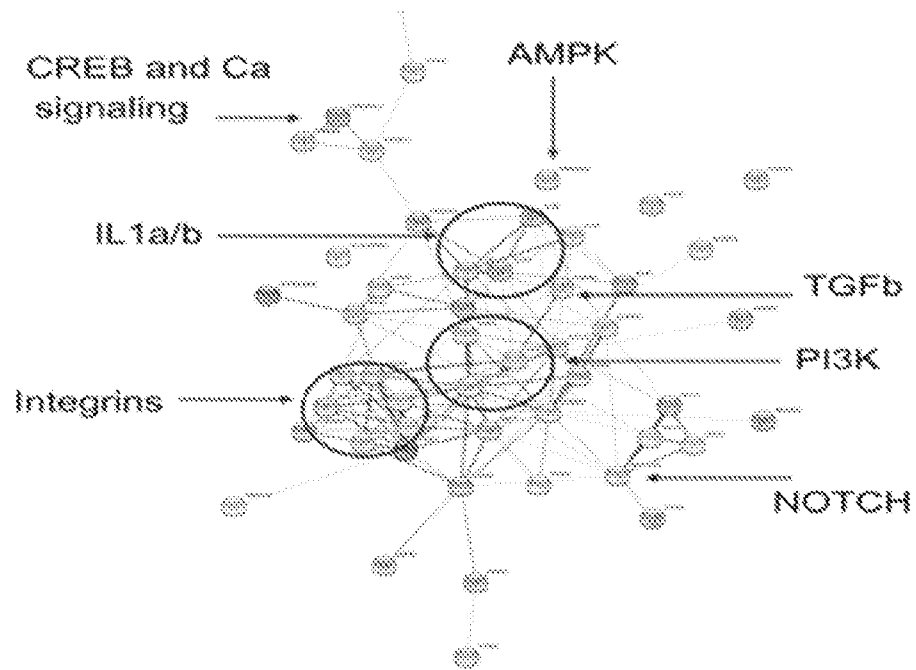


FIG. 17B

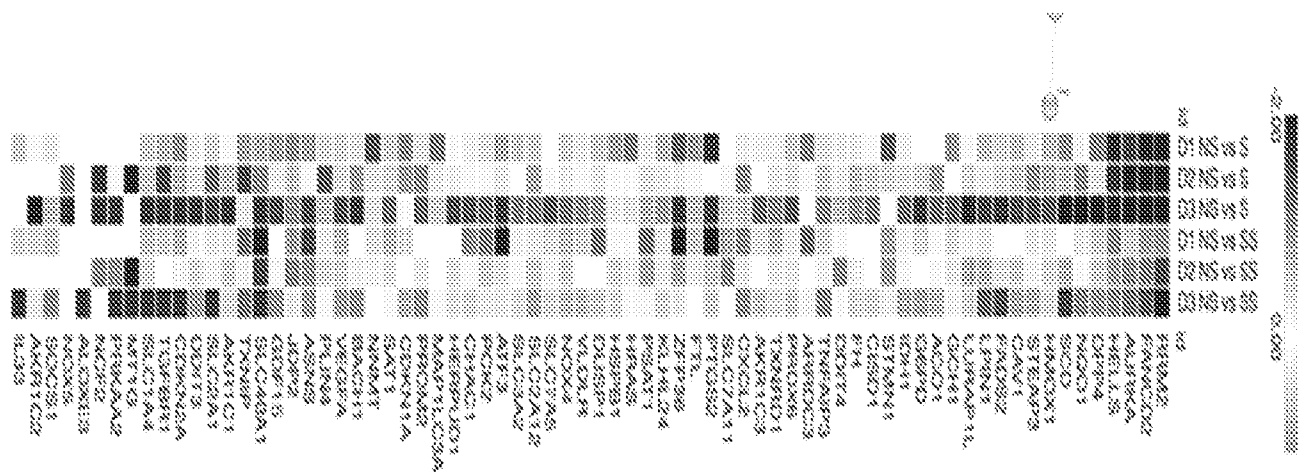


FIG. 17C

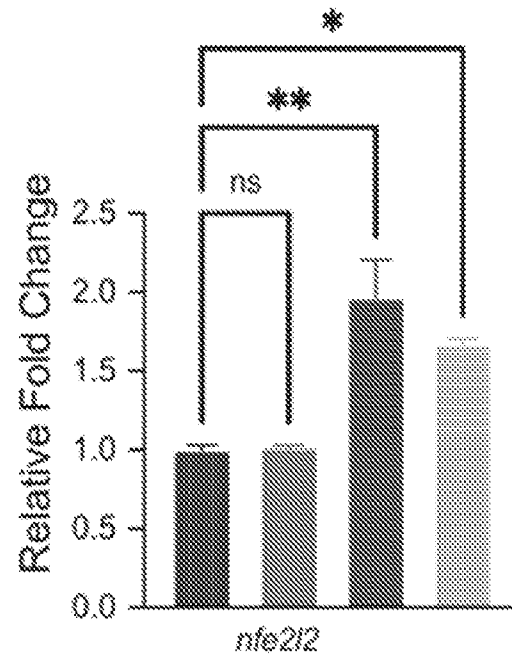


FIG. 17D

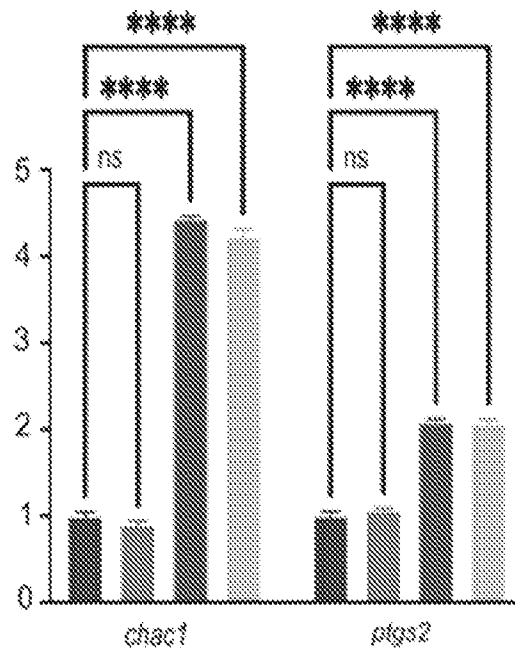


FIG. 17E

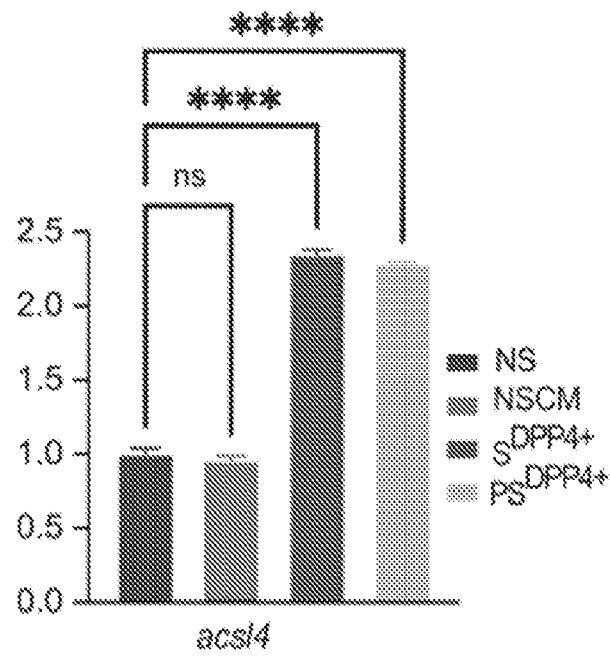
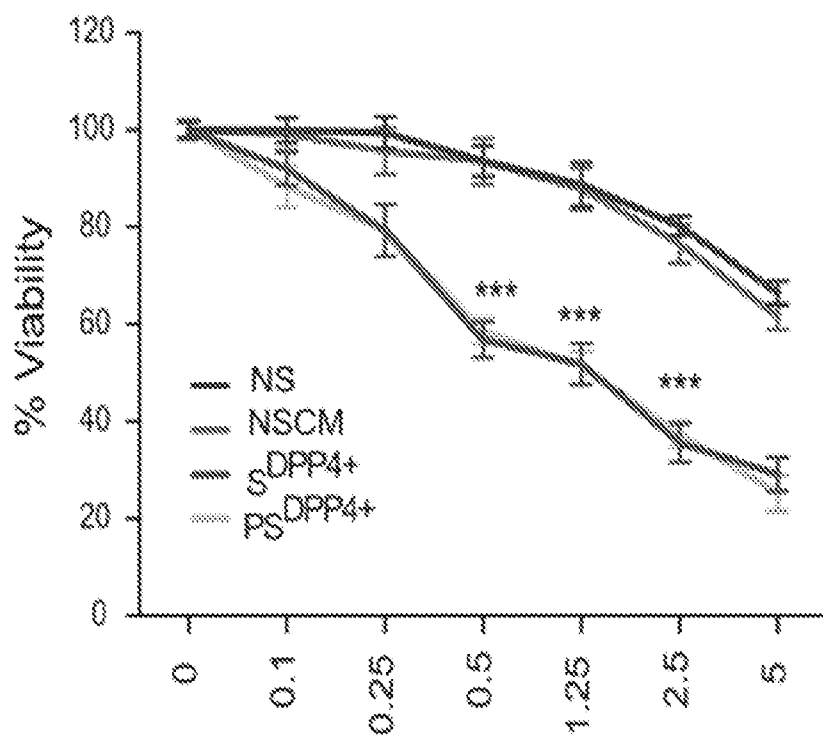
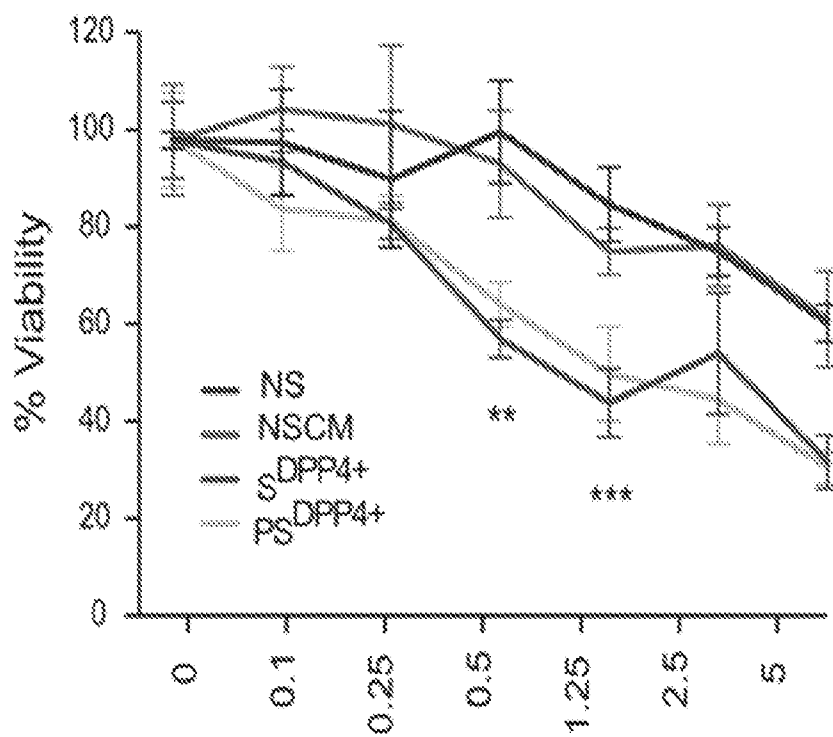


FIG. 17F

FIG. 18A - FIN56 (μM)FIG. 18B - FIN56 (μM)

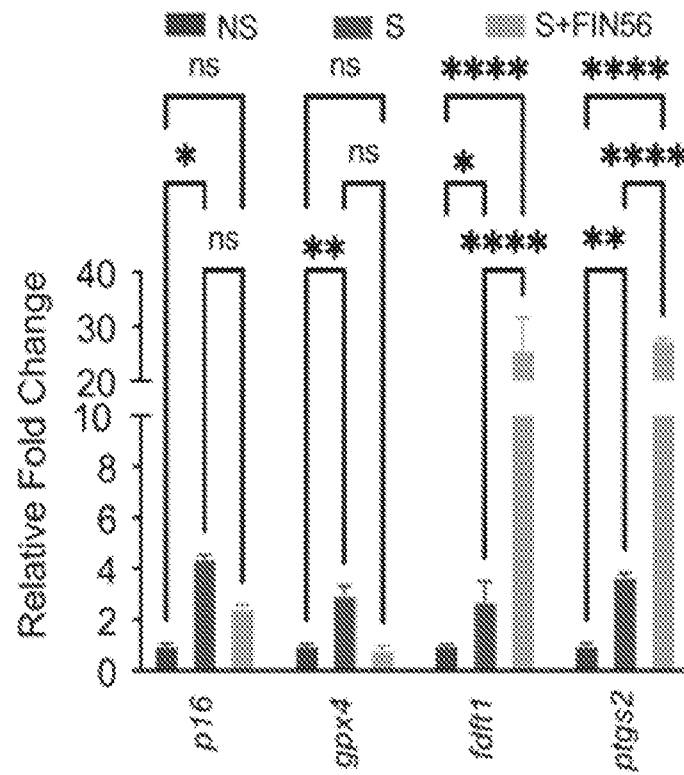


FIG. 18C

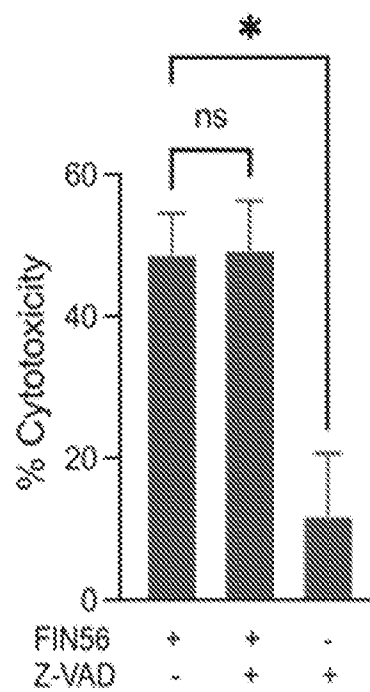


FIG. 18D

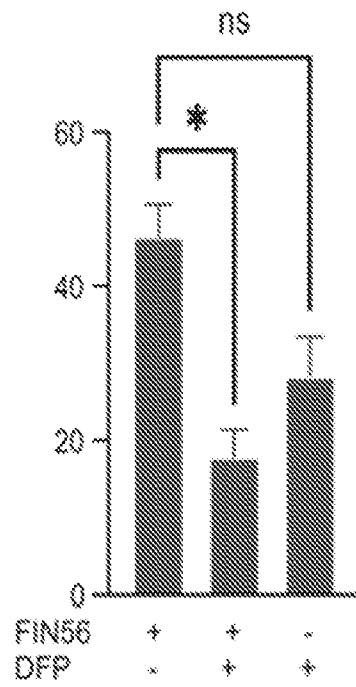


FIG. 18E

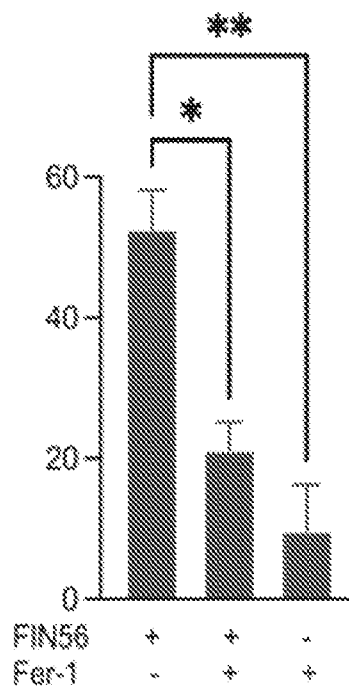


FIG. 18F

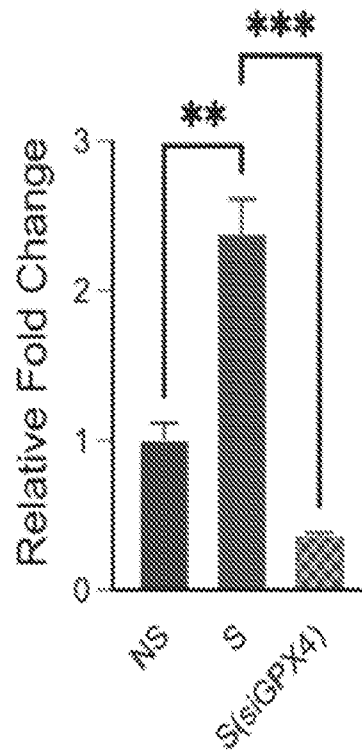


FIG. 18G

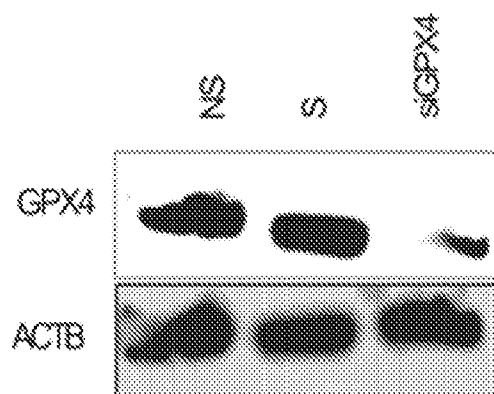


FIG. 18H

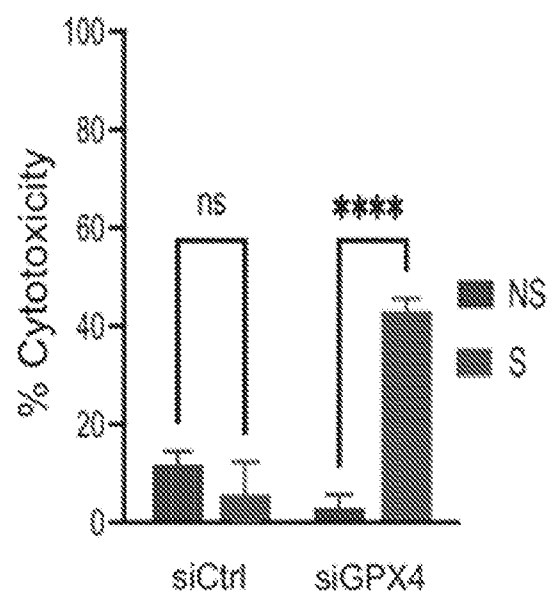


FIG. 18I

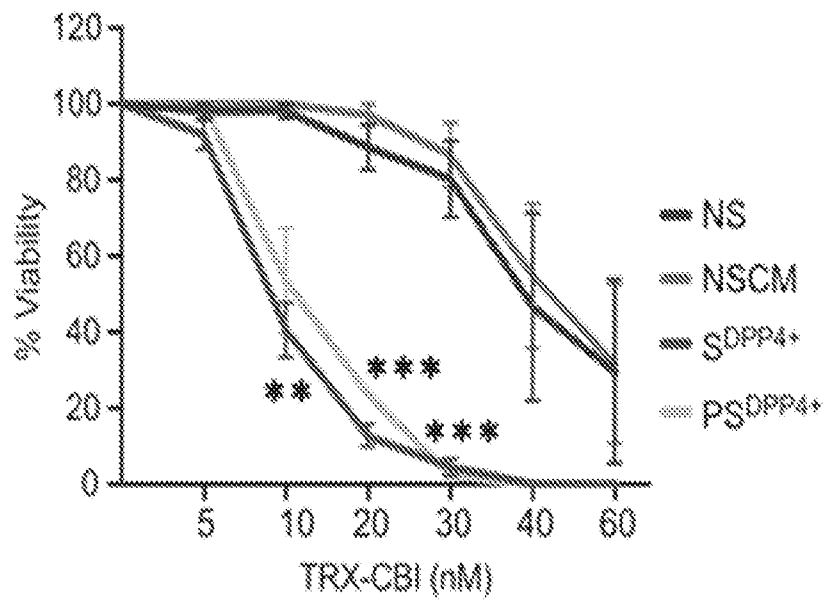


FIG. 19A

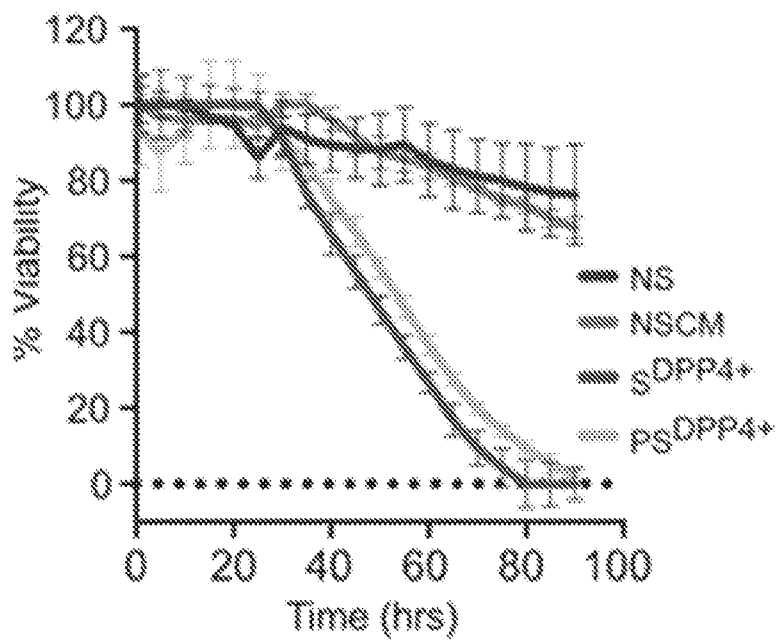


FIG. 19B

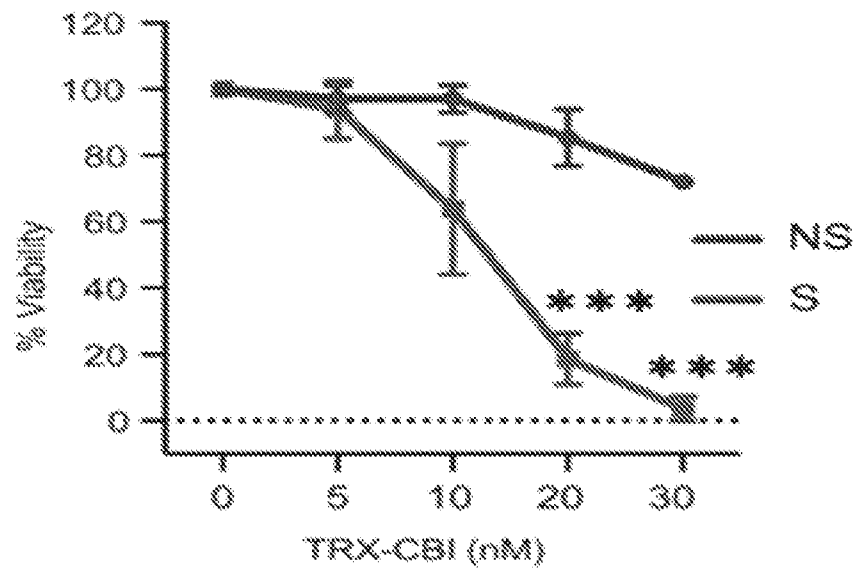


FIG. 19C

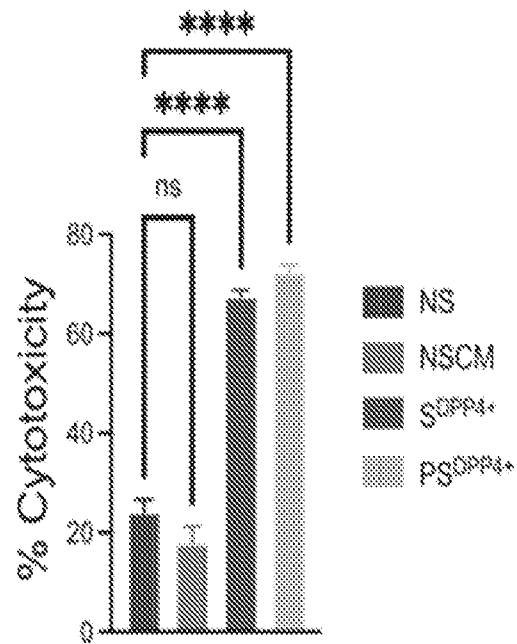


FIG. 19D

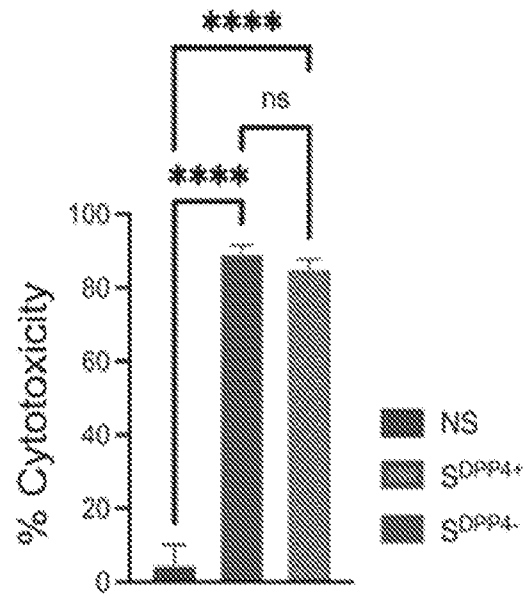


FIG. 19E

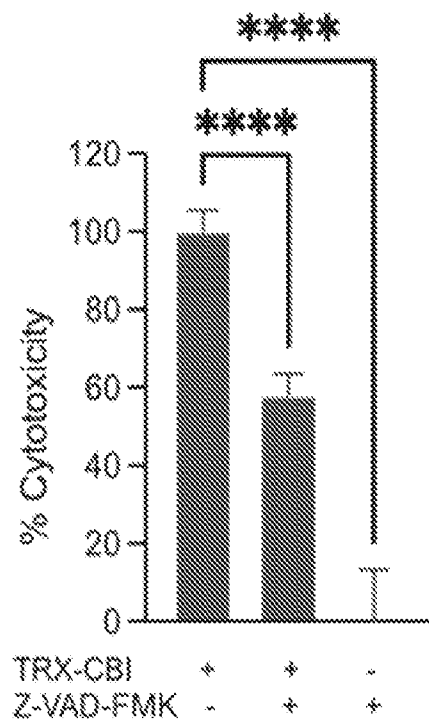


FIG. 19F

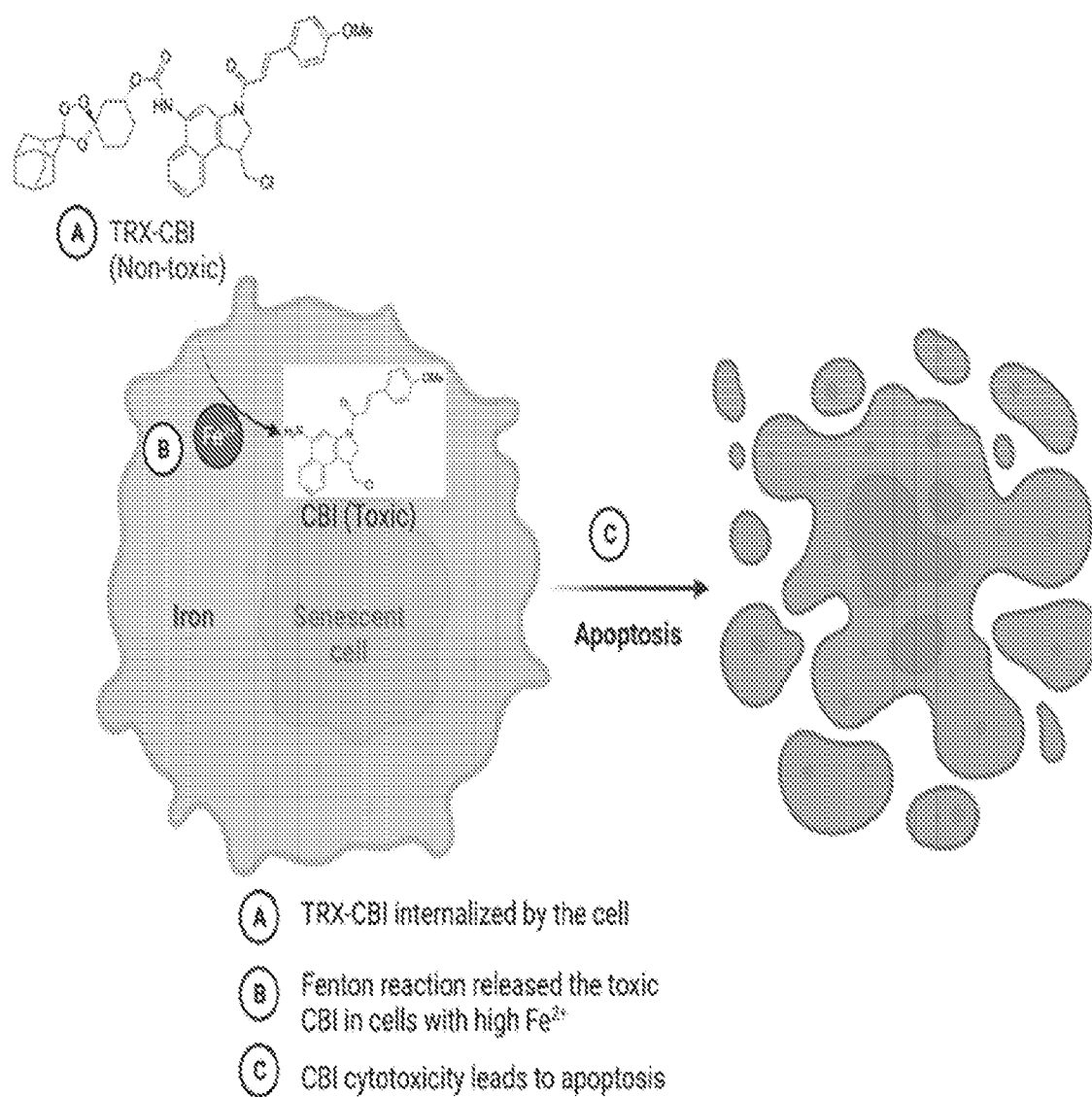


FIG. 19G