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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 November 2022 (17.11.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/241228 A2

(51) International Patent Classification:
H02K 7/18 (2006.01)

(21) International Application Number:
PCT/US2022/029213

(22) International Filing Date:
13 May 2022 (13.05.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/188,573 14 May 2021 (14.05.2021) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, 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, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, 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, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: VARIANTS OF SIRT6 FOR USE IN PREVENTING AND/OR TREATING AGE-RELATED DISEASES

(57) Abstract: The present invention relates to an isolated nucleic acid molecule encoding a variant of sirtuin 6 (SIRT6) having at least 75% identity with sequence SEQ ID NO: 1, the variant having at least one mutation selected in the group comprising or consisting of a N308K substitution and a A313S substitution with respect to sequence SEQ ID NO: 1. The invention provides means for the regulation of ageing, and/or senescence, and/or lifespan in an individual. The invention further provides means for the repair of double strand breaks in a cell. Finally, the invention also provides means for the prevention and/or the treatment of an age-related disease.

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VARIANTS OF SIRT6 FOR USE IN PREVENTING AND/OR TREATING AGE-RELATED DISEASES

[0001] Statement regarding federally sponsored research or development. This invention was made with government support under AG056278, AG027237, AG047200 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF INVENTION

[0002] The present invention relates to lifespan, longevity and age-related diseases. More particularly, the invention relates to variants of sirtuin 6 (SIRT6), and therapeutic uses thereof, for the regulation of ageing and for treating and/or preventing age-related diseases.

BACKGROUND OF INVENTION

[0003] Living to a hundred years-old has a strong genetic component, and is yet extremely rare, with a prevalence in developed nations of at most 1 per 6,000. Genome-wide association studies have so far only identified APOE as a significative biomarker associated with extreme longevity in humans, likely because of a gross lack of statistical power.

[0004] However, a great deal of diseases, referred to as age-related diseases, occur often as the individual gets older.

[0005] Examples of such age-related diseases include, *e.g.*, cardiovascular diseases, strokes, hypertension, cancer, type 2 diabetes, Parkinson's disease, Alzheimer's disease and other dementia, chronic obstructive pulmonary disease (COPD), osteoarthritis, osteoporosis, cataract, age-related macular degeneration, hearing loss.

[0006] It is now believed that age-related diseases are the consequence of a decreased capacity of the body to repair DNA damages and to maintain the integrity of telomeres.

[0007] To date, a family of proteins called the sirtuins (SIRTs), has been associated with various mechanisms involved in DNA damage repair, telomeric integrity, senescence, and longevity.

[0008] Among the SIRTs proteins, SIRT6, encoded by the SIRT6 gene regulates the expression of telomere reverse transcriptase required for telomere elongation, as well as deacetylate histone 3 lysine 9 (H3K9) and deacetylate histone 3 lysine 56 (H3K56) resulting in maintaining the telomeric integrity. In addition, SIRT6 gene was shown to be recruited to the damaged sites and promote DNA repair through deacetylating the repair proteins such as poly (ADP-ribose) polymer-ase (PARP)-1, Ku70, NBS, and Werner (WRN) helicase. Especially, SIRT6 acts as a transcriptional regulator to suppress gene expression by stabilizing the chromatin structure.

[0009] Furthermore, SIRT6 modulates cellular senescence through the deacetylation of a variety of signaling molecules such as FOXO and p53. SIRT6 regulates the RelA subunit of NFκB by modifying the cellular senescence-related gene expression. Finally, SIRT6 also reduces cytotoxicity and therefore decreases the damage that causes premature senescence.

[0010] Therefore, SIRT6 may constitute a good target to prevent and/or treat age-related diseases.

[0011] There is still a need to provide means to prevent and/or treat age-related diseases. In particular, there is a need to prevent and/or treat senescence-related disorder, in particular premature senescence. There is also a need to improve DNA damage repairs involved in senescence, including premature senescence, and decreased longevity.

SUMMARY

[0012] A first aspect of the invention relates to an isolated nucleic acid molecule encoding a variant of sirtuin 6 (SIRT6) having at least 75% identity with sequence **SEQ ID NO: 1**, the variant having at least one mutation selected in the group comprising or

consisting of a substitution N308K and a substitution A313S with respect to sequence **SEQ ID NO: 1**.

[0013] In some embodiments, the nucleic acid molecule is of sequence selected in the group comprising or consisting of **SEQ ID NO: 6**, **SEQ ID NO: 7** and **SEQ ID NO: 8**.

[0014] Another aspect of the invention pertains to an isolated polypeptide encoded by a nucleic acid molecule as defined herein.

[0015] In certain embodiments, wherein the polypeptide is of sequence selected in the group comprising or consisting of **SEQ ID NO: 2**, **SEQ ID NO: 3** and **SEQ ID NO: 4**.

[0016] A further aspect of the invention relates to a vector comprising the isolated nucleic acid molecule as defined herein.

[0017] In some embodiments, the vector is a viral vector, in particular an adeno-associated viral vector (AAV), an exosome-associated AAV vector (exo-AAV), an adenoviral vector, a retroviral vector, or a herpes virus vector.

[0018] In one aspect, the invention relates to a suspension comprising a vector as defined herein.

[0019] The invention also pertains to a cell expressing the polypeptide as defined herein, the cell being preferably transfected with an isolated nucleic acid molecule, or a vector as defined herein.

[0020] Another aspect of the invention relates to a pharmaceutical composition comprising (i) an isolated nucleic acid molecule, or an isolated polypeptide, or a vector as defined herein, and (ii) a pharmaceutically acceptable excipient.

[0021] The invention relates to the use of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector as defined herein, in the regulation of ageing, and/or senescence, and/or lifespan in an individual, preferably a mammalian individual, more preferably a human individual.

[0022] A further aspect of the invention pertains to the use of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according as defined herein, in the repair of double strand break in a cell, preferably a mammalian cell, more preferably a human cell.

[0023] In some aspects, the invention also relates to an isolated nucleic acid molecule, or an isolated polypeptide, or a vector as defined herein, for use in the prevention and/or the treatment of an age-related disease.

[0024] In certain embodiments, the individual is a mammalian individual, preferably a human individual.

[0025] In some embodiments, the age-related disease is selected in the group comprising or consisting of progeria, Werner syndrome, neurodegenerative disease, Alzheimer's disease, cancer, cardiovascular disease, obesity, type 2 diabetes, hypercholesterolemia, hypertension, ocular disorders, cataracts, glaucoma, osteoporosis, blood clotting disorders, arthritis, hearing loss and stroke.

[0026] In another aspect, the invention also relates to a kit comprising (i) an isolated nucleic acid molecule, or an isolated polypeptide, or a vector as defined herein, and (ii) means to administer the isolated nucleic acid molecule, the isolated polypeptide, or the vector.

DEFINITIONS

[0027] In the present invention, the following terms have the following meanings:

[0028] "**About**", when preceding a figure, means plus or less 10% of the value of said figure. It is to be understood that the value to which the term "about" refers is itself also specifically, and preferably, disclosed.

[0029] "**Comprise**" is intended to mean "**contain**", "**encompass**" and "**include**". In some embodiments, the term "**comprise**" also encompasses the term "**consist of**".

[0030] "**Sirtuin 6**" also referred to as "**SIRT6**", is intended to refer to the polypeptide with the Entrez Gene number 51548, and also non-limitatively relates to the NAD-Dependent Protein Deacetylase Sirtuin-6, Regulatory Protein SIR2 Homolog 6, SIR2-Like Protein 6, SIR2L6, Sirtuin (Silent Mating Type Information Regulation 2, S. Cerevisiae, Homolog) 6, Sirtuin (Silent Mating Type Information Regulation 2 Homolog) 6, Sir2-Related Protein Type 6, Sirtuin Type 6 and EC 2.3.1.286.

[0031] "**Isolated**" refers to a nucleic acid molecule or polypeptide a that is removed from the initial biological context that has allowed to generate this nucleic acid molecule or polypeptide a. In practice the biological context comprises at least a cell, or one or more enzyme(s).

[0032] "**Nucleic acid**", also referred to as "**polynucleotide**", refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Nucleic acid" or "polynucleotide" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double- stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "Nucleic acid" or "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "nucleic acid" or "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "nucleic acid" or "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

[0033] "**Polypeptide**" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides,

oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acid residues other than the 20 gene-encoded amino acid residues.

[0034] "**Age-related disease**" refers to the physiological or pathological conditions that correlate with an individual getting older or prematurely older, and which prevalence is above the average prevalence observed within the general population. Non-limitative examples of age-related diseases include progeria, Werner syndrome, neurodegenerative diseases, cardiovascular diseases, strokes, hypertension, cancer, type 2 diabetes, Parkinson's disease, dementia, chronic obstructive pulmonary disease (COPD), osteoarthritis, osteoporosis, cataract, age-related macular degeneration, hearing loss, and the like.

[0035] "**Suspension**" refers to a liquid mixture in which the active principle, such as the nucleic acid molecules, polypeptides, or vectors according to the invention, is/are floating in a liquid medium.

[0036] "**Treating**" or **"treatment"** or **"alleviation"** refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder, in particular an age-related disease. Those in need of treatment include those already with said disorder as well as those prone to develop the disorder or those in whom the disorder is to be prevented. An individual is successfully "treated" for an age-related disease if, after receiving a therapeutic amount of the active principle, in particular the nucleic acid molecules, polypeptides, or vectors according to the present invention, the individual shows observable and/or measurable reduction in or absence of one or more of the symptoms associated with the age-related disease; reduced morbidity and mortality, and improvement in quality of life issues. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to physician or authorized personnel.

[0037] "**Preventing**" refers to keeping from happening, and/or lowering the chance of the onset of, or at least one adverse effect or symptom of, an age-related disease, disorder or condition associated with a deficiency in or absence of an organ, tissue or cell function.

[0038] "**Therapeutically efficient amount**" refers to the level or the amount of the active principle that is aimed at, without causing significant negative or adverse side effects to the target, (1) delaying or preventing the onset of an age-related disease, disorder, or condition; (2) slowing down or stopping the progression, aggravation, or deterioration of one or more symptoms of an age-related disease, disorder, or condition; (3) bringing about ameliorations of the symptoms of an age-related disease, disorder, or condition; (4) reducing the severity or incidence of an age-related disease, disorder, or condition; or (5) curing an age-related disease, disorder, or condition. A therapeutically effective amount may be administered prior to the onset of an age-related disease, disorder, or condition, for a prophylactic or preventive action. Alternatively, or additionally, the therapeutically effective amount may be administered after the onset of an age-related disease, disorder, or condition, for a therapeutic action. In one embodiment, a therapeutically effective amount of the active principle is an amount that is effective in reducing at least one symptom of an age-related disease, disorder or condition.

[0039] "**Individual**" refers to an animal, preferably a mammal, more preferably a human. In one embodiment, the individual is a male. In another embodiment, the individual is a female. In one embodiment, an individual may be a "patient", *i.e.* a warm-blooded animal, more preferably a human, who/which is awaiting the receipt of, or is receiving medical care or was/is/will be the object of a medical procedure, or is monitored for the development of cancer. In one embodiment, the individual is an adult (for example a subject above the age of 18). In another embodiment, the individual is a child (for example a subject below the age of 18).

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] **Figure 1** is a scheme showing the screening strategy for variants in genome maintenance (GM) genes in Ashkenazi Jewish (AJ) centenarians.

[0041] **Figure 2** is a plot showing the purified SIRT6 proteins turn-over rate. SILAC analysis on HEK293 cells expressing SIRT6 variants.

[0042] **Figure 3** is a graph showing the thermostability of purified SIRT6 proteins (WT: wild type SIRT6 polypeptide; Cent: SIRT6 variant polypeptide with N308K and A313S mutations). Data represents two replicates with two technical replicates each using SIRT6 from the Roc and Ichor preps.

[0043] **Figures 4A-4B** are a combination of graphs. **Fig. 4A** shows the fluorescence lifetime τ measured from FLT signals by fitting the data to Equation 1 (see Methods). Each of the two different SIRT6 biosensors shows a decreased fluorescence lifetime compared to the GFP-only control, indicating highly significant FRET. Compared with the biosensor with C-terminal GFP, the intra-GFP biosensor shows a greater lifetime change (more FRET, shorter distance R, more structural sensitivity). The centSIRT6 of this biosensor was also tested. It shows a significant increase in lifetime τ (1.96 ± 0.02 ns), compared to the wild type SIRT6 (1.75 ± 0.01 ns). Error bars indicate SEM (n = 3-5). **Fig 4B** shows the FRET efficiency E and the distance R (in Angstrom; Å) were determined from lifetime data (Equations. 2 and 3, Methods), revealing that the distance R, measure for the intra-GFP biosensor, was significantly greater for the centSIRT6 by 3.0 ± 0.4 Å.

[0044] **Figure 5A-5B** are a combination of graphs. **Fig. 5A** shows the Michaelis-Menten kinetic parameters using differential concentrations of myristoylated peptide. **Fig. 5B** shows the Michaelis-Menten kinetic parameters using differential concentrations of NAD+. Reactions were conducted in triplicate. Closed circles: WT; closed squares: Cent.

[0045] **Figure 6** is a graph showing the tryptophan fluorescence curves for SIRT6 variants with titrated concentrations of NAD+.

[0046] **Figure 7A-7B** is a combination of plots showing the deacetylase activity on H3K9ac (**Fig. 7A**) and H3K18ac (**Fig. 7B**) residues, with reduced activity in centSIRT6 allele (the deacetylase activity is inversely proportional to the relative abundance of acetylated compounds). Designer histones saturated with the corresponding acetylated histone residue were incubated with purified SIRT6 and 1 mM NAD+ for 1 h prior to resolution by SDS-PAGE gel and staining with acetyl-specific histone antibodies. All

reactions were conducted with an n=3; error bars show s.d. Statistics were calculated using Students t-test, two tailed. Asterisk indicate $p<0.05$.

[0047] **Figure 8A-8B** is a combination of plots showing the deacetylation kinetics on H3K9ac (**Fig. 8A**) and H3K18ac (**Fig. 8B**) residues of SIRT6 variants. Designer histones were incubated with purified SIRT6 and 5 mM NAD+, then resolved by SDS-PAGE and analyzed by immunoblotting with acetyl-specific histone antibodies. All reactions were conducted with an n=3; error bars show s.d. Statistics were calculated using Students t-test, two tailed. Asterisk indicate $p<0.05$.

[0048] **Figure 9A-9B** is a combination of plots showing the *in vitro* deacetylation rates on H3K9ac (**Fig. 9A**) and H3K18ac (**Fig. 8B**) residues of purified SIRT6 proteins with histones purified from HeLa cells.

[0049] **Figure 10** is a plot showing the quantitative mass spec of histone H3 peptide purified from human cells expressing different SIRT6 alleles, which did not reveal a difference in acetylation levels. Relative fraction represents the portion of the peptide encompassing H3K9-17 compared to the summed total of all of the peptide quantitation values for the same region. The average and standard deviation of three different preps is plotted.

[0050] **Figure 11** is a photograph showing the whole cell histone H3 acetylation levels in cumate-inducible SIRT6 human fibroblasts, assessed by Western blot. Cu, cumate; PQ, paraquat. Cumate dosage required for equivalent SIRT6 protein abundance was determined by Western blot, and administered accordingly to respective cell lines.

[0051] **Figure 12** is a plot showing the self-ribosylation of SIRT6 using radiolabeled NAD+. Recombinant SIRT6 was incubated with P32-labeled NAD+ and then run on an SDS-PAGE. Signal was measured using a PhosphoImager. All experiments were repeated at least three times; error bars show s.d. Significance was determined by Student's t-test, two tailed. Asterisk indicate $p<0.05$. Asterisks indicate significance over HPRT control.

[0052] **Figure 13** is a plot showing the self-ribosylation of SIRT6 with titration of NAD+. mADPr-specific antibody was used to detect ribosylated wild type SIRT6 (closed circles) and centSIRT6 (closed squares) proteins. Plot was fit the Michaelis-Menten equation with Kaleidagraph software. Km NAD was 142+29 for centSIRT6 and 106+45 for wild type SIRT6 and maximal signal was ~2× greater for centSIRT6 compared to the wild type SIRT6.

[0053] **Figure 14** is a graph showing the activation of PARP1 by SIRT6 variants. SIRT6 protein was incubated with human PARP1 protein and then analyzed by immunoblotting with poly-ADPr antibody. Poly-ADPr activity of PARP1 results in a wide range of product size. Activity was assessed by quantifying poly-ADPr signal in whole lanes for each sample. All experiments were repeated at least three times; error bars show s.d. Significance was determined by Student's t-test, two tailed. Asterisk indicate $p<0.05$. Lower asterisks indicate significance over HPRT control. Upper asterisks indicated significance over wild type SIRT6.

[0054] **Figure 15** is a graph showing the qRT-PCR analysis of LINE1 expression in cumate-inducible SIRT6 fibroblasts. Primers assessed both 5' (ORF1; closed bars) and 3' (ORF2; dashed bars) LINE1 sequences from the L1MdA1 family of active LINE1 retrotransposons. Assessment of both regions was conducted to mitigate contributions from partial insertion sequences in coding genes. All experiments were repeated at least three times. Error bars represent s.d. Significance was determined by Student's t-test, two tailed, unless otherwise stated. Asterisk indicate $p<0.05$.

[0055] **Figure 16A-16B** is a combination of graphs showing the stimulation of NHEJ (**Fig. 16A**) and HR (**Fig. 16B**) by SIRT6 variants. Reporter cell lines were co-transfected with SIRT6-expressing plasmid, I-Sce1 plasmid, and DsRed transfection control. After 72 hr recovery, reactivation of the GFP reporter was measured by flow cytometry. Stimulation of NHEJ or HR was calculated as radio of GFP+/DsRed+ positive cells.

[0056] **Figure 17** is a graph showing the basal γH2AX foci in cumate-inducible SIRT6 fibroblasts under uninduced (white bars) or induced (black bars) conditions. Foci were scored in at least 80 cells per condition.

[0057] **Figure 18** is a plot showing the DNA repair kinetics in cumate-inducible SIRT6 fibroblasts. Cells were grown on slides and irradiated with 2 Gy gamma radiation, followed by immunostaining for γH2AX. Irradiation was conducted when the cells were at 75% confluency on slides. Cells were fixed and foci scored at t=0.5 h, 2 h, 4 h, 6 h, and 24 h post-irradiation. Foci were scored in at least 80 cells per genotype per timepoint. Asterisk indicates significant difference from the wild type SIRT6 ($p<0.05$).

[0058] **Figure 19A-19B** is a combination of plots showing the oxidative stress resistance. Cumate-inducible SIRT6 fibroblasts were induced for SIRT6 expression and exposed to paraquat for 24 hours. Resistance was determined by apoptosis staining 48 hours after exposure.

[0059] **Figure 20A-20C** is a combination of graphs. **Fig. 20A** is showing the number of adherent cells after transfection with SIRT6 variants. Cells were transfected with SIRT6 plasmids encoding different SIRT6 alleles and cell numbers were counted after 72 hours. HCA2 are normal human foreskin fibroblasts. a: Control (CT); b: WT; c: N308K SIRT6 variant; d: A313S SIRT6 variant; e: Cent (N308K A313S variant). Asterisk indicates significant difference from the wild type SIRT6 ($p<0.05$). **Fig. 20B-20C** are showing the apoptosis staining of cancer cell lines HT1080 (**Fig. 20B**) and HeLa (**Fig. 20C**) 48 hours after transfection. Cells were stained with Annexin V/PI and analyzed by flow cytometry. Significance was determined by two-way ANOVA. All experiments were repeated at least three times. Error bars represent s.d. Significance was determined by Student's t-test, two tailed, unless otherwise stated. Asterisk indicate $p<0.05$.

[0060] **Figure 21** is a photograph showing the immunoprecipitation (IP) experiments on lysates from cumate-induced fibroblasts expressing wild type (WT) or centSIRT6 (Cent) alleles with antibodies to SIRT6, LMNA, and mADPr. SIRT6 expression was induced 48 hours prior to IP. The IP experiments were repeated three times. One representative set of IPs is shown.

[0061] **Figure 22** is a graph showing SIRT6 IP followed by Western blot with antibodies to LMNA, in which CentSIRT6 (Cent) shows stronger interaction with LMNA compared to the wild type SIRT6 (WT). Quantification of the IP experiment shown in **Figure 21**.

[0062] **Figure 23** is a graph showing LMNA IP from cumate-inducible SIRT6 fibroblasts followed by Western blot with antibodies to SIRT6, in which LMNA shows enhanced interaction with centSIRT6 (Cent) compared to the wild type (WT). Quantification of the IP experiment shown in **Figure 21**.

[0063] **Figure 24** is a graph showing the quantification of the IP experiment shown in **Figure 21**. SIRT6 IP from cumate-inducible SIRT6 fibroblasts followed by Western blot with antibody to mADPr residues shows that centSIRT6 displays enhanced mADPr.

[0064] **Figure 25** is a graph showing the quantification of the IP experiment shown in **Figure 21**. IP with mADPr antibody using extract from cumate-induced SIRT6 fibroblasts, followed by Western blot with antibodies to LMNA, shows that LMNA displays enhanced mADPr signal in cells expressing centSIRT6 (Cent), as compared to wild type (WT).

[0065] **Figure 26** is a scheme showing protein-protein interactions profiles of SIRT6 and LMNA. SIRT6 and LMNA are colored dark grey and featured as central points in two opposing nodes of interactions. A third node (upper middle) shows interaction partners that are shared by SIRT6 and LMNA. Proteins whose interactions are enhanced by the centSIRT6 allele are colored light grey. Proteins that interacted equally with wild type and centSIRT6 alleles are uncolored. H15 is a special case because it showed increased interaction with the centSIRT6 and decreased interaction with LMNA in the presence of the centSIRT6 allele. Proteins known to be ribosylated in previous reports are shown as hexagons.

[0066] **Figure 27A-27B** is a combination of histograms. **Fig. 27A** shows SIRT6 expression in cumate-inducible telomerase-immortalized HCA2 human fibroblast cell lines. SIRT6 alleles were integrated in the genome of SIRT6 knockout HCA2 cells using PiggyBac Transposon Vector System. Different dosages of cumate and resulting SIRT6 abundance were used to determine the dose needed to achieve equivalent SIRT6 expression for each cell line. Cells were normalized by count and total protein. Subsequent experiments utilizing cumate-inducible SIRT6 fibroblasts were controlled for SIRT6 abundance using these data. For each cell line, the box indicates the corresponding

cumate dosage for equivalent expression (WT=60 μg/ml, N308K=30 μg/ml, A313S=30 μg/ml, and Cent=7.5 μg/ml). These concentrations were used in experiments with these cells. **Fig. 27B** shows qRT-PCR expression analysis of SIRT6 using standardized dosages of cumate (dosage corresponds to boxes in **Fig. 27A**).

[0067] **Figure 28** is a histogram showing Self-ribosylation of SIRT6 using biotin-labeled NAD+. Recombinant SIRT6 was incubated with NAD+ conjugated with a biotin residue and then run on an SDS-PAGE. Each allele was assessed relative to its 0hr time point and normalized to SIRT6 total protein loading controls.

[0068] **Figure 29A-29F** is a combination of photographs, graphs and histograms showing the analysis of CRISPR human MSC cell lines. **Fig. 29A** and **29B** show DNA double strand repair efficiency in wild type and centSIRT6 ("Cent #1" and "Cent #2") hMSCs. DSB repair reporter constructs were integrated into hMSCs. After 72 hr recovery, reactivation of the GFP reporter was measured by flow cytometry. Stimulation of NHEJ or HR was calculated as radio of GFP+/DsRed+ positive cells. **Fig. 29C** shows cell viability in MMS-treated wild type and centSIRT6 ("Cent") hMSCs. hMSCs were treated with MMS for 48 hours and cell viability was evaluated by MTS assay. Data were normalized to the control group (0 mM). n = 6. **Fig. 29D** shows immunofluorescence staining of 53BP1 in wild type and centSIRT6 ("Cent") hMSCs under MMS treatment. Numbers of 53BP1 foci in the nuclei of wild type and centSIRT6 hMSCs with or without MMS (0.25 mM) treatment were quantified. >600 nuclei from 10 images were scored. **Fig. 29E** shows qRT-PCR analysis of SIRT6 expression in wild type and centSIRT6 ("Cent #1" and "Cent #2") hMSCs (P2). Data normalized to Actin and were presented as mean ± SEM, NS, not significant. **Fig. 29F** shows this quantification. Error bars represent s.d. Significance was determined by Student's t-test, two tailed. Asterisk indicate $p<0.05$.

[0069] **Figure 30A-30C** is a combination of photograph and histograms illustrating overexpression of SIRT6 (wild type, SIRT6 N308K or centSIRT6 ("Cent")) in human Werner Syndrome immortalized fibroblasts. **Fig. 30A** shows a proliferation assay of the same cells, using Hoechst fluorescence staining at 24h, 48h and 72h. Data are showed as mean + SEM. **Fig. 30B** shows a telomere length assay showing significant difference in all the transfected cells when compared to not transfected control cells (* = versus

control). **Fig. 30C** shows that telomerase activity assay identified a significant decrease in centSIRT6 group ("Cent") when compared to not transfected control cells.

[0070] **Figure 31A-31B** is a combination of photograph and histogram showing that SIRT6 overexpression (wild type, SIRT6 N308K or centSIRT6 ("Cent")) induces cell death in hepatocellular carcinoma cell lines, after 12 days LV transfection and 3 days after hygromycin selection. **Fig. 31A** shows that in human hepatocellular carcinoma (HCC) cells, SIRT6 WT/SIRT6 N308K/centSIRT6 ("Cent") overexpression was lethal, with a larger effect observed for SIRT6 N308K/centSIRT6 ("Cent"). **Fig. 31B** represents the quantification of cell viability illustrated in **FIG. 31A**.

[0071] **Figure 32** is a graph showing that SIRT6 overexpression (wild type, SIRT6 N308K or centSIRT6 ("Cent")) increase differentiation of 3T3-L1 pre-adipocytes (quantification of immunofluorescence).

[0072] **Figure 33A-33E** is a combination of photograph and histograms showing the effect of SIRT6 overexpression (wild type, SIRT6 N308K and centSIRT6 ("Cent")) in hepatic stellate cells (LX2). **Fig. 33A** shows the viability of LX2 cells after 24h of transfection with SIRT6 (WT or mutants). **Fig. 33B-33E** shows gene expression (mRNA) in LX2 cells overexpressing or not SIRT6 (WT or mutants) after 24h TGF-β 20ng treatment for Vimentin (**Fig. 33B**), TIMP1 (**Fig. 33C**), COL1A1 (**Fig. 33D**) and αSMA (**Fig. 33E**) (N = 4; * = $p < 0.05$ versus respective control).

[0073] **Figure 34A-34C** is a combination of histograms showing the effect of SIRT6 overexpression (wild type, SIRT6 N308K and centSIRT6 ("Cent")) on spheroids made from Immortalized Human Hepatocytes (IHH) and human hepatic stellate cells (LX2). Spheroids are formed from IHH cells and with 5% the total cell mass of LX2. **Fig. 34A** shows the quantification of collagen I in IHH-LX2 spheroids overexpressing or not centSIRT6 ("Cent") (quantification of immunofluorescence). **Fig. 34B-34C** shows IHH-LX2 spheroids gene expression relative to GAPDH for αSMA, COL1A1, TIMP1, VIMENTIN and MMP2, either in absence of Free Faty Acids (FFA) (**Fig. 34B**) or in presence of FFA (**Fig. 34C**) (* $p<0.05$; ** $p<0.01$; *** $p<0.001$ versus respective control).

DETAILED DESCRIPTION

[0074] The inventors used herein an alternative, candidate functional association approach to directly identify rare longevity gene variants enriched in the genome of a cohort of Ashkenazi Jewish (AJ) centenarians.

[0075] Based on previous evidences of DNA damage as a driver of ageing, the inventors performed targeted sequencing of 301 genes involved in Genome Maintenance (GM), a major longevity assurance system, in 496 AJ centenarians and 572 controls.

[0076] Among the ten GM genes containing nominally significant missense genetic variants enriched in the centenarian genome, was SIRT6, a deacylase and mono-ADP ribosyl transferase (mADPr) enzyme-6, involved in both DNA double-strand breaks (DSB) repair and longevity in model organisms.

[0077] The inventors selected two genetically linked variants in the same allele (N308K and A313S), for further functional analysis. Characterization of this SIRT6 centenarian allele (centSIRT6) demonstrated it to be a stronger suppressor of LINE1 retrotransposons, confer enhanced stimulation of DNA DSB repair, and more robust cancer cell killing compared to the wild type allele.

[0078] Surprisingly, centSIRT6 displayed weaker deacetylase activity compared to the wild type. Conversely, its mADPr activity was strongly enhanced. FRET-based analysis demonstrated the centSIRT6 had a more open conformation. centSIRT6 displayed a stronger interaction with Lamin A/C (LMNA), which correlated with enhanced ribosylation of LMNA.

[0079] This invention relates to an isolated nucleic acid molecule encoding a variant of sirtuin 6 (SIRT6) having at least 75% identity with sequence **SEQ ID NO: 1**, the variant having at least one mutation selected in the group comprising or consisting of a N308K substitution and an A313S substitution with respect to sequence **SEQ ID NO: 1**.

[0080] As used herein the expression "at least 75% identity" encompasses 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity.

[0081] The level of identity of 2 polypeptides may be performed by using any one of the known algorithms available from the state of the art. Illustratively, the amino acid identity percentage may be determined using the CLUSTAL W software (version 1.83), the parameters being set as follows:

- for slow/accurate alignments: (1) Gap Open Penalty: 10.00; (2) Gap Extension Penalty:0.1; (3) Protein weight matrix: BLOSUM;

- for fast/approximate alignments: (4) Gap penalty: 3; (5) K-tuple (word) size: 1; (6) No. of top diagonals: 5; (7) Window size: 5; (8) Scoring Method: PERCENT.

[0082] In certain embodiments, the isolated nucleic acid molecule encodes a variant of SIRT6 having at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95%, most preferably at least 96%, 97%, 98% or 99% identity with sequence **SEQ ID NO: 1**, the variant having at least one mutation selected in the group comprising or consisting of a N308K substitution and an A313S substitution with respect to sequence **SEQ ID NO: 1**.

[0083] Within the scope of the invention the sequence **SEQ ID NO: 1** refers to the 361 amino acid residues sequence of wild type SIRT6 polypeptide. In practice, substitutions N308K and A313S refer to the mutations of the codon encoding the naturally occurring Asn (N) amino acid residue at position 308 in the SIRT6 polypeptide, and the Ser (S) amino acid residue at position 313 in the SIRT6 polypeptide, respectively.

[0084] Within the scope of the invention the sequence **SEQ ID NO: 5** refers to the 1,068 nucleotides (bp) sequence of wild type SIRT6 polypeptide.

[0085] In some embodiments, the naturally occurring Asn (N) amino acid residue at position 308 in the SIRT6 polypeptide is encoded by codon “aac” at positions 922 to 924 of **SEQ ID NO: 5**. In some embodiments, the naturally occurring Ser (S) amino acid residue at position 313 in the SIRT6 polypeptide is encoded by codon “gcc” at positions 937 to 939 of **SEQ ID NO: 5**.

[0086] In certain embodiments, the N308K substitution is represented by a mutation of codon “aac” at positions 922 to 924 of **SEQ ID NO: 5** into codon “aag” or codon “aaa”,

preferably into codon “aag”. In other words, the N308K substitution is represented by a mutation of nucleotide “c” at positions 924 of **SEQ ID NO: 5** into nucleotide “g” or nucleotide “a”, preferably into nucleotide “g”.

[0087] In certain embodiments, the A313S substitution is represented by a mutation of codon “gcc” at positions 937 to 939 of **SEQ ID NO: 5** into a codon selected in a group consisting of codons “tcc”, “tct”, “tca” and “tcg”, preferably codon “tcc”. In other words, the A313S substitution is represented by one or two mutation(s) selected in a group consisting of a mutation of nucleotide “g” at positions 937 of **SEQ ID NO: 5** into nucleotide “t”; a mutation of nucleotide “g” at positions 937 of **SEQ ID NO: 5** into nucleotide “t” and of nucleotide “c” at positions 939 of **SEQ ID NO: 5** into nucleotide “t”; a mutation of nucleotide “g” at positions 937 of **SEQ ID NO: 5** into nucleotide “t” and of nucleotide “c” at positions 939 of **SEQ ID NO: 5** into nucleotide “a”; and a mutation of nucleotide “g” at positions 937 of **SEQ ID NO: 5** into nucleotide “t” and of nucleotide “c” at positions 939 of **SEQ ID NO: 5** into nucleotide “g”.

[0088] In some embodiments, the nucleic acid molecule is of sequence selected in the group comprising or consisting of **SEQ ID NO: 6**, **SEQ ID NO: 7** and **SEQ ID NO: 8**.

[0089] As used herein, sequence **SEQ ID NO: 6** refers to the nucleic acid sequence of the variant of SIRT6 with N308K substitution, in particular, with mutation of codon “aac” at positions 922 to 924 of **SEQ ID NO: 5** into codon “aag”.

[0090] As used herein, sequence **SEQ ID NO: 7** refers to the nucleic acid sequence of the variant of SIRT6 with A313S substitution, in particular, with mutation of codon “gcc” at positions 922 to 924 of **SEQ ID NO: 5** into codon “tcc”.

[0091] As used herein, sequence **SEQ ID NO: 8** refers to the nucleic acid sequence of the variant of SIRT6 with N308K and A313S substitutions, in particular, with mutation of codon “aac” at positions 922 to 924 of **SEQ ID NO: 5** into codon “aag” and with mutation of codon “gcc” at positions 922 to 924 of **SEQ ID NO: 5** into codon “tcc”.

[0092] In some embodiments, the variant of SIRT6 encoded by the isolated nucleic acid molecule as defined herein has a deacylase and/or mono-ADP ribosyl transferase (mADPr) activity.

[0093] In practice, the deacylase activity and mono-ADP ribosyl transferase (mADPr) activity may be assayed accordingly to any suitable from the state in the art, or a method adapted therefrom. Illustratively, deacylase activity may be assayed by contacting *in vitro* the variant of SIRT6 with histones, in the presence of NAD^+, $MgCl_2$, DTT and performing a Western blot analysis using anti-H3K9ac and anti-H3K18ac antibodies. Illustratively, mono-ADP ribosyl transferase (mADPr) activity may be assayed by contacting *in vitro* the variant of SIRT6 with PARP1, in the presence of $ZnCl_2$, $MgCl_2$, NAD^+, DTT, salmon sperm DNA and performing a Western blot analysis using anti-PADPR antibodies.

[0094] In certain embodiments, the variant of SIRT6 has at most about 90%, preferably at most about 50%, more preferably at most about 25% deacylase activity as compared to wild type SIRT6 (of sequence **SEQ ID NO: 1**). Within the scope of the invention, the expression "at most about 90%" encompasses about 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 1% or less.

[0095] In some embodiments, the variant of SIRT6 has at most least 100%, preferably at least about 200%, more preferably at least about 300% mono-ADP ribosyl transferase (mADPr) activity as compared to wild type SIRT6 (of sequence **SEQ ID NO: 1**). Within the scope of the invention, the expression "at least about 100%" encompasses about 100%, 120%, 140%, 160%, 180%, 200%, 220%, 240%, 260%, 280%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750% or more.

[0096] Another aspect of the invention relates to an isolated polypeptide encoded by a nucleic acid molecule according to the instant invention.

[0097] This invention relates to an isolated polypeptide being a variant of SIRT6 having at least 75% identity with sequence **SEQ ID NO: 1**, the variant having at least one mutation selected in the group comprising or consisting of a substitution N308K and a substitution A313S with respect to sequence **SEQ ID NO: 1**.

[0098] As used herein the expression "at least 75% identity" encompasses 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% identity.

[0099] In certain embodiments, the isolated polypeptide being a variant of SIRT6 has at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95%, most preferably at least 96%, 97%, 98% or 99% identity with sequence **SEQ ID NO: 1**, the variant having at least one mutation selected in the group comprising or consisting of a substitution N308K and a substitution A313S with respect to sequence **SEQ ID NO: 1**.

[0100] In some embodiments, the polypeptide is of sequence selected in the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**.

[0101] As used herein, sequence **SEQ ID NO: 2** refers to the amino acid sequence of the variant of SIRT6 with N308K substitution. In some embodiments, the polypeptide of sequence **SEQ ID NO: 2** is encoded by a nucleic acid molecule of sequence **SEQ ID NO: 6**.

[0102] As used herein, sequence **SEQ ID NO: 3** refers to the amino acid sequence of the variant of SIRT6 with A313S substitution. In some embodiments, the polypeptide of sequence **SEQ ID NO: 3** is encoded by a nucleic acid molecule of sequence **SEQ ID NO: 7**.

[0103] As used herein, sequence **SEQ ID NO: 4** refers to the amino acid sequence of the variant of SIRT6 with N308K and A313S substitutions. In some embodiments, the polypeptide of sequence **SEQ ID NO: 4** is encoded by a nucleic acid molecule of sequence **SEQ ID NO: 8**.

[0104] In certain embodiments, the polypeptide is a recombinant polypeptide. As used herein, the term "recombinant polypeptide" refers to a polypeptide encoded by an engineered nucleic acid and synthesized upon transformation of said engineered nucleic acid into a microorganism or transfection in an eukaryotic cell for synthesis purposes.

[0105] A further aspect of the invention relates to a vector comprising the isolated nucleic acid molecule according to the invention.

[0106] In some embodiments, the vector in a minicircle nucleic acid, a plasmid, a cosmid or a bacterial artificial chromosome.

[0107] As used herein, the term "minicircle nucleic acid" encompasses non-viral vectors that merely comprise a gene expression cassette and are free of viral and/or bacterial backbone DNA elements from standard plasmids.

[0108] As used herein, the term "plasmid" is intended to refer to a small extra-genomic DNA molecule, most commonly found as circular double stranded DNA molecules that may be used as a cloning vector in molecular biology, to make and/or modify copies of DNA fragments up to about 15 kb (*i.e.*, 15,000 base pairs). Plasmids may also be used as expression vectors to produce large amounts of proteins of interest encoded by a nucleic acid sequence found in the plasmid downstream of a promoter sequence.

[0109] A used herein, the term "cosmid" refers to a hybrid plasmid that contains cos sequences from Lambda phage, allowing packaging of the cosmid into a phage head and subsequent infection of bacterial cell wherein the cosmid is cyclized and can replicate as a plasmid. Cosmids are typically used as cloning vector for DNA fragments ranging in size from about 32 to 52 kb.

[0110] As used herein, the term "bacterial artificial chromosome" or "BAC" refers to extra-genomic nucleic acid molecule based on a functional fertility plasmid that allows the even partition of said extra-genomic DNA molecules after division of the bacterial cell. BACs are typically used as cloning vector for DNA fragment ranging in size from about 150 to 350 kb.

[0111] In practice, the vector comprising the nucleic acid molecule encoding a variant of SIRT6 may be in the form of a plasmid, in particular resulting from the cloning of a nucleic acid of interest into a nucleic acid vector. In some embodiments, non-limitative suitable nucleic acid vectors are pBluescript vectors, pET vectors, pETduet vectors,

pGBM vectors, pBAD vectors, pUC vectors. In one embodiment, the plasmid is a low copy plasmid. In one embodiment, the plasmid is a high copy plasmid.

[0112] In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is selected in a group comprising or consisting of an adenovirus; an adeno-associated virus (AAV); an exosome-associated AAV (exo-AAV); an alphavirus; a herpesvirus; a retrovirus, such as, *e.g.*, a lentivirus or a non-integrative lentivirus; vaccinia virus; a baculovirus; or virus like particles such as, *e.g.*, particles derived from Hepatitis B virus, Parvoviridae, Retroviridae, Flaviviridae, Paramyxoviridae or bacteriophages.

[0113] In certain embodiments, the vector is a viral vector, in particular an adeno-associated viral vector (AAV), an exosome-associated AAV vector (exo-AAV), an adenoviral vector, a retroviral vector, or a herpes virus vector.

[0114] In some embodiments, the adeno-associated viral vector (AAV) is AAV serotype 2 or AAV serotype 5.

[0115] In certain embodiments, the vector, in particular the viral vector, is an exo-AAV vector. As used herein, exo-AAV refers to a vector wherein an adeno-associated virus (AAV) vector, or parts thereof, is associated with an extracellular vesicle (also referred to as exosome), wherein the AAV vector is partially fused, embedded or internalized in the extracellular vesicle. The extracellular vesicle may express specific proteins or markers, for example for targeting purposes.

[0116] In certain embodiments, the vector, in particular the viral vector, does not cross the blood-brain barrier. In some alternative embodiments, the vector, in particular viral vector crosses the blood-brain barrier. In practice, the choice of the viral vector may depend of the organ or tissue that is targeted. For example, for brain cancer, neurodegenerative disease, Alzheimer's disease and progeria, a vector that crosses the blood-brain barrier may be preferably selected. On the contrary for obesity, cardiovascular diseases, type 2 diabetes, hypercholesterolemia, ocular diseases, a vector that does not cross the blood-brain barrier may be preferably selected.

[0117] In practice, assessing whether a vector, in particular a viral vector, crosses the blood/brain barrier may be performed by any suitable method acknowledged from the state of the art, or a method adapted therefrom. Illustratively, this assessment may be performed by the means of one of the two gold-standard experimental measures of blood/brain barrier permeability, namely, (1) logBB, which is intended to measure the concentration of a compound in the brain divided by concentration in the blood; and (2) logPS, which measures the permeability surface-area product.

[0118] In some embodiments, the vector, in particular the viral vector, comprises a promoter sequence suitable for gene expression in mammalian individuals, preferably in human individuals.

[0119] Non-limitative examples of promoter sequence suitable for gene expression in mammalian individuals, preferably in human individuals, include CMV (human cytomegalovirus) promoter, EF1a (human elongation factor 1 alpha) promoter, SV40 (Simian vacuolating virus 40) promoter, PGK1 (phosphoglycerate kinase) promoter, Ubc (human ubiquitin C) promoter, and the like.

[0120] In certain embodiments, the promoter sequence is preferably the CMV promoter.

[0121] In some embodiments, the vector, in particular the viral vector, further comprises a nucleic acid sequence that facilitates the nuclear localization of the polypeptide encoded by the nucleic acid molecule according to the invention into a target recipient cell. In practice, these nuclear localization signal (NLS) have been abundantly discussed in the state of the art.

[0122] In certain embodiments, the vector, in particular the viral vector, may comprise a S/MAR (for scaffold/matrix attachment region) nucleic acid sequence. As used herein, the S/MAR nucleic acid sequence, also referred to SAR (for scaffold-attachment region) or MAR (for matrix-associated region), is intended to refer to a nucleic acid sequence that is naturally found in the DNA of eukaryotic chromosomes and that promotes attachment to the nuclear matrix. In some embodiments, the S/MAR nucleic acid sequence may serve as an origin of replication. In practice, vectors comprising a S/MAR nucleic acid sequence may behave as extra-chromosome in a transfected cell and be advantageously transmitted

to its progeny. Illustratively, suitable S/MAR nucleic acid sequence may be determined as described in Narwade *et al.* (Nucleic Acids Research, 2019; 47(14):7247–7261).

[0123] In one aspect, the invention relates to a suspension comprising a vector according to the invention.

[0124] In some embodiments, the suspension further comprises a fluid including one or more ingredients selected from the group consisting of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and a combination thereof.

[0125] In certain embodiment, the suspension is formulated for intravenous infusion. In practice, the suspension formulated for intravenous infusion may comprise saline (*e.g.*, 0.9% NaCl); lactated Ringers; 5% dextrose; a colloid, such as, *e.g.*, albumin; the like; and any combination thereof.

[0126] In one aspect, the invention pertains to a cell expressing the polypeptide, the cell being preferably transfected with an isolated nucleic acid molecule, or a vector according to the instant invention.

[0127] In certain embodiments, the cell is a eukaryote cell, preferably an animal cells, more preferably a mammalian cell. As used herein "mammalian cell" includes non-human mammalian cells and human cells. In some embodiments, the cell is a human cell.

[0128] In some embodiments, the cell is selected in the group comprising or consisting of nerve cells, bone cells, breast cells, red blood cells, white blood cells, cartilage cells, epithelial cells, endothelial cells, skin cells, muscle cells, bladder cells, kidney cells, liver cells, prostate cells, cervix cells, ovarian cells, pulmonary cells, retinal cells, conjunctival cells, corneal cells, fat cells, and the like.

[0129] It is to be understood that the cell according to the invention has been transfected with an isolated nucleic acid molecule according to the invention, or transduced with an isolated nucleic acid molecule according to the invention, or contacted with the vector or the suspension containing the nucleic acid molecule according to the invention. Therefore, the cell contains the nucleic acid molecules either integrated or not in its genome. In practice, because the vector is an expression system, the nucleic acid molecule

encoding the variant of SIRT6 is present within the cell in a form allowing its expression and its final location, *i.e.*, the cell nucleus and cytoplasm.

[0130] Another aspect of the invention relates to a pharmaceutical composition comprising (i) an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention, and (ii) a pharmaceutically acceptable excipient.

[0131] In some embodiments, a suitable pharmaceutically acceptable carrier according to the invention includes any and all conventional solvents, dispersion media, fillers, solid carriers, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. In certain embodiments, suitable pharmaceutically acceptable carriers may include, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and a mixture thereof. In some embodiments, pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the cells. The preparation and use of pharmaceutically acceptable carriers are well known in the art.

[0132] One aspect of the invention relates to the use of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention, in the regulation of ageing, and/or senescence, and/or lifespan in an individual, preferably a mammalian individual, more preferably a human individual.

[0133] As used herein, the term "regulating the senescence" is intended to refer to the process of decreasing, reducing or stopping the senescence mechanism, in particular premature senescence.

[0134] As used herein, the term "regulating the lifespan" is intended to refer to the process of increasing or improving the lifespan, in other words increasing or improving the duration of the life time course.

[0135] The invention further relates to a method for the regulation of ageing, and/or senescence, and/or lifespan in an individual, preferably a mammalian individual, more preferably a human individual, in need thereof, comprising the administration of a

therapeutically efficient amount of an isolated nucleic acid molecule, or an polypeptide isolated, or a vector according to the instant invention.

[0136] A further aspect of the invention pertains to the use of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention, in the repair of double strand breaks in a cell, preferably a mammalian cell, more preferably a human cell.

[0137] The invention relates also to a method for the repair of double strand breaks in a cell, preferably a mammalian cell, more preferably a human cell, comprising the administration of a therapeutically efficient amount of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention.

[0138] As used herein, "repair of double strand breaks" refers to the process allowing to recover DNA integrity by repair DNA damages consisting in the occurrence of a break in both strands of the double strand DNA nucleic acid. In practice, the repair of double strand breaks involves repair mechanisms such as non-homologous end joining (NHEJ) and homologous repair (HR).

[0139] In practice, efficiency of double strand breaks repair may be assessed by any suitable method from the state of the art, or a method adapted therefrom. Illustratively, double strand break repairs efficiency may be assessed by the methods described in Seluanov *et al.* (J Vis Exp, 2010; doi:10.3791/2002).

[0140] Within the scope of the invention, the nucleic acid molecule, the polypeptide, the vector, the suspension or the pharmaceutical composition according to the invention are intended to increase, improve or ameliorate the efficiency of the DSB repair in the target cell, as compared to the efficiency of the DSB repair in the absence of any treatment, as a reference level.

[0141] As used herein, the term "increase, improve or ameliorate the efficiency of the DSB repair" encompasses at least 1.2-fold increase as compared to a reference level. Within the scope of the invention, the term "at least 1.2-fold" encompasses, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.5, 5.0, 5.5, 6.0,

6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000-fold, or more.

[0142] In another aspect, the invention relates to an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention, for use in the prevention and/or the treatment of an age-related disease.

[0143] The invention also relates to the to the use of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention, for the preparation or the manufacture of a medicament for the prevention and/or the treatment of an age-related disease.

[0144] In some further aspect, the invention pertains to a method for the prevention and/or the treatment of an age-related disease in an individual in need thereof, comprising the administration of a therapeutically efficient amount of an isolated nucleic acid molecule, or an isolated polypeptide, or a vector according to the instant invention.

[0145] In certain embodiments, the individual in need thereof is a mammalian individual, preferably a human individual.

[0146] In some embodiments, the age-related disease is selected in the group comprising or consisting of progeria, Werner syndrome, neurodegenerative disease, Alzheimer's disease, cancer, cardiovascular disease, obesity, type 2 diabetes, hypercholesterolemia, hypertension, ocular disorders, cataracts, glaucoma, osteoporosis, blood clotting disorders, arthritis, hearing loss and stroke.

[0147] In one embodiment, the age-related disease is progeria or Werner syndrome.

[0148] In some embodiments, the age-related disease is cancer. In certain embodiments, the cancer is a blood cancer or a solid cancer.

[0149] As used herein, the term “blood cancer”, also referred to as “hematologic cancer”, encompasses any cancer involving uncontrolled proliferation of blood cells, in particular white blood cells. Blood cancers includes leukemia, lymphoma (Hodgkin and non-Hodgkin lymphomas) and myeloma.

[0150] In certain embodiments, the cancer is a blood cancer. In some embodiments, said cancer is a blood cancer selected in the group consisting of Hodgkin's disease, immunoblastic lymphadenopathy, lymphoma, chronic lymphocytic leukemia, acute leukemia, and the like.

[0151] As used herein, the term "solid cancer" encompasses any cancer (also referred to as malignancy) that forms a discrete tumor mass, as opposed to cancers (or malignancies) that diffusely infiltrate a tissue without forming a mass.

[0152] In certain embodiments, the cancer is a solid cancer. In some embodiments, the solid cancer is selected in the group consisting of fibrosarcoma, melanoma, breast carcinoma, colon carcinoma, renal carcinoma, adrenocortical carcinoma, testicular teratoma, skin sarcoma, fibrosarcoma, lung carcinoma, adenocarcinoma, liver carcinoma (*i.e.*, hepatocarcinoma), glioblastoma, prostate carcinoma, ovarian cancer and pancreatic carcinoma.

[0153] In some embodiments, the age-related disease is selected in the group comprising or consisting of Hutchinson Gilford progeria, Werner syndrome, Alzheimer's disease and cancer.

[0154] In some embodiments, the age-related disease is selected in the group comprising or consisting of Hutchinson Gilford progeria, Werner syndrome, Alzheimer's disease and fibrosarcoma.

[0155] In some embodiments, the age-related disease is selected in the group comprising or consisting of Hutchinson Gilford progeria, Werner syndrome, Alzheimer's disease and hepatocarcinoma.

[0156] In one embodiment, the age-related disease is hepatocarcinoma. In one embodiment, the age-related disease is fibrosarcoma.

[0157] In certain embodiments, the progeria is the Hutchinson Gilford progeria.

[0158] In one embodiment, the age-related disease is Werner syndrome.

[0159] In some embodiments, the nucleic acid molecule, the polypeptide, the vector, the suspension, or the pharmaceutical composition according to the invention is to be administered to an individual in need thereof by any suitable route, *i.e.*, by a dermal administration, by an oral administration, a topical administration or a parenteral administration, *e.g.*, by injection, including a sub-cutaneous administration, a venous administration, an arterial administration, in intra-muscular administration, an intra-ocular administration and an intra-auricular administration.

[0160] In certain embodiments, the nucleic acid molecule, the polypeptide, the vector, the suspension, or the pharmaceutical composition according to the invention is to be administered to an individual in need thereof by a dermal administration. In certain embodiments, the nucleic acid molecule, the polypeptide, the vector, the suspension, or the pharmaceutical composition according to the invention is associated with a composition enabling and/or facilitating dermal administration, *e.g.*, by increasing dermal tropism or by increasing dermal barrier penetration. In some embodiments, the dermal administration enables a prolonged liberation of the nucleic acid molecule, the polypeptide, the vector, the suspension, or the pharmaceutical composition according to the invention.

[0161] In certain embodiments, the nucleic acid molecule, the polypeptide, the vector, the suspension, or the pharmaceutical composition according to the invention is to be administered to an individual in need thereof by an intravenous administration, in particular by intravenous infusion or intravenous injection.

[0162] Within the scope of the instant invention, the therapeutically effective amount of the nucleic acid molecule, the polypeptide, the vector, the suspension, or the pharmaceutical composition according to the invention, to be administered may be determined by a physician or an authorized person skilled in the art and can be suitably adapted within the time course of the treatment.

[0163] In certain embodiments, the therapeutically effective amount to be administered may depend upon a variety of parameters, including the material selected for administration, whether the administration is in single or multiple doses, and the

individual's parameters including age, physical conditions, size, weight, gender, and the severity of the age-related disease to be treated.

[0164] In certain embodiments, a therapeutically effective amount of the isolated polypeptide, or the pharmaceutical composition comprising the isolated polypeptide according to the invention, agent may range from about 0.001 mg to about 3,000 mg, per dosage unit, preferably from about 0.05 mg to about 100 mg, per dosage unit.

[0165] Within the scope of the instant invention, the expression "from about 0.001 mg to about 3,000 mg" includes, from about 0.001 mg, 0.002 mg, 0.003 mg, 0.004 mg, 0.005 mg, 0.006 mg, 0.007 mg, 0.008 mg, 0.009 mg, 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.07 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1,000 mg, 1,100 mg, 1,150 mg, 1,200 mg, 1,250 mg, 1,300 mg, 1,350 mg, 1,400 mg, 1,450 mg, 1,500 mg, 1,550 mg, 1,600 mg, 1,650 mg, 1,700 mg, 1,750 mg, 1,800 mg, 1,850 mg, 1,900 mg, 1,950 mg, 2,000 mg, 2,100 mg, 2,150 mg, 2,200 mg, 2,250 mg, 2,300 mg, 2,350 mg, 2,400 mg, 2,450 mg, 2,500 mg, 2,550 mg, 2,600 mg, 2,650 mg, 2,700 mg, 2,750 mg, 2,800 mg, 2,850 mg, 2,900 mg, 2,950 mg and 3,000 mg per dosage unit.

[0166] In certain embodiments, the isolated polypeptide or the pharmaceutical composition comprising the isolated polypeptide according to the invention, may be at dosage levels sufficient to deliver from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, preferably from about 0.1 mg/kg to about 40 mg/kg, preferably from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, and more preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day. Within the scope of the instant invention, the expression "from about 0.001 mg/kg to about 100 mg/kg" includes about 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3

mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg and 100 mg/kg.

[0167] In some embodiments, the therapeutically efficient amount of the isolated nucleic acid molecule, vector, or pharmaceutical composition according to the invention is ranging from about 10^1 to about 10^{15} copies per ml. In practice, a therapeutically efficient amount includes about 10^1, 5×10^1, 10^2, 5×10^2, 10^3, 5×10^3, 10^4, 5×10^4, 10^5, 5×10^5, 10^6, 5×10^6, 10^7, 5×10^7, 10^8, 5×10^8, 10^9, 5×10^9, 10^{10}, 5×10^{10}, 10^{11}, 5×10^{11}, 10^{12}, 5×10^{12}, 10^{13}, 5×10^{13}, 10^{14}, 5×10^{14} and 10^{15} copies per ml. In certain embodiments, the therapeutically efficient amount is from about 10^1 to about 10^{15} copies per cm^3, which includes about 10^1, 5×10^1, 10^2, 5×10^2, 10^3, 5×10^3, 10^4, 5×10^4, 10^5, 5×10^5, 10^6, 5×10^6, 10^7, 5×10^7, 10^8, 5×10^8, 10^9, 5×10^9, 10^{10}, 5×10^{10}, 10^{11}, 5×10^{11}, 10^{12}, 5×10^{12}, 10^{13}, 5×10^{13}, 10^{14}, 5×10^{14} and 10^{15} copies per cm^3. In some embodiments, the therapeutically efficient amount is from about 10^1 to about 10^{15} copies per dose, which includes about 10^1, 5×10^1, 10^2, 5×10^2, 10^3, 5×10^3, 10^4, 5×10^4, 10^5, 5×10^5, 10^6, 5×10^6, 10^7, 5×10^7, 10^8, 5×10^8, 10^9, 5×10^9, 10^{10}, 5×10^{10}, 10^{11}, 5×10^{11}, 10^{12}, 5×10^{12}, 10^{13}, 5×10^{13}, 10^{14}, 5×10^{14} and 10^{15} copies per dose.

[0168] In certain embodiments, the isolated nucleic acid molecule, isolated polypeptide, vector, suspension, or pharmaceutical composition according to the instant invention is to be co-administered, or sequentially administered, with a drug suitable for preventing and/or treating an age-related disease, in particular a disease selected in the group consisting of progeria, Werner syndrome, neurodegenerative disease, Alzheimer's disease, cancer, cardiovascular disease, obesity, type 2 diabetes, hypercholesterolemia, hypertension, ocular disorders, cataracts, glaucoma, osteoporosis, blood clotting disorders, arthritis, hearing loss and stroke.

[0169] As used herein, the term “co-administered” refers to a simultaneous administration of the active principles. As used herein, the term “sequentially administered” refers to an administration of a first active principle before or after the administration of a second active principle.

[0170] Another aspect of the invention relates to a kit comprising (i) an isolated nucleic acid, an isolated polypeptide molecule, a vector, or a suspension according to the instant invention, and (ii) means to administer the isolated nucleic acid molecule, the isolated polypeptide, the vector, or the suspension.

[0171] In some embodiments, the means to administer the isolated nucleic acid molecule, the isolated polypeptide, the vector, or the suspension include a syringe or a catheter.

[0172] Another aspect of the invention relates to a method for increasing adipogenic differentiation in a subject, comprising administering an effective amount of a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the amino acid sequence selected from the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**, or a nucleic acid molecule encoding thereof. In some embodiments, the nucleic acid molecule has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the nucleic acid sequence selected from the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**.

[0173] Another aspect of the invention relates to an *in vitro* method for increasing adipogenic differentiation of a cell, or population of cells, comprising exposing the cell, or population of cells, to an effective amount of a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the amino acid sequence selected from the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**, or a nucleic acid molecule encoding thereof. In some embodiments, the nucleic acid molecule has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the nucleic acid sequence selected from the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**. In some embodiments, the cell, or population of cells, is a pre-adipocyte.

[0174] Another aspect of the invention relates to a method for decreasing α-SMA expression in a subject, comprising administering an effective amount of a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence

identity with the amino acid sequence selected from the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**, or a nucleic acid molecule encoding thereof. In some embodiments, the nucleic acid molecule has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the nucleic acid sequence selected from the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**.

[0175] Another aspect of the invention relates to an *in vitro* method for decreasing α-SMA expression in a cell, or population of cells, comprising exposing the cell, or population of cells, to an effective amount of a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the amino acid sequence selected from the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**, or a nucleic acid molecule encoding thereof. In some embodiments, the nucleic acid molecule has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the nucleic acid sequence selected from the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**. In some embodiments, the cell, or population of cells, is a pre-adipocyte.

[0176] Another aspect of the invention relates to a method for decreasing collagen expression in a subject, comprising administering an effective amount of a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the amino acid sequence selected from the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**, or a nucleic acid molecule encoding thereof. In some embodiments, the nucleic acid molecule has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the nucleic acid sequence selected from the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**.

[0177] In some embodiments, collagen is selected from the group comprising or consisting of type I collagen, type II collagen, type III collagen, type V collagen and type IX collagen. In some embodiments, collagen is type I collagen. In some embodiments, the type I collagen is type I alpha 1 collagen or type I alpha 2 collagen. In some

embodiments, the type I collagen is type I alpha 1 collagen, and is encoded by COL1A1 gene.

[0178] Another aspect of the invention relates to an *in vitro* method for decreasing collagen expression in a cell, or population of cells, comprising exposing the cell, or population of cells, to an effective amount of a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the amino acid sequence selected from the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**, or a nucleic acid molecule encoding thereof. In some embodiments, the nucleic acid molecule has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5 or 100% sequence identity with the nucleic acid sequence selected from the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**. In some embodiments, the cell, or population of cells, is a pre-adipocyte.

[0179] In some embodiments, collagen is selected from the group comprising or consisting of type I collagen, type II collagen, type III collagen, type V collagen and type IX collagen. In some embodiments, collagen is type I collagen. In some embodiments, the type I collagen is type I alpha 1 collagen or type I alpha 2 collagen. In some embodiments, the type I collagen is type I alpha 1 collagen, and is encoded by COL1A1 gene.

EXAMPLES

[0180] The present invention and disclosure are further illustrated by the following examples.

EXAMPLE 1

1. Materials and Methods

1.1. Study subjects and sample collection

[0181] This study group consisted of 1,068 Ashkenazi Jewish (AJ) samples: 496 AJ centenarians and 572 AJ controls that were previously collected as part of longevity study

at the Albert Einstein College of Medicine. A centenarian is defined as a healthy individual living independently at 95 years of age or older and a control is defined as an individual without a family history of unusual longevity; parents of controls survived to the age of 85 years or less. Informed written consent was obtained in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine. Genomic DNAs were extracted from blood samples and amplified using Illustra GenomiPhi V2 DNA Amplification kits (GE healthcare Life Sciences®).

1.2. Two rounds of targeted sequencing

[0182] In stage 1, 301 candidate genes involved in genome maintenance were selected and sequenced in 51 centenarians and 51 controls. The complete list of 301 candidate genes is provided in **Table 1**.

[0183] **Table 1**: List of 301 genome maintenance genes sequenced in first round

AATF	LIG1	POU2F3	SIRT4	UBE3A
AICDA	LIG3	POU3F1	SIRT5	UBE3B
ALKBH2	LIG4	POU3F2	SIRT6	UBE3C
ANAPC10	LMNA	POU3F3	SIRT7	UBE4A
ANAPC11	MBD4	POU3F4	SKP1	UBE4B
ANAPC13	MDM2	POU4F1	SKP2	UBOX5
ANAPC4	MEN1	POU4F2	SLC25A24	UBR5
ANAPC5	MGMT	POU4F3	SMEK1	UIMC1
ANAPC7	MGRN1	POU5F2	SMUG1	UNG
APC	MID1	POU6F1	STRAP	URM1
APC2	MLH1	POU6F2	STUB1	WRN
APEX1	MLH3	PPIL2	SUMO1	WWP1
APEX2	MNAT1	PRDX2	SUMO1P1	WWP2
ATM	MPG	PRKDC	SUMO2	XAB2
ATMIN	MRE11A	PRPF19	SUMO3	XPA
ATR	MSH2	RAD1	SUMO4	XPC
ATRIP	MSH3	RAD17	SYVN1	XRCC1
ATRX	MSH4	RAD18	TBP	XRCC2
BARD1	MSH6	RAD21	TCEB1	XRCC3
BLM	MUTYH	RAD23A	TCEB2	XRCC4
BRCA1	NBN	RAD23B	TDG	XRCC5
BRCA2	NEDD4	RAD50	TDP1	XRCC6
BRCC3	NEIL1	RAD51	TERF1	
BRIP1	NEIL2	RAD51B	TERF2	
BTRC	NEIL3	RAD51C	TERF2IP	

BUB1	NHEJ1	RAD51D	TERT
C19orf40	NHLRC1	RAD52	TNKS
CBL	NTHL1	RAD9A	TP53
CDC16	NUDT1	RAG1	TP53BP1
CDC20	OGG1	RBX1	TPP1
CDC23	PALB2	RCHY1	TREX1
ERCC8	PAPD7	RECQL	TREX2
EXO1	PARK2	RECQL4	TRIM32
FAM175A	PARP1	RECQL5	TRIP12
FANCA	PARP2	REV1	TXN
FANCB	PCNA	REV3L	UBA1
FANCC	PIAS1	RFC1	UBA2
FANCD2	PIAS4	RFC2	UBA3
FANCE	PINX1	RFC3	UBE2A
FANCF	PML	RFC4	UBE2B
FANCG	PMS1	RFC5	UBE2C
FANCI	PMS2	RFWD2	UBE2D2
FANCL	PNKP	RNF168	UBE2F
FANCM	POLA1	RNF7	UBE2G1
FBXO4	POLB	RNF8	UBE2G2
FEN1	POLD1	RPA1	UBE2H
GABARAPL2	POLD2	RPA2	UBE2I
GADD45A	POLE	RPA3	UBE2J1
GADD45B	POLE3	RPA4	UBE2J2
GTF2H1	POLE4	SAE1	UBE2K
GTF2H2	POLG	SCARA3	UBE2L3
GTF2H3	POLH	SENP1	UBE2L6
GTF2H5	POLI	SENP2	UBE2M
H2AFX	POLK	SENP3	UBE2N
HDAC9	POLL	SENP5	UBE2O
HERC1	POLM	SENP6	UBE2Q1
HERC2	POLN	SENP7	UBE2S
HLTF	POLQ	SENP8	UBE2T
HMGB1	POT1	SIAH1	UBE2U
HUS1	POU1F1	SIRT1	UBE2V2
HUWE1	POU2F1	SIRT2	UBE2W
ITCH	POU2F2	SIRT3	UBE2Z

[0184] SureSelectXT Target enrichment platform (Agilent®) was applied to design and capture genomic regions covering exons, exon-intron junctions, and 2 kb proximal promoter sequences of the candidate genome maintenance genes. Individually indexed libraries were sequenced by the Illumina® HiSeq2000. Sequencing reads were aligned

using BWA and variants were called by GATK. All variants in the 102 samples were characterized and functionally annotated using ANNOVAR. To prioritize candidate genes, UMINP (Univariate minP) gene-based association with permutation (n=100) was performed, which considered the effect of at least one significant single variant and identified 122 genes showing a nominal p-value less than 0.05 (**Table 2**).

[0185] **Table 2**: List of 122 genes showing significant association with longevity in gene-based UMINP analysis

Gene	p-value	Gene	p-value
AATF	< 0.01	**POU3F4**	< 0.01
AICDA	< 0.01	**POU4F2**	< 0.01
ANAPC11	< 0.01	**POU4F3**	< 0.01
ANAPC5	< 0.01	**POU5F2**	< 0.01
APC2	< 0.01	**PPIL2**	< 0.01
APEX2	< 0.01	**PRKDC**	< 0.01
ATM	< 0.01	**RAD17**	< 0.01
ATRX	< 0.01	**RAD23B**	< 0.01
BARD1	< 0.01	**RAD50**	< 0.01
BLM	< 0.01	**RAD51**	< 0.01
BRCA1	< 0.01	**RAD51D**	< 0.01
BUB1	< 0.01	**RAD52**	< 0.01
CDC26	< 0.01	**RAD9A**	< 0.01
CDC34	< 0.01	**RAG1**	< 0.01
CUL7	< 0.01	**RBX1**	< 0.01
DCLRE1A	< 0.01	**RECQL4**	< 0.01
DCLRE1B	< 0.01	**RFC5**	< 0.01
DCLRE1C	< 0.01	**RNF168**	< 0.01
DDB1	< 0.01	**RNF8**	< 0.01
DMC1	< 0.01	**SAE1**	< 0.01
E2F1	< 0.01	**SENP1**	< 0.01
EXO1	< 0.01	**SENP8**	< 0.01
FAM175A	< 0.01	**SIRT1**	< 0.01
FANCB	< 0.01	**SIRT2**	< 0.01
FANCD2	< 0.01	**SIRT4**	< 0.01
FANCF	< 0.01	**SIRT7**	< 0.01
FANCI	< 0.01	**SKP2**	< 0.01
FANCM	< 0.01	**STRAP**	< 0.01
FEN1	< 0.01	**SUMO1**	< 0.01
GADD45B	< 0.01	**SUMO2**	< 0.01
HERC2	< 0.01	**SYVN1**	< 0.01
HMGB1	< 0.01	**TERF2**	< 0.01

HUS1	< 0.01	**TNKS**	< 0.01
HUWE1	< 0.01	**TP53BP1**	< 0.01
ITCH	< 0.01	**TREX1**	< 0.01
LIG1	< 0.01	**UBA3**	< 0.01
MBD4	< 0.01	**UBE2B**	< 0.01
MDM2	< 0.01	**UBE2C**	< 0.01
MEN1	< 0.01	**UBE2J2**	< 0.01
MGMT	< 0.01	**UBE2O**	< 0.01
MGRN1	< 0.01	**UBE2Q1**	< 0.01
MNAT1	< 0.01	**UBE2U**	< 0.01
MPG	< 0.01	**UBE2V2**	< 0.01
MRE11A	< 0.01	**UBE2W**	< 0.01
MSH3	< 0.01	**UBOX5**	< 0.01
NEIL3	< 0.01	**UIMC1**	< 0.01
NTHL1	< 0.01	**UNG**	< 0.01
OGG1	< 0.01	**URM1**	< 0.01
PALB2	< 0.01	**XPA**	< 0.01
PINX1	< 0.01	**XPC**	< 0.01
PMS1	< 0.01	**XRCC1**	< 0.01
POLA1	< 0.01	**CDH1**	0.01
POLD1	< 0.01	**CREBBP**	0.01
POLE	< 0.01	**PIAS4**	0.01
POLH	< 0.01	**SIRT5**	0.01
POLM	< 0.01	**ATR**	0.03
POLN	< 0.01	**SENP3**	0.03
POT1	< 0.01	**SIRT6**	0.03
POU2F1	< 0.01	**NBN**	0.04
POU2F3	< 0.01	**POU3F2**	0.04
POU3F3	< 0.01	**RFC4**	0.04

[0186] Gene ontology (GO) enrichment analysis was implemented using the clusterProfiler package and identified the enrichment of 122 UMINP-significant genes in DNA double-strand breaks repair functions.

[0187] In stage 2, 106 candidate genes involved in DNA double-strand breaks repair were selected and sequenced in 496 centenarians and 572 controls. The list of 106 DSB repair genes is provided in **Table 3**.

[0188] **Table 3**: List of 106 DSB repair genes sequenced in second round

APLF	FANCB	NSMCE1	RAD51C	SMARCAL1
APTX	FANCD2	NUCKS1	RAD51D	SMC5
ATM	FANCM	OGG1	RAD52	SPIDR
BABAM1	GEN1	OTUB1	RAD54B	SPO11
BARD1	GINS2	PALB2	RAD54L	SWI5
BLM	GINS4	PARP1	RBBP8	TDP1
BRCA1	HELB	PARP2	REC8	TDP2
BRCA2	HELQ	PARP3	RECQL	TERF2IP
CDC7	HUS1	PAXIP1	RECQL5	TP53
CHEK1	INO80	PNKP	REV3L	TP53BP1
CHEK2	KAT5	POLA1	RMI1	UBE2N
DCLRE1A	LIG3	POLB	RMI2	WRAP53
DCLRE1B	LIG4	POLM	RNF168	WRN
DCLRE1C	MEIOB	POLQ	RNF8	XRCC1
DDX11	MGMT	POT1	RPA3	XRCC2
DNA2	MLH1	PRDM9	RTEL1	XRCC4
EME1	MMS19	PRKDC	SETD2	XRCC5
ERCC1	MMS22L	RAD50	SETMAR	XRCC6
ERCC4	MSH2	RAD51	SETX	
ERCC6	MUS81	RAD51AP1	SFR1	
ERCC8	NHEJ1	RAD51AP2	SIRT6	
FAN1	NIPBL	RAD51B	SLX4	

[0189] A 25-sample pooling target capture sequencing was performed. Briefly, 25 samples from the same group were pooled together for equimolar concentration and totally 20 pools of centenarians and 23 pools of controls were used to generate indexed libraries according to the Illumina® TruSeq DNA sample preparation low-throughput (LT) protocol. Captured libraries were further generated using SeqCap EZ Choice Enrichment Kit (Roche®) which enriched genomic regions covering exons, exon-intron junctions, and 2 kb proximal promoter sequences of the candidate DSB genes and sequenced by Illumina® HiSeq2000. Sequencing reads were aligned using BWA and variants were called using the software CRISP (Comprehensive Read analysis for Identification of Single Nucleotide Polymorphisms), which was specifically developed to call variants in pooled DNA sequence data. All variants in the 1,068 samples were characterized and functionally annotated using ANNOVAR. To test for longevity associations, we performed different subtypes of SKAT (Sequencing Kernel Association Test) analyses, such as SKAT for rare variants, SKAT-C for all variants (common and

rare), and SKAT-O for variants with directionality (enrichment only in centenarians or in controls) (**Table 4**).

[0190] **Table 4**: List of 10 longevity-associated DSB repair genes with their centenarian-enriched missense variants (part 1)

Gene	SKAT-C	SKAT-O	SKAT
PARP2	0.00142	0.01563	0.01362
RAD51B	0.0041	0.04227	0.025
SIRT6	0.03423	0.33932	0.20718
TERF2IP	0.042	0.00703	0.02925
ATM	0.04499	0.05524	0.03293
MLH1	0.06486	0.07838	0.03947
BRCA2	0.07093	0.0099	0.03295
ERCC6	0.28236	0.01455	0.01379
MMS22L	0.39608	0.04202	0.36627
TP53	0.47502	0.00147	0.00488

1.3. Cell lines

[0191] HEK293 SIRT6 overexpression lines were generated by transfecting HEK293 cells with linearized CMV-SIRT6 plasmids via jetPRIME transfection reagent and selecting stably integrated clones. To generate normal human fibroblasts expressing WT and centenarian alleles of SIRT6 under control of cumate-inducible promoter (cumate SIRT6 fibroblasts), constructs containing SIRT6 alleles under control of cumate promoter were integrated in the genome of telomerase immortalized SIRT6-KO human HCA2 foreskin fibroblasts via the PiggyBac Transposon Vector System. Endogenous SIRT6 was knocked out in these cells using CRISPR/Cas-9. NHEJ and HR reporter assays were conducted using telomerase-immortalized HCA2 human fibroblast cell lines containing integrated reporter constructs (I9A and H15C). HT1080 and HeLa cell lines were used to assess anti-tumor activity.

1.4. Cell culture

[0192] All cell lines were maintained in humidified incubators at 5% CO_2, 5% O_2, at 37°C. Cells were grown in Eagle's minimum essential medium with 15% fetal bovine serum and 1× penicillin/streptomycin, with the exception of the HEK293, HT1080 and

HeLa cells, which were culture in DMEM with D-Glucose and L-Glutamine. The cell lines are routinely tested for mycoplasma contamination.

1.5. Western blotting

[0193] Cells and reactions were collected using a 2× Laemmli solution and incubated on ice for 15 min, during which time the samples were passed through a large gauge needle several times and vortexed every 5 min. Samples were then spun at 14,000 RPM to remove debris and the supernatant transferred to a new tube. Samples were heated in boiling water for 20 min before being centrifuged at 14,000 RPM for 1min and loaded into a BioRad® Criterion 4-20% gel. After transfer to PDVF membrane and blocked (5% dehydrated milk) for 2 h at RT (room temperature), membranes were incubated overnight antibodies in 2.5% blocking buffer at 4°C. Membranes were washed 3× with TBST for 10 min each before secondary antibody in 1×TBST was added for 2 h incubation at RT. Membranes were washed 3× with TBST for 10 min and then imaged.

[0194] The following antibodies were used: H3 (Abcam® ab500)-1:5,000, H3K9ac (Abcam® ab4441)-1:1,000, H3K18ac (Abcam® ab1191)-1:1,000, β-tubulin (Abcam® ab6046)-1:10,000, SIRT6 (Cell Signaling® #12486)-1:1,000, AbD33204 (Rabbit, REF)-1:500, Lamin A/C-1:1,000 (Abcam® ab108595, Millipore® 05-714), γH2AX (Millipore® 05-636), PARP1(Abcam® ab227244)-1:1,000, mADPr 1:500 (AbD33204 and AbD33205).

1.6. Immunoprecipitation (IP)

[0195] Cells were plated and grown with equivalent dosages of cumate for 48 h prior to IP. In brief, cells were collected via trypsin and centrifugation and then lysed on ice using IP Buffer (20 mM HEPES pH=8, 0.2 mM EDTA, 5% glycerol, 150 mM NaCl, 1% NP40) + COMplete® protease inhibitor for 10 min. Lysate was sonicated at 25% output for 10 pulses and then debris pelleted by centrifugation at 4°C at 13,000 RPM for 10 min. Supernatant was transferred to a new tube and precleared with A/G Sepharose beads at 4°C for 1 h on a rotator. Beads were removed by centrifugation and transfer of cleared sample to new tubes. 50 µl of sample was reserved as input control. Samples were incubated overnight at 4°C with 3 µg of antibody (SIRT6; Cell Signaling®) #12486;

PARP1-Abcam® ab227244; Lamin A/C-Millipore® 05-714; AbD33204, AbD33205, then with 30 µl of 25% Sepharose for 2 h at 4°C. Samples were spun down and beads washed 5× with IP buffer. Final resuspension with 100 µl IP buffer.

1.7. DNA Repair Assays

[0196] Both NHEJ and HR efficiency were assessed as previously described in Seluanov *et al.* (J Vis Exp, 2010; doi:10.3791/2002). In brief, I-Sce1, SIRT6 or HPRT control, and dsRED plasmid were transfected into telomerase-immortalized human foreskin fibroblasts containing chromosomally integrated NHEJ or HR reporter cassettes (I9A or H15C) cells. Cells were allowed 3 days to recover prior to analysis by flow cytometry. Efficiency was calculated as the ratio of GFP events over dsRED events.

1.8. Transfections

[0197] Transfections were carried out by plating cells at a density of 500,000 cells/10 cm plate two days prior to transfection. Transfections were carried out using the Amaxa® Nucleofector with Normal Human Dermal Fibroblast transfection solution following manufacture's protocol. For cancer cell transfections, jetPRIME transfection reagent was used to deliver plasmids into cells.

1.9. Quantitative RT-PCR

[0198] Total RNA was isolated from cells at 80% confluence using Trizol Reagent and then treated with DNase. cDNA was synthesized using Superscript III (Life Technologies®) cDNA kit with the OligodT primer. qRT-PCR was performed on the BioRad® CFX Connect Real Time machine with SYBR™ Green Master Mix (BioRad®) using 30 ng of cDNA per reaction with 3× reactions/sample. All primer sets (see **Table 5** below) were tested for specificity and efficiency. Actin was assayed using Quantum mRNA Actin Universal primers (Thermo Fisher Scientific® AM1720).

[0199] **Table 5:** Primers used for the qRT-PCR

Primer name	**Sequence**	**SEQ ID NO:**
LINE1 ORF1 Fwd	ATGGCGAAAGGCAAACGTAAG	**SEQ ID NO: 9**
LINE1 ORF1 Rev	ATTTTCGGTTGTGTTGGGGTG	**SEQ ID NO: 10**
LINE1 ORF2 Fwd	GCAGGGGTTGCAATCCTAGTC	**SEQ ID NO: 11**
LINE1 ORF2 Rev	CTGGGTGCTCCTGTATTGGGT	**SEQ ID NO: 12**

1.10. Immunofluorescence and apoptosis

[0200] γH2AX immunostaining was carried out as previously described in Mao *et al.* (Science, 2011; Vol. 332:1443-1446). Anti- γH2AX antibody was purchased from Millipore® (05-636). Apoptosis in fibroblasts was measured using the Annexin V Staining Kit (Roche®).

1.11. Gamma Irradiation

[0201] Cells were grown to 75% confluency prior to treatment on coated slides. A Cs-137 irradiator was used to deliver 2 Gy of radiation to cells. Cells were transported in a 37°C container and media were replaced post exposure.

1.12. Paraquat Treatment

[0202] Cells were maintained to 75% confluency, at which point fresh media lacking sodium pyruvate and containing paraquat was added. Cells were maintained for 24 h in treated media, followed by replacement with fresh media lacking sodium pyruvate. 48 h post treatment, the cells stained and evaluated for apoptosis.

1.13. SIRT6 Protein Purification

[0203] His-tagged SIRT6 cDNA was cloned into pET11a vectors and transformed into Rosetta-Gami *E. coli* cells. Cells were grown in presence of antibiotics and then protein production was induced with 0.5 mM IPTG 2 h before harvest. Cells were spun down and

lysed on ice for 1 h using a in a solution of 50 mM Tris-HCl (pH=7.5), 300 mM NaCl, 10% glycerol, and 10 mM imidazole with EDTA-free protease inhibitor (Sigma Aldrich®, #P8849) and 1mg/ml egg white lysozyme followed by sonication with a Branson instrument. After removal of cell debris by centrifugation, lysate was incubated with Ni2+ -NTA agarose overnight. Solution with beads was placed in gravity column and washed with lysis solution, followed by 2 volumes of wash buffer (lysis buffer + 30 mM imidazole). Finally, protein was eluted with elution buffer (lysis buffer + 500 mM imidazole) and fractions were collected. Protein concentration was assessed by bicinchoninic acid (BCA) assay and run on an SDS-page gel. The 3-4 highest concentration fractions were pooled and dialyzed against storage buffer (50 mM Tris-HCl pH=7.5, 150 mM NaCl, 1 mM DTT, 5% glycerol).

1.14. Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC)

[0204] Turnover experiments were performed using HEK293 cell lines stably expressing SIRT6 WT or centSIRT6. Cells were cultured for one week until confluent in MEM for SILAC (Thermo Fisher Scientific®) supplemented with 10% dialyzed FBS (Gibco®), L-glutamine, L-arginine $^{13}C_6$ $^{15}N_4$ (Cambridge Isotopes®), and L-lysine $^{13}C_6$ $^{15}N_2$ (Cambridge Isotopes®). The media were changed to normal culture medium and cell pellets were harvested at 0, 2, 4, 6, 8, 12, and 24 h post-media change. Cell pellets were lysed in buffer containing 8 M urea, 75 mM NaCl, 50 mM Tris, pH 8.5, with protease inhibitor cocktail (Roche®). Pellets were vortexed for 30 s and sonicated 5× 10 s with 1 min rest on ice in between sonication steps. Lysate was centrifuged at 15,000 ×g for 10 min and then supernatant was collected.

1.15. SIRT6 deacylation activity

[0205] The substrate for this reaction is a synthetic TNF-derived peptide first developed by Schuster *et al.* (Sci Rep, 2016; Vol. 6:22643) was synthesized by Genscript®. Reactions were performed with 150 mM NaCl, 20 mM Tris, 5% glycerol, 1mM β-mercaptoethanol, 2 μM SIRT6, and substrate concentration ranges of 7-1000 μM NAD+ and 2-88 μM peptide. Reactions were performed at 37°C and fluorescence was measured using 310/405 nm excitation/emission spectra on a Tecan Spark 20M plate reader.

Readings were taken every 30 s and initial rates were calculated from the relative fluorescence increase over a minimum of 6 min.

1.16. Histone Analysis

[0206] Histones were purified from cumate SIRT6 fibroblasts (SIRT6 KO primary human foreskin fibroblasts constitutively expressing the catalytic subunit of telomerase as well as various alleles of SIRT6 via a cumate-inducible promoter) using an established acid extraction method followed by propionylation of lysines prior to trypsin digestion to enhance coverage. A data independent analysis (DIA) mass spectrometry (MS) method was employed for quantitation of modified peptides after samples were separated by nano-LC using EpiProfile® or, alternatively, after direct infusion followed by a one-minute acquisition and analysis with EpiProfileLite®.

1.17. Analysis of SIRT6 and LMNA protein-protein interactions by mass spec

[0207] Cumate SIRT6 fibroblasts were used to compare interactomes for centSIRT6 and the wild type SIRT6. Nuclear extracts were prepared using hypotonic lysis buffer. Nuclei isolated from approximately 2.5×10^7 cells were resuspended in 1 ml of extraction buffer (50 mM TrisHCl pH 7.5, 150 mM NaCl, 10% glycerol, 5 mM DTT, 0.25% NP-40, 1× Roche® Complete protease inhibitors, as well as kinase/phosphatase inhibitors- 1 mM $NaVO_4$, 10 mM beta-glycerophosphate, 1 mM sodium pyrophosphate, 1 mM NaF) and nuclei were disrupted by passage through a 27-gauge needle followed by sonication (3 pulses at constant 20% power) with a Branson® Sonifier on ice. Next, samples were filtered through 0.2 μm MWCO filter to remove any insoluble material (and reduce non-specific binding). Proteins were quantified using the BCA assay and equal amount of wild type SIRT6 and centSIRT6 derived extract were divided into 5 replicates each. Three replicates of extract received 4 μg of anti-SIRT6 antibody (Cell Signaling® #1248) or anti-LaminA (Lamin A/C-Millipore® 05-714) and 75 μl of Miltenyi® protein G μMACS magnetic particles. Two of the replicates were mixed with normal rabbit IgG (Cell Signaling® #2729) as controls for non-specific binding to the particles. Samples were rotated at 4°C for 6 h followed by separation by the magnet. Samples were washed with 3 additional ml of extraction buffer and eluted with 100 μl of boiling elution buffer (5%

SDS with 50 mM Tris-HCl pH 7.5). After removal of SDS with S-columns (Protifi®; Huntington, NY) and trypsin digestion, peptides were resuspended in MS-grade water and labeled with tandem mass tags (TMT 10-plex; Thermo Fisher Scientific®). Samples were resolved by nano-electrospray ionization on an Orbitrap Fusion Lumos MS instrument (Thermo Fisher Scientific®) in positive ion mode using a 30 cm home-made column packed with 1.8 μm C-18 beads to resolve the peptides. Solvent A was 0.1% formic acid and solvent B was 100% acetonitrile (CAN) with 0.1% formic acid. The length of the run was 2 h with a 90 min gradient. CID (35% collision energy) was used for MS2 fragmentation. HCD (60% collision energy) was used for MS3 detection of TMT groups. Other details of the run parameters may be found in the embedded run report of the RAW data file uploaded to the ProteomeXchange database. Peptide assignments were made using Proteome Discoverer and Sequest and MS3 ions were used for quantitation. False discovery rates (FDR) were estimated using a Decoy Database Search with Target FDR (strict) set to 0.01 and Target FDR (relaxed) set to 0.05. Validation was based on q-value. In the consensus step, ions with a co-isolation threshold above 30% were excluded. Normalization between replicates was achieved using the total protein approach where peptide counts for a single protein were divided by the sum of all proteins in the lane. Proteins appearing in specific antibodies (either anti-SIRT6 or anti-Lamin A) compared to normal IgG serum with Student t-test $p < 0.05$) were considered as interactions. Similarly, proteins showing higher levels in centSIRT6 versus wild type SIRT6 were based upon $p<0.05$.

1.18. SIRT6-NAD+ binding by Tryptophan Fluorescence

[0208] 1 μM of SIRT6 protein in 50 μl of buffer (50 mM Tris-HCl pH=7.5 and 150 mM NaCl) was combined with a range of NAD+ (0-2 mM). Samples were then placed in a 384 Corning® Flat Black plate and analyzed in a Tecan Spark® 20M plate reader. Fluorescence was measured across 300-400 nm range in 2 nm steps using a 280 nm excitation. Background quenching by NAD+ of SIRT6 denatured by 7 M urea was subtracted from the spectra as described previously in Pan *et al.* (J Phys Chem B, 2011; Vol. 115:3245-3253).

1.19. Deacetylase Assay

[0209] 3 μg of SIRT6 protein was combined with 0.5 μg histones, 1 or 5 mM NAD^+, 30 mM Tris-HCl pH=8, 4 mM $MgCl_2$, 1mM DTT and ddH_2O up to 50 μl. All reagents were prepared on ice and moved to 37°C for the duration of the incubation. Reaction was quenched with direct application of 1 volume of 2× Laemmli buffer with BME. Samples were boiled for 15 min before being run in Western analysis using anti-H3K9ac and anti-H3K18ac antibodies.

1.20. PARP1 Ribosylase Assay

[0210] 5 μg of SIRT6 protein was combined with 100 fm PARP1, 20 mM Tris-HCl pH=8, 10 μM $ZnCl_2$, 10 μM $MgCl_2$, 10% glycerol, 300 μM NAD+, 1 mM DTT, 0.1 μg/ml salmon sperm DNA and ddH_2O up to 50 μl. All reagents were prepared on ice and moved to 30°C for 30 min. Reaction was quenched with direct application of 1 volume of 2× Laemmli buffer with BME. Samples were boiled for 15 min before being run in Western analysis using anti-PADPR antibody.

1.21. Self-Ribosylation Assay

[0211] Three μg of SIRT6 was combined with 50 mM Tris-HCl pH=7.5, 1 mM DTT, 10 μM $ZnCl_2$, 150 mM NaCl, 25uM NAD+, 1 μl [^{32}P]- NAD+ (800 Ci/mmol, 5m Ci/ml; Perkin Elmer® BLU023x250UC) and ddH_2O up to 20 μl. All reagents were combined on ice in a master mix except for SIRT6. Master mix was aliquoted into reaction tubes and SIRT6 was added and incubated at 37°C for 3 h. Reaction were quenched with 1 volume of 2× Laemmli buffer with BME. Samples were boiled and then run on SDS-PAGE gel and then transferred to PDPF membrane. Self-ribosylation was assayed using phosphoimager.

1.22. Thermostability

[0212] 4 μM SIRT6 protein was combined with 1× SYPRO™ Orange dye in storage buffer (50 mM Tris-HCl pH=7.5, 150 mM NaCl, 1mM DTT, 5% glycerol) to 50 μl. Samples were placed in qRT-PCR plate and run on a BioRad® CFX Connect Real Time machine using a Melt Curve protocol (30°C-75°C, 0.5°C steps in 10 s intervals).

1.23. FRET

[0213] Mutagenesis to generate the centenarian mutant (N308K/A313S) was performed using NEB Q5 site-directed mutagenesis kit, following protocols for primer design.

[0214] HEK293-6E cells were transfected using 293fectin® reagent according to the manufacturer's instructions (Thermo Fisher Scientific®). The SIRT6 biosensors were expressed using mammalian expression vector pTT5 (National Research Council, Canada). After two days in culture, approximately 30 million were produced, generating transiently transfected cell lines expressing the biosensor at high levels. The cell line was maintained using F17 medium (Sigma Aldrich®) + (200 nM/mL) GlutaMAX®.

[0215] On each day of FRET assays, approximately 30 million cells from each transfection were harvested, washed three times in PBS with no Mg or Ca (Thermo Fischer Scientific®; Waltham, MA) by centrifugation at 300 × g, filtered using 70-µm cell strainers (Corning®; Corning, NY), and diluted to 1-2 million cells/mL using a Countess™ Automated cell counter (Invitrogen®; Carlsbad, CA). On each day of experiments, cell viability was assessed using the trypan blue assay. After resuspension and dilution in PBS, the cells were continuously and gently stirred with a magnetic bar at room temperature to keep the cells in suspension and prevent clumping.

[0216] An in-depth description of the fluorescence instrumentation has been published previously in Schaaf *et al.* (SLAS Discov, 2017; Vol. 22:262-273). For FLT measurements, the observed fluorescence waveform was convolved with the instrument response function to determine F(t) (Equation 1), the average energy transfer efficiency E was calculated from the average FLTs of donor τ_D and donor-acceptor, τ_{DA}, using Equation 2, and the donor-acceptor distance R was calculated using Equation 3.

$$F(t) = \sum_{i=1}^{2} A_i \, exp\left(-t/\tau_i\right) \qquad \text{(Equation 1)}$$

$$FRET\ E = 1 - \frac{\tau_{DA}}{\tau_D} \qquad \text{(Equation 2)}$$

$$R = R_0 [(1/E) -1]^{1/6} \qquad \text{(Equation 3)}$$

1.24. Quantification and statistical analysis

[0217] Unless otherwise noted, the Student's t-test was used to determine statistical significance between groups. All tests were two-tailed and p-values were considered significant below a 0.05 threshold. Cell culture experiments utilized at least two separately derived cell lines for each genotype and were performed in triplicate unless noted otherwise. *In vitro* assays were done in triplicate using two or more separate protein isolation preps.

Results

[0218] While there is evidence that genome maintenance (GM) is a major longevity assurance system, with genetic defects in GM genes leading to reduced life span and premature ageing in humans and mice and a correlation of more efficient DNA repair with longer maximum lifespan across animal species, the role of GM in human extreme longevity has never been systematically studied.

[0219] To investigate whether GM plays a role in human extreme longevity, a panel of 301 genome maintenance genes (**Table 1**) in pathways including nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR), non-homologous end-joining (NHEJ), telomere maintenance, and genes involved in the regulation of these pathways, was assembled.

[0220] Two rounds of targeted sequencing were then performed to identify rare variants in these genes in DNA from peripheral blood of Ashkenazi Jewish (AJ) centenarians and AJ individuals between 60 and 70, unrelated to the probands and without centenarian relatives (**Figure 1**). In the first round, 51 AJ centenarians and 51 AJ controls were sequenced and identified 122 genes that showed association with longevity (**Table 2**). Gene ontology analysis showed that these genes were enriched for DNA double strand breaks (DSB) repair functions. DSB repair genes were next sequenced in a larger population of 496 AJ centenarians and 572 controls (**Table 4**).

[0221] SIRT6, a gene functionally related not only to DSB repair but also to longevity in model organisms was especially interesting. SIRT6 knockout mice show premature ageing and genomic instability whereas mice overexpressing SIRT6 display an extended lifespan (see Mostoslavsky *et al.*; Cell, 2006; Vol. 124:315-329). Higher SIRT6 activity is associated with more efficient DSB repair and longer maximum lifespan across mammalian species. At the molecular level, SIRT6 is involved in DNA repair, telomere maintenance, silencing of the repetitive elements including LINE1 retrotransposons, regulation of glucose homeostasis, inflammation and pluripotency, and tumor suppression.

[0222] The SIRT6 allele found enriched in centenarians contained two missense mutations within the highly flexible C-terminus, converting a polar asparagine 308 to a charged lysine (N308K) and a hydrophobic alanine 313 to a polar serine (A313S). Genotyping in centenarian carriers confirmed that these two SIRT6 variants occurred as linked double variants, which we named centSIRT6. This allele was very rare (minor allele frequency of 0.001%) in the whole exome sequence data of 50,726 adult participants of predominantly European ancestry in the Geisinger Health System. It was next tested whether centenarian SIRT6 allele alters the biological activities of SIRT6 protein.

[0223] SILAC analysis of HEK293 cells expressing different SIRT6 alleles showed no difference in the protein turnover rates between the WT and centSIRT6 (**Figure 2**). To assess the biochemical properties of the different SIRT6 alleles, recombinant versions of each allele were purified. Consistent with similar half-lives of wild type and centSIRT6 *in vivo*, it was observed no notable differences in the thermal stability of the SIRT6 alleles *in vitro* (**Figure 3**), suggesting that centSIRT6 does not grossly alter the folding or the ambient stability. As centSIRT6 mutations change the polarity and charge of the residues, SIRT6 FRET biosensors fused to the middle and the C-terminus of SIRT6 were used to assess for more subtle changes to protein structure that centSIRT6 mutations might cause (**Figure 4A-4B**). The centSIRT6 decreased FRET by $\Delta E = 6.4 \pm 0.1$ % relative to the wild type SIRT6, corresponding to an increase in the donor-acceptor distance R by 3.0 ± 0.4 Å. This increase indicates centSIRT6 mutations shift the SIRT6 structure toward a more open conformation.

[0224] SIRT6 deacylase activity was tested on a myristolated peptide to determine Km for NAD+ and peptide for wild type and centSIRT6. While the centenarian allele displayed a slight reduction in activity (Vmax) compared to the wild type ($p = 0.02$) (**Figure 5A-5B**), Km was not significantly different between wild type and centSIRT6 for both substrates (**Table 6**).

[0225] **Table 6**: Kinetic parameters Km and Vmax

	Km_{NAD} (μM)	Km_{PEP} (μM)	Vmax (RFU/S	$Vmax/Km_{PEP}$
WT	55.5 ± 5.4	61.1 ± 12.4	37.2 ± 4.1	0.61
Cent	48.4 ± 3.7	46.0 ± 11.3	24.3 ± 2.9	0.53

[0226] Quenching of SIRT6 intrinsic Trp fluorescence by NAD+ binding also showed that centSIRT6 has similar affinity for NAD+ to the wild type allele (**Figure 6**). Overall, these data indicate that centSIRT6 confers a slight reduction in deacylase catalytic efficiency (as defined by Vmax /Km) (**Table 6**).

[0227] The centSIRT6 displayed significantly lower deacetylase activity on recombinant nucleosomes with saturated H3K9ac and H3K18ac modifications (**Figure 7A-7B**). Additionally, centSIRT6 allele displayed significantly slower histone deacetylase kinetics (**Figure 8A-8B**). Similar to the synthetic histones, the centSIRT6 allele deacetylated both H3K9ac and H3K18ac residues on nucleosomes purified from HeLa cells, at a significantly reduced rate compared to the wild type SIRT6 (**Figure 9A-9B**). Taken together, the combined data from deacylase and histone deacetylase experiments indicate that the centSIRT6 variant has reduced deacylase/deacetylase activity.

[0228] To test the effect of the centSIRT6 allele on histone deacetylation activity *in vivo*, telomerase-immortalized human HCA2 fibroblast cell lines were generated in a SIRT6 KO background containing a cumate switch promoter driving expression of SIRT6 WT, N308K, A313S, and the centSIRT6 alleles (cumate SIRT6 fibroblasts). Each cell line was induced with cumate to drive equal levels of SIRT6 expression (**Figures 27A-27B**). Histone post-translational modifications from purified histones were quantified from these cells by mass spectrometry using peptide standards as described previously (Sidoli et al. Journal of visualized experiments: JoVE, 2016; doi:10.3791/54112). Cells expressing the centSIRT6, N308K, and A313S SIRT6 alleles showed similar global

histone post translational modification (PTM) levels at all sites known to be SIRT6 substrates including H3K9ac, H3K18ac, and H3K56ac (**Figure 10**). Similarly, global levels of H3K9ac and H3K56ac measured by Western blot did not show significant changes, even when challenged with paraquat-induced oxidative damage (**Figure 11**). These results suggest that reduced deacylation activity of centSIRT6 is compensated *in vivo* by additional protein factors or PTMs that help maintain SIRT6 deacetylation activity at close to the wild type levels.

[0229] In addition to its deacetylase activity, SIRT6 has mono-ADP ribosylation (mADPr) activity, able to mono-ADP ribosylate itself and other proteins. mADPr activity is critical to SIRT6 role in DNA damage response and LINE1 repression. To assess the mADPr activity, two known SIRT6 substrates were used: PARP1 and SIRT6, itself. The centSIRT6 demonstrated a significantly higher auto-ribosylation rate with radio labeled NAD+ than the wild type SIRT6 (**Figure 12**). The single SNP alleles and the combined centSIRT6 displayed a similar increase in self-mADPr compared to the wild type, suggesting a general increase that is not cumulative between the two SNPs (**Figures 12 and 28**). Following this, an antibody with specificity to mADPr residues was utilized to examine the self-ribosylation efficiency using a titration of NAD+ (Bonfiglio *et al.*; Cell, 2020; Vol. 183:1086-1102). As with the radiolabeled NAD+, centSIRT6 displayed almost 2-fold higher maximal mADPr activity and displayed a higher Km NAD of 142+29, compared to 106+45 for wild type SIRT6 (**Figure 13**). Thus, the overall efficiency as defined by mADPrmax/ Kapp NAD was 0.037 for centSIRT6 compared to 0.023 for wild type SIRT6, which also equates to a nearly two-fold difference. In trans, mADPr activity was assessed by incubation with human PARP1, which was previously shown to be ribosylated by SIRT6. Similar to self-ribosylation, the centSIRT6 displayed higher PARP1 ribosylation activity (**Figure 14**). Curiously, while both single mutants had elevated activity, the A313S allele was more similar to the centSIRT6 than N308K (**Figure 14**). These data show that centSIRT6 displays enhanced mADPr activity.

[0230] The loss of silencing of these elements contributes to age-related sterile inflammation and drives progeroid phenotypes in SIRT6 KO mice. To assess the capacity of the centSIRT6 allele to silence LINE1 transposons, the cumate-inducible fibroblasts

expressing alleles of SIRT6 were used. qRT-PCR analysis of both 5'- and 3'-biased regions of an evolutionarily active family of LINE1s both showed that the centSIRT6 allele enhances LINE1 suppression compared to the wild type SIRT6, as did the N308K allele (**Figure 15**). The A313S allele showed a slight trend towards stronger suppression, but did not show a significant improvement over the wild type SIRT6 when assessed for the 3' bias. These results show that centSIRT6 is more efficient at silencing LINE1 elements, which are functionally implicated in longevity.

[0231] To quantify the differences between the SIRT6 alleles at promoting DSB repair, *in vivo* GFP-based assays that measure nonhomologous end joining (NHEJ) and homologous recombination (HR) repair of a chromosomal DSB induced by I-SceI enzyme were employed. Different alleles of SIRT6 were transiently expressed in the NHEJ and HR reporter telomerase-immortalized human foreskin fibroblast cell lines. It was found that equivalent amounts of the centSIRT6 stimulated NHEJ and HR 2.5-fold and 2-fold greater respectively, than the wild type SIRT6 (**Figure 16A-16B**). Similarly, it was observed in the cumate SIRT6 fibroblasts expressing centSIRT6 that basal levels of γH2AX foci were reduced by 50% relative to those observed in either single alleles or the wild type SIRT6 (**Figure 17**). Taken together, these data indicate that the centSIRT6 allele elicits enhanced DNA repair activity.

[0232] It was next compared whether different SIRT6 alleles have different effects upon stress. Using the cumate SIRT6 fibroblasts, the cells were exposed to γ-radiation and the resolution of DSBs was assessed over time via γH2AX immunostaining. It was observed that, while both wild type and centSIRT6-expressing cell lines had similar levels of γH2AX foci immediately after exposure, the centSIRT6-expressing cells showed an improved recovery rate (**Figure 18**). Furthermore, it was found that the centSIRT6-expressing cells were more resistant to oxidative stress-induced apoptosis (**Figure 19A-19B**). In contrast, in the absence of cumate induction, all cell lines showed no significant differences in survival after oxidative stress (**Figure 19A**). Taken together, these results indicate that the centSIRT6 improves DSB repair and resistance to DNA damage.

[0233] To further confirm that centSIRT6 enhances genome maintenance, a knock-in human embryonic stem cells carrying centSIRT6 allele was generated. The two

centenarian mutations were introduced using CRISPR CAS9, and confirmed by sequencing. Two independent knock-in clones were generated and differentiated into MSCs. The MSCs were then transfected with linearized NHEJ and HR GFP reporters. Cells harboring centSIRT6 allele showed higher efficiency of repair (**Figures 29A-29B**). These cells were also showed increased survival and lower number of 53BP1 foci upon treatment with methyl methanesulfonate (MMS), a potent DNA-damaging agent (**Figures 29C-29D**). Interestingly, the centSIRT6 knock-in cells showed higher protein and mRNA levels of SIRT6 (**Figures 29E-29F**), suggesting that centenarian mutations increase mRNA stability, in addition to modulating SIRT6 enzymatic activities. As it was impossible to compare equal levels of SIRT6 in CRISPR knock-in cells, the majority of *in vivo* assays relied on cumate cells.

[0234] In order to assess the tumor cell killing capacity of the centSIRT6 allele, SIRT6 alleles were transfected into two common tumor cell lines, HT1080 fibrosarcoma cells and HeLa cells. The centSIRT6 showed ~2-fold fewer adherent surviving cells in both cancer cell lines (**Figure 20A**). The centSIRT6 allele triggered at least 2-fold higher levels of apoptosis than the wild type SIRT6 allele (**Figure 20B-20C**). These data indicate that, centSIRT6 confers a more robust anti-tumor activity than its wild type equivalent.

[0235] Since the centSIRT6 mutations are located in the flexible C-terminus of SIRT6 and may influence protein-protein interactions, the interactomes of the centSIRT6 and the wild type alleles were compared. Antibodies against SIRT6 were used to immuno-precipitate SIRT6-interacting proteins from cumate SIRT6 fibroblasts and to analyze them by mass spectrometry with tandem-mass tags (TMT) to permit accurate quantitation. Several interacting proteins were enriched by the centSIRT6 allele (**Table 7**).

[0236] **Table 7**: Proteins showing stronger interaction with centSIRT6 compared to the wild type SIRT6.

Name	Description	Peptides	p value
RBP56	TATA-binding protein-associated factor 2N	5	0.0002
LMNA	Prelamin-A/C	38	0.0025
RL35	60S ribosomal protein L35	4	0.0056
CEBPB	CCAAT/enhancer-binding protein beta	1	0.0119

VIME	Vimentin	40	0.0188
NUMA1	Nuclear mitotic apparatus protein 1	2	0.0227
NOL6	Nucleolar protein 6	2	0.0250
FRG1	Protein FRG1	2	0.0336
A0A0B4 J2E5	Uncharacterized protein	3	0.0354
PHRF1	PHD/RING finger domain-containing protein 1	3	0.0445
H15	Histone H1.5	11	0.0516
H31	Histone H3.1	3	0.0534
NOL10	Nucleolar protein 10	1	0.0581

[0237] Proteins were prepared from cumate-induced SIRT6 cell lines and immuno-precipitated with SIRT6 antibody; rabbit pre-immune serum was used as a control. Prior to analysis by mass spectrometry, samples were labeled with tandem-mass tags.

[0238] Most notable proteins showing stronger interaction with centSIRT6 than the wild type SIRT6 were LMNA and vimentin (VIME), which showed 38 and 40 peptides respectively. Both of these proteins are known to interact with SIRT6, and LMNA was shown to stimulate SIRT6 activity. No loss or gain of interactions was apparent in the centSIRT6 set, indicating that the effect of the novel allele could be in enhancing existing SIRT6 functions.

[0239] LMNA is a nuclear scaffold protein playing a central role in nuclear organization and is also implicated in ageing. Aberrant processing of LMNA results in human premature ageing syndrome, Hutchison Gilford Progeria, while LMNA SNPs have been identified in human centenarians. Fibroblasts isolated from centenarians were also shown to have increased levels of pre-LMNA, suggesting that modulated functions of LMNA are associated with both premature ageing and exceptional longevity. To confirm the mass spec data on SIRT6 and LMNA interactions, co-IP were performed with SIRT6 antibodies followed by Western blot with LMNA antibodies using cumate SIRT6 fibroblasts. It was found that centSIRT6, indeed, associates more strongly with LMNA than the wild type SIRT6 allele (**Figure 21** and **Figure 22**) and the effect was reciprocal as assessed by co-IP with LMNA antibodies followed by Western blot with SIRT6 antibodies (**Figure 21** and **Figure 23**). Taken together these results demonstrate that centSIRT6 interacts more strongly with LMNA than its wild type counterpart.

[0240] Given the enhanced mADPr activity of the centSIRT6 allele, it was next assessed the ribosylation status of LMNA using the mADPr antibodies. SIRT6 IPs performed on the cumate SIRT6 fibroblasts, using the mADPr specific antibody, showed that centSIRT6 was more ribosylated *in vivo* (**Figure 24**); as demonstrated *in vitro* with the purified SIRT6 proteins (**Figure 13**). IP with mADPr specific antibody and subsequent Western blot with an anti-LMNA antibody revealed increased ribosylation of LMNA in the presence of the centSIRT6 allele (**Figure 25**). LMNA has been reported to be ADP ribosylated *in vivo* on several residues.

[0241] It was hypothesized that the centSIRT6 allele may influence the interaction of LMNA with other proteins. To test this, LMNA from the cumate fibroblasts expressing either the wild type SIRT6 or centSIRT6 alleles were immuno-precipitated and quantitative protein interaction analysis using TMT labeling followed by mass spectrometry were performed. While many proteins showed similar interaction with LMNA, regardless of the SIRT6 allele present, a large group of proteins showed enhanced interaction with LMNA in the presence of centSIRT6 (**Figure 26**). LMNA-interaction partners enhanced by sentSIRT6 included ELAVL1, FUS, PCBP2, SAFB, SRSF1, SRSF3, TFIP11, THRAP3, BCLAF1, and U2AFBP all proteins that facilitate the coordination of the DNA damage response with RNA processing. Many of the proteins identified in SIRT6 and LMNA IPs are ADP-ribosylated (shown as hexagons in **Figure 26**), suggesting that ADP-ribosylation by SIRT6 or SIRT6-activated PARP1 may play a role in mediating these interactions. Taken together, these data show that centSIRT6 has enhanced capability to promote DNA repair and suppress LINE1 elements, which is likely mediated by its enhanced mADPr activity and stronger interaction with LMNA.

Discussion

[0242] The discovery of beneficial SIRT6 mutations associated with enhanced mADPr activity in a centenarian cohort suggests that increased SIRT6 activity benefits human longevity. While increased SIRT6 activity correlates with longer lifespan in model organisms, it has not been clear which of SIRT6's enzymatic activities are central to longevity. Interestingly, loss of SIRT6 deacetylation activity in humans or SIRT6 knockout in monkeys lead to severe developmental phenotypes rather than premature

ageing, suggesting that, at least in primates, deacetylation activity of SIRT6 is required for development but not necessarily for adult longevity. The change in the balance of deacetylation and mADPr enzymatic activities leads to enhanced function of SIRT6 in DNA repair, LINE1 suppression and cancer cell killing, which require mADPr activity. The centSIRT6 variant also shows enhanced binding to LMNA which may further promote its function in DNA repair and chromatin organization via direct stimulation of SIRT6 enzymatic activity, and by coordinating interactions of LMNA with other components of the LMNA complex.

EXAMPLE 2

Materials and Methods

Cell culture

[0243] 3T3-L1, LX2, HepG2, Hep3B (all purchased from American Type Culture Collection - ATCC, Manassas) and WSF (purchased from The Coriell Institute for Medical Research, NJ) cells were grown in basal media cosnsisting of high glucose (4.5g/l) DMEM (LM-D1108/500, Biosera) supplemented with 10% bovine fetal calf serum (F9665, Sigma-Aldrich), 15 mM hepes buffer (L0180, Biowest), GlutaMAX™ Supplement (100x, 35050-038, Gibco), 100 U/ml penicillin and 100 mg/ml streptomycin solution (L0022, Biowest), 100nM sodium pyruvate (11360, Gibco) and non-essential amino acids (NEAA 11140, 100x, Gibco) in 5% CO2/humidified atmosphere at 37°C. The medium was routinely changed every 2 days and the cells were sub-cultured using TrypLE Express (1x, Gibco) when reaching 90% confluence.

[0244] IHH cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in Williams E medium (12551032, Gibco) containing 2g/l glucose and supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin solution, 20 mU/ml insulin and 50 nM dexamethasone in 5% CO2/humidified atmosphere at 37°C. The cell culture medium was changed every 2 days and the cells were sub-cultured using TrypLE Express when reaching 90% confluence.

[0245] For the purpose of microscopy analyses, all appropriate cell lines were seeded on

glass coverslips coated with 0.2% gelatin with no further modification of standard cultivation protocols. For the evaluation of the effect of SIRT6 variants and their overexpression on tissue fibrosis, appropriate cell lines were treated or not with recombinant TGF-β1 protein (100-21C, Peprotech, US), as a potent inductor of fibrotic process, at concentration of 10 and 20 ng/ml.

Lentivirus infection and establishing SIRT6 cell lines

[0246] The cells, after reaching confluence, were split and seeded into 24-well plate with growth surface area of $2cm^2$, where 50×10^4 cells per well were seeded. Immediately after seeding, cells were infected with Lentivirus containing SIRT6 constructs: LV2-EMPTY-IresKat2S, LV2-SIRT6(wt)-IresKat2S, LV2-SIRT6(N308K)-IresKat2S and LV2-SIRT6(centSIRT6)-IresKat2S, in basal DMEM media supplemented with 4ug/mL polybrene transfection reagent (TR-1003, Sigma-Aldrich) with MOI of 1-2. Cells were cultivated with Lentivaral constructs for the period of 24 hours, then the fresh medium was added and cells were cultivated for another 48 hours. Then the cells were treated with selection media comprising of basal DMEM media with 500µg/mL of hygromycin (H3274, Merck) for the period of 5 days (every other day the media was changed for the fresh one). After selection, remaining cells were expanded and checked for the presence of fluorescent Kat2S signal by fluorescence microscope ex/em 588/635. SIRT6 overexpression was confirmed by western-blotting.

3T3-L1 differentiation to adipocytes

[0247] Differentiation of 3T3-L1 adipocytes was induced 2 days post- seeding at 100% confluence by incubating cells for the period of 72 hours in differentiation media consisting of standard basal high-glucose DMEM media additionally supplemented with 500 µM IBMX (I5879, Sigma-Aldrich), 1µM rosiglitazone (I5879, Sigma-Aldrich), 250nM dexamethasone (D4902, Sigma-Aldrich), 10nM 3,3′,5′-Triiodo-L-thyronine (T6397, Sigma-Aldrich), 17µM D-Pantothenic acid (21210, Sigma-Aldrich), 10µg/ml human transferrin (T8158, Sigma-Aldrich) and 1µg/ml insulin (I9278, Sigma-Aldrich). Then the cells are kept in the maintenance media consisting of basal high-glucose DMEM media supplemented only with 25 nM dexamethasone and 0.1µg/ml insulin for the period

of at least 4 days. Adipogenic differentiation and/or accumulation of intracellular lipids was evaluated using fluorescence labeling by BODIPY dye (1 µg/ml) for 30 minutes (ex/em 493/503) and DAPI counterstain 1 ug/mL for 30 min. Fluorescence was then captured by Agilent BioTek FLx800 Microplate Fluorescence Reader equipped with appropriate excitation/emission filters.

Cell viability assay

[0248] Cells were seeded into 96-well plates at 2000 viable cells per well and after 24, 48 and 72 hours, the culture media was removed and replace with medium containing Hoechst dyes at 1 ug/mL for nuclear staining. After 20 minutes of incubation at 37°C, the cells were twice washed with PBS and the fluorescence signal was measured with Agilent BioTek FLx800 Microplate Fluorescence Reader at ex/em 355/488 nm. The ratio between the signal at different time points and T0 was used as relative percentage of viable cells.

Gene expression measurement

[0249] Genomic RNA was isolated by column separation techniques using an RNeasy mini Kit (74106, Qiagen, Germany), according to manufacturer's instructions. At least 4 biological replicates were prepared for each treatment group. After quantification of isolated total RNA on NanoDrop 1000 spectrophotometer (ThermoFisher Scientific), 1µg of total isolated RNA was used to prepare cDNA using a High-Capacity cDNA Reverse Transcription Kit (4368814, ThermoFisher Scientific). Real Time-PCR was performed in at least two technical replicates using a StepOnePlus™ Real-Time PCR System (Applied Biosystems) and SYBR™ Select Master Mix (4472908, ThermoFisher Scientific). The PCR reaction was held in 10ul volume and 250 ng cDNA per well was used as the input quantity. The primer sequences used in this study are listed in **Table 8**.

[0250] **Table 8**: Primer sequences used in the study

Gene		**Sequence (5′-3′)**	**SEQ ID NO:**
αSMA	F	AAAAGACAGCTACGTGGGTGA	13
	R	GCCATGTTCTATCGGGTACTTC	14
COL1A1	F	GTGCGATGACGTGATCTGTGA	15
	R	CGGTGGTTTCTTGGTCGGT	16
TIMP1	F	ACCACCTTATACCAGCGTTATGA	17

	R	GGTGTAGACGAACCGGATGTC	18
VIMENTIN	F	AGTCCACTGAGTACCGGAGAC	19
	R	CATTTCACGCATCTGGCGTTC	20
MMP2	F	TACAGGATCATTGGCTACACACC	21
	R	GGTCACATCGCTCCAGACT	22
GAPDH	F	GGTGCGTGCCCAGTTGA	23
	R	TACTTTCTCCCCGCTTTTT	24
Actinβ	F	CATGTACGTTGCTATCCAGGC	25
	R	CTCCTTAATGTCACGCACGAT	26

Telomere Length

[0251] Quantitative RT-PCR was used to determine changes in average telomere length of LV transfected WSF cells and untreated WSF based on ScienCell's Relative Human Telomere Length Quantification qPCR Assay Kit (#8909). The kit directly compares the average telomere length of the samples. The telomere primer set recognizes and amplifies telomere sequences. The single copy reference (SCR) primer set recognizes and amplifies a 100 bp-long region on human chromosome 17, and serves as reference for data normalization. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) no non-specific amplification. Each primer set has been validated by qPCR with melt curve analysis and gel electrophoresis for amplification specificity and by template serial dilution for amplification efficiency. Genomic DNA (gDNA) was isolated from using the Qiagen DNA Isolation Kit (Qiagen, Hilden, Germany). Each PCR reaction contained genomic DNA sample (0.01 μg/μL), telomere primer, 2x qPCR master mix, and nuclease-free water. Primer-probe real-time PCR was performed using StepOnePlus™ Real-Time PCR System (Applied Biosystems). All reactions were performed in duplicate, and template controls were included in each run. Amplification was performed under the following conditions: denaturation for 10 min at 95°C followed by 32 cycles of denaturation for 20 sec at 95°C, annealing for 20 sec at 52°C, and extension for 45 sec at 72°C. The average telomere length was calculated by following the manufacturer's instructions.

Quantification of telomerase activity

[0252] Relative telomerase activity of LV WSF transfected cell lines and normal WSF cells was assessed by qPCR in StepOnePlus™ Real-Time PCR System (Applied

Biosystems) using SYBR® Green assay kits (ScienCell's Telomerase Activity Quantification qPCR Assay Kit [TAQ], Carlsbad, CA, USA) following the recommendations of the manufacturers. Three million cells were harvested for each sample. For each experiment, two controls were used: a negative telomerase and positive cell lysates (Cat #8928e). PCR was performed in a final volume of 20 µL using 1 µL of post-telomerase reaction sample, 2 µL of TPS, 10 µL of 2x qPCR FastStart Essential Green Master Mix (Roche Diagnostics International) and 7 µL of nuclease-free water. The PCR conditions were as follows: 95°C for 10 min followed by 36 cycles of: 95°C for 20 sec, 52°C for 20 sec and 72°C for 45 sec. All reactions were run in three replicates. Data analysis was conducted according to manufacturer's instructions.

Immunoblotting analyses

[0253] Cells were harvested from cultivation plates using TrypLE Express, washed with 1x PBS and centrifuged at 300g. After discarding supernatant, obtained pellet was resuspended 1x Rippa lysis buffer (20-188, Millipore, USA) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (100X, Thermo Fisher) and lysed on ice (4°C) for 30 minutes with vigorous vertexing every 10min. After spinning at 10 000g for 10min, supernatant was moved to new vial and concentration of protein was measured by Pierce™ BCA Protein Assay Kit (23225, Thermo Fisher) according to manufacturer's instruction. Equal amount of protein sample (at least 20µg) was mixed with 1x Laemmli Sample buffer (1610747, 4x, Bio-Rad) and after boiling at 95°C for 5 min and cooling on ice, equal volume of proteins (40µl) was loaded on 10% Mini-PROTEAN® TGX Stain-Free™ Protein Gels (4568034, Bio-Rad) and separated by electrophoresis running at 120 volts for 45 minutes. Protein transfer was performed on PVDF membranes using Trans-Blot Turbo RTA Mini 0.45 µm LF PVDF Transfer Kit (1704274, Bio-Rad) and Bio-Rad Trans-Blot Turbo Transfer System at 1.3A and 25V for 10 min. Membranes were then blocked with 5 % bovine serum albumin (BSA, P6154, BioWest) dissolved in TBST buffer (20 mM Tris–HCl, pH 7.6, 140 mM NaCl, 0.1 % Tween 20) for at least 30 minutes and incubated with the specific primary antibodies (see below) diluted in TBST blocking solution, at appropriate dilutions. Following three washes in TBST buffer, membranes were incubated with a secondary antibody conjugated with horseradish peroxidase diluted

in TBST blocking buffer. After three further washes with TBST, proteins were revealed by Clarity Western ECL Substrate (1705061, Bio-Rad) and the signal detected on Bio-Rad ChemiDoc XRS+ imaging systems. For quantitative measurement, the scanned membranes were analyzed using the Image Lab™ Software (Bio-Rad).

[0254] Antibodies used in this study: Cell Signaling Technology (MA, USA) - rabbit anti-Akt (1:1000), rabbit anti-Phospho-Akt (Ser473) (1:1000), rabbit anti Vimentin (1:1000), rabbit anti Histone H3 (D1H2, 1:1000), Abcam (UK) - mouse anti-β-actin HRP conjugated antibody (AC-15; 1:2000), mouse anti-GAPDH monoclonal HRP conjugated antibody (1:2000), rabbit anti Collagen I (1:1000), rabbit anti αSMA (1:1000), rabbit anti SIRT6 antibody (1:1000, EPR18463), ThermoFischer Scientific (CA, USA) - mouse IgG1 GAPDH monoclonal HRP conjugated antibody (1:2000), secondary goat Anti-rabbit IgG HRP-linked (1:2000);

3D Spheroid Culture

[0255] For the generation of the cell spheroids, cells were seeded into BIOFLOAT™ 96-well round bottom ultra-low attachment plates for cell culture at 10000 viable cells per well. Each IHH LV transfect cell line (CTL/EMPTY, WT, N308K and centSIRT6) was co-cultured with either normal LX2 or overexpressing centSirt6 LX2 cells with 20:1 ratio, to reproduce the physiological proportion in the liver parenchyma, where hepatocytes are major cell type with only ≈ 5% hepatic stellate cells. The spheroids were grown in basal high glucose DMEM media supplemented as described above. The plates were incubated for 3 days at 37°C in a humidified atmosphere at 5% CO2, after which spheroids were treated or not with free fatty acid solution (FFA, L9655, Sigma-Aldrich) and kept in cultivation for 48 hours. Spheroids were then collected, washed twice with PBS, fixed in 4% paraformaldehyde + Eosin solution for 20 minutes, incubated in 10% sucrose for 20 minutes and incorporated into tissue freezing media (14020108926, Leica Biosystems, USA) for cutting and subsequent immuno-histological analysis.

Histological Immuno-staining

[0256] Samples of embedded and snap frozen spheroids in tissue freezing media were cut to 7μm at -20°C with a cryotome (Leica Microsystems) and stored at -80°C for further

use. To evaluate the effect of SIRT6 variants and their overexpression on liver tissue fibrosis, spheroid histological sections were immunolabeled to detect Collagen 1A. Slides were washed once in 1xPBS to dissolve tissue freezing media and blocked in 1xPBS supplemented with 0.2% Tween-20 and 5% BSA. Primary antibody rabbit anti-Collagen I (1:500, ab34710, Abcam) was diluted in DAKO antibody diluent (S202230-2, Agilent technologies) and incubated overnight in humid chamber at lab temperature. After three consecutive washes with 1xPBS, mix of secondary antibody (1:500) donkey anti-rabbit IgG coupled with Alexa Fluor™ 647 was applied and incubated for at least 1h. After washing three times with 1xPBS, slides were counterstained with DAPI (1ug/ml) solution for 15 minutes and mounted in Mowiol hardening media. After hardening (overnigth at 4°C), images were captured using an Axio scan Z.1 (ZEISS) equipped with a Hamamatsu ORCA-Flash 4.0 camera and ImageJ software (NIH, USA) analysis program was used to evaluate all immunofluorescence images. Fibrosis, *i.e.*, collagene 1A abundance in spheroid samples, was evaluated as the % of the total spheroid area delineated by DAPI fluorescence at 100x magnification, when at least 5 spheroids per each condition/cell line were used in three consecutive and independent experiments.

Results

[0257] SIRT6 wild type or SIRT6 carrying one or two mutations associated with human exceptional longevity (SIRT6 N308K, centSIRT6) were overexpressed in a variety of cell lines, and assessed how this affected their specific cellular functions.

Werner Syndrome model

[0258] In human Werner Syndrome immortalized fibroblasts, SIRT6 WT/SIRT6 N308K/centSIRT6 did not affect cell viability, proliferation or telomere length (**Figure 30A-30B**). However, centSIRT6 decreased telomerase activity (**Figure 30C**).

[0259] Although it may seem counter-beneficial, Werner Syndrome immortalized fibroblasts are poorly characterized immortalized lines that might have carcinogenic properties. Therefore, without being bound by any theory, and in the event that this is the case, decreasing telomerase activity would be a beneficial effect.

Human hepatocellular carcinoma model

[0260] In human hepatocellular carcinoma (HCC) cells, SIRT6 WT/SIRT6 N308K/centSIRT6 overexpression was lethal, with a larger effect observed for SIRT6 N308K/centSIRT6 (**Figure 31A-31B**). Therefore, mutated SIRT6 may be useful for treating cancer.

Adipogenic differentiation

[0261] It has been reported that SIRT6 deficiency results in hypoglycemia and impairment of energy homeostasis, suggesting that SIRT6 could play an important role in regulating adipocyte differentiation. Aged adipocytes become hypertrophic, in particular under a high fat diet intake, becoming insulin resistant and producing a wide pattern of pro-inflammatory cytokines, contributing to the increase of circulating triglycerides and free fatty acids, which together affects other organs and results in the comorbidities typically associated with obesity. On the other hand, the increase of adipogenesis allows the formation of new adipocytes that are more sensitive to insulin, showing increased capacity of fatty storage and enhanced levels of adipokines, that contribute to metabolic homeostasis and lowering of inflammation levels.

[0262] Here, the results demonstrate that SIRT6 plays a role in the increase of adipogenic differentiation (**Figure 32**), consistent with the role of this protein in energy homeostasis. The SIRT6 mutants (SIRT6 N308K and centSIRT6) retain this property, which is of interest for the treatment of ageing and ageing-related diseases.

Hepatic stellate cells model

[0263] α-SMA expression is considered a reliable marker of hepatic stellate cell (HSC) activation and a key biomarker for liver fibrosis. In the liver, HSCs play a central role in physiological tissue repair as well as in the development of fibrosis, transdifferentiating from "quiescent" HSC to a myofibroblastic phenotype in response to Transforming Growth Factor beta (TGF-β), inflammatory processes and ROS.

[0264] SIRT6 WT/SIRT6 N308K/centSIRT6 overexpression did not affect cell viability or proliferation (**Figure 33A**). Upon LX2 activation with TGFbeta, there was a blockage

of the activation of some fibrogenic markers (vimentin, TIMP1) by lentiviral infection, irrespective of the genotype (**Figure 33B-33C**). Collagen mRNA expression was increased by TGFbeta, with no effect of SIRT6 WT/SIRT6 N308K/centSIRT6 overexpression (**Figure 33D**). However, centSIRT6 significantly decreased the mRNA expression of aSMA, a major fibrogenic marker (**Figure 33E**).

[0265] The lower gene expression levels of α-SMA in centSIRT6 LX2 compared to control suggests a potential anti-fibrotic effect of centSIRT6 in preventing liver fibrosis and/or promoting its resolution.

Hepatic spheroids model

[0266] Together with α-SMA, Collagen (COL1A1) represent one of the key fibrosis markers. Its production in liver is increased mainly by activated hepatic stellate cells.

[0267] Spheroids formed from IHH cells and with 5% the total cell mass of LX2 (human hepatic stellate cells) were generated. Spheroids were treated or not with free fatty acids (FFA), and assessed collagen production by immunofluorescence (**Figure 34A**).

[0268] In IHH/LX2 spheroids with IHH transfected with centSIRT6, a decrease of internal collagen content compared to control (Empty group) was observed, suggesting that at the basal levels, in absence of stressor factors, IHH centSIRT6 inhibit the collagen production (**Figure 34B**).

[0269] It is known that exposure to free fatty acids (FFAs) promotes oxidant stress and indirect activates LX2 increasing collagen biosynthesis. Surprisingly, in presence of FFAs, increased levels of internal collagen in IHH centSIRT6 spheroids compared to control were observed (**Figure 34C**). Increased levels of free fatty acids together with enhanced beta-oxidation induced by centSIRT6 overexpression may have provided more ATP necessary for the acquisition of the myofibroblastic phenotype of fibroblasts, overcoming the inhibitory effect on collagen production exerted by centSIRT6 overexpression observed on the basal level.

SEQUENCES USED HEREIN

SEQ ID NO: 1 – Wild type SIRT6 amino acid sequence

[0270] MSVNYAAGLSPYADKGKCGLPEIFDPPEELERKVWELARLVWQSSSVV
FHTGAGISTASGIPDFRGPHGVWTMEERGLAPKFDTTFESARPTQTHMALVQLE
RVGLLRFLVSQNVDGLHVRSGFPRDKLAELHGNMFVEECAKCKTQYVRDTVV
GTMGLKATGRLCTVAKARGLRACRGELRDTILDWEDSLPDRDLALADEASRN
ADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLRIHGYVDEV
MTRLMKHLGLEIPAWDGPRVLERALPPLPRPPTPKLEPKEESPTRINGSIPAGPK
QEPCAQHNGSEPASPKRERPTSPAPHRPPKRVKAKAVPS

SEQ ID NO: 2 – SIRT6 N308K variant amino acid sequence

[0271] MSVNYAAGLSPYADKGKCGLPEIFDPPEELERKVWELARLVWQSSSVV
FHTGAGISTASGIPDFRGPHGVWTMEERGLAPKFDTTFESARPTQTHMALVQLE
RVGLLRFLVSQNVDGLHVRSGFPRDKLAELHGNMFVEECAKCKTQYVRDTVV
GTMGLKATGRLCTVAKARGLRACRGELRDTILDWEDSLPDRDLALADEASRN
ADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLRIHGYVDEV
MTRLMKHLGLEIPAWDGPRVLERALPPLPRPPTPKLEPKEESPTRIKGSIPAGPK
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SEQ ID NO: 3 – SIRT6 A313S variant amino acid sequence

[0272] MSVNYAAGLSPYADKGKCGLPEIFDPPEELERKVWELARLVWQSSSVV
FHTGAGISTASGIPDFRGPHGVWTMEERGLAPKFDTTFESARPTQTHMALVQLE
RVGLLRFLVSQNVDGLHVRSGFPRDKLAELHGNMFVEECAKCKTQYVRDTVV
GTMGLKATGRLCTVAKARGLRACRGELRDTILDWEDSLPDRDLALADEASRN
ADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLRIHGYVDEV
MTRLMKHLGLEIPAWDGPRVLERALPPLPRPPTPKLEPKEESPTRINGSIPSGPKQ
EPCAQHNGSEPASPKRERPTSPAPHRPPKRVKAKAVPS

SEQ ID NO: 4 – SIRT6 N308K A313S variant (centSIRT6) amino acid sequence

[0273] MSVNYAAGLSPYADKGKCGLPEIFDPPEELERKVWELARLVWQSSSVV

FHTGAGISTASGIPDFRGPHGVWTMEERGLAPKFDTTFESARPTQTHMALVQLE
RVGLLRFLVSQNVDGLHVRSGFPRDKLAELHGNMFVEECAKCKTQYVRDTVV
GTMGLKATGRLCTVAKARGLRACRGELRDTILDWEDSLPDRDLALADEASRN
ADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLRIHGYVDEV
MTRLMKHLGLEIPAWDGPRVLERALPPLPRPPTPKLEPKEESPTRIKGSIPSGPKQ
EPCAQHNGSEPASPKRERPTSPAPHRPPKRVKAKAVPS

SEQ ID NO: 5 – Wild type SIRT6 nucleic acid sequence

[0274] atgtcggtgaattacgcggcggggctgtcgccgtacgcggacaagggcaagtgcggcctcccggagatcttcg
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SEQ ID NO: 6 – SIRT6 N308K variant nucleic acid sequence

[0275] atgtcggtgaattacgcggcggggctgtcgccgtacgcggacaagggcaagtgcggcctcccggagatcttcg
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SEQ ID NO: 7 – SIRT6 A313S variant nucleic acid sequence

[0276] atgtcggtgaattacgcggcggggctgtcgccgtacgcggacaagggcaagtgcggcctcccggagatcttcg
accccccggaggagctggagcggaaggtgtgggaactggcgaggctggtctggcagtcttccagtgtggtgttccacacgg
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acctgcagcccaccaagcacgaccgccatgctgacctccgcatccatggctacgttgacgaggtcatgacccggctcatgaa
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accccaagctggagcccaaggaggaatctcccacccggatcaacggctctatcccctccggccccaagcaggagccctg
cgcccagcacaacggctcagagcccgccagccccaaacgggagcggcccaccagccctgccccccacagacccccaa
aagggtgaaggccaaggcggtccccagctga

SEQ ID NO: 8 – SIRT6 N308K A313S variant (centSIRT6) nucleic acid sequence

[0277] atgtcggtgaattacgcggcggggctgtcgccgtacgcggacaagggcaagtgcggcctcccggagatcttcg
accccccggaggagctggagcggaaggtgtgggaactggcgaggctggtctggcagtcttccagtgtggtgttccacacgg
gtgccggcatcagcactgcctctggcatccccgacttcaggggtccccacggagtctggaccatggaggagcgaggtctgg
cccccaagttcgacaccacctttgagagcgcgcggcccacgcagacccacatggcgctggtgcagctggagcgcgtgggc
ctcctccgcttcctggtcagccagaacgtggacgggctccatgtgcgctcaggcttccccagggacaaactggcagagctcc
acgggaacatgtttgtggaagaatgtgccaagtgtaagacgcagtacgtccgagacacagtcgtgggcaccatgggcctgaa

ggccacgggccggctctgcaccgtggctaaggcaaggggctgcgagcctgcaggggagagctgagggacaccatccta
gactgggaggactccctgcccgaccgggacctggcactcgccgatgaggccagcaggaacgccgacctgtccatcacgct
gggtacatcgctgcagatccggcccagcgggaacctgccgctggctaccaagcgccggggaggccgcctggtcatcgtca
acctgcagcccaccaagcacgaccgccatgctgacctccgcatccatggctacgttgacgaggtcatgacccggctcatgaa
gcacctggggctggagatccccgcctgggacggcccccgtgtgctggagagggcgctgccacccctgccccgcccgccc
acccccaagctggagcccaaggaggaatctcccacccggatcaagggctctatcccctccggccccaagcaggagccctg
cgcccagcacaacggctcagagcccgccagccccaaacgggagcggcccaccagccctgccccccacagacccccccaa
aagggtgaaggccaaggcggtccccagctga

SEQ ID NO: 9 - LINE1 ORF1 Forward-primer

[0278] atggcgaaaggcaaacgtaag

SEQ ID NO: 10 - LINE1 ORF1 Reverse-primer

[0279] attttcggttgtgttggggtg

SEQ ID NO: 11 - LINE1 ORF2 Forward-primer

[0280] gcaggggttgcaatcctagtc

SEQ ID NO: 12 - LINE1 ORF2 Reverse-primer

[0281] ctgggtgctcctgtattgggt

SEQ ID NO: 13 - αSMA Forward-primer

[0282] aaaagacagctacgtgggtga

SEQ ID NO: 14 - αSMA Reverse-primer

[0283] gccatgttctatcgggtacttc

SEQ ID NO: 15 - COL1A1 Forward-primer

[0284] gtgcgatgacgtgatctgtga

SEQ ID NO: 16 - COL1A1 Reverse-primer

[0285] cggtggtttcttggtcggt

SEQ ID NO: 17 - TIMP1 Forward-primer

[0286] accaccttataccagcgttatga

SEQ ID NO: 18 - TIMP1 Reverse-primer

[0287] ggtgtagacgaaccggatgtc

SEQ ID NO: 19 - VIMENTIN Forward-primer

[0288] agtccactgagtaccggagac

SEQ ID NO: 20 - VIMENTIN Reverse-primer

[0289] catttcacgcatctggcgttc

SEQ ID NO: 21 - MMP2 Forward-primer

[0290] tacaggatcattggctacacacc

SEQ ID NO: 22 - MMP2 Reverse-primer

[0291] ggtcacatcgctccagact

SEQ ID NO: 23 - GAPDH Forward-primer

[0292] ggtgcgtgcccagttga

SEQ ID NO: 24 - GAPDH Reverse-primer

[0293] tactttctccccgcttttt

SEQ ID NO: 25 - Actinβ Forward-primer

[0294] catgtacgttgctatccaggc

SEQ ID NO: 26 - Actinβ Reverse-primer

[0295] ctccttaatgtcacgcacgat

CLAIMS

1. An isolated nucleic acid molecule encoding a variant of sirtuin 6 (SIRT6) having at least 75% identity with sequence **SEQ ID NO: 1**, the variant having at least one mutation selected in the group comprising or consisting of a substitution N308K and a substitution A313S with respect to sequence **SEQ ID NO: 1**.

2. The isolated nucleic acid molecule according to claim **1**, wherein the nucleic acid molecule is of sequence selected in the group comprising or consisting of **SEQ ID NO: 6, SEQ ID NO: 7** and **SEQ ID NO: 8**.

3. An isolated polypeptide encoded by a nucleic acid molecule according to claim **1** or **2**.

4. The isolated polypeptide according to claim **3**, wherein the polypeptide is of sequence selected in the group comprising or consisting of **SEQ ID NO: 2, SEQ ID NO: 3** and **SEQ ID NO: 4**.

5. A vector comprising the isolated nucleic acid molecule according to claim **1** or **2**.

6. The vector according to claim **5**, wherein the vector is a viral vector, in particular an adeno-associated viral vector (AAV), an exosome-associated AAV vector (exo-AAV), an adenoviral vector, a retroviral vector, or a herpes virus vector.

7. A suspension comprising a vector according to claim **5** or **6**.

8. A cell expressing the polypeptide according to claim **3** or **4**, the cell being preferably transfected with an isolated nucleic acid molecule according to claim **1** or **2**, or a vector according to claim **5** or **6**.

9. A pharmaceutical composition comprising (i) an isolated nucleic acid molecule according to claim **1** or **2**, or an isolated polypeptide according to claim **3** or **4**, or a vector according to claim **5** or **6**, and (ii) a pharmaceutically acceptable excipient.

10. Use of an isolated nucleic acid molecule according to claim **1** or **2**, or an isolated polypeptide according to claim **3** or **4**, or a vector according to claim **5** or **6**, in the

regulation of ageing, and/or senescence, and/or lifespan in an individual, preferably a mammalian individual, more preferably a human individual.

11. Use of an isolated nucleic acid molecule according to claim **1** or **2**, or an isolated polypeptide according to claim **3** or **4**, or a vector according to claim **5** or **6**, in the repair of double strand break in a cell, preferably a mammalian cell, more preferably a human cell.

12. An isolated nucleic acid molecule according to claim **1** or **2**, or an isolated polypeptide according to claim **3** or **4**, or a vector according to claim **5** or **6**, for use in the prevention and/or the treatment of an age-related disease.

13. The isolated nucleic acid molecule, or isolated polypeptide, or vector, for use according to claim **12**, wherein the individual is a mammalian individual, preferably a human individual.

14. The isolated nucleic acid molecule, or isolated polypeptide, or vector, for use according to claim **12** or **13**, wherein the age-related disease is selected in the group comprising or consisting of progeria, Werner syndrome, neurodegenerative disease, Alzheimer's disease, cancer, cardiovascular disease, obesity, type 2 diabetes, hypercholesterolemia, hypertension, ocular disorders, cataracts, glaucoma, osteoporosis, blood clotting disorders, arthritis, hearing loss and stroke.

15. A kit comprising (i) an isolated nucleic acid molecule according to claim **1** or **2**, or an isolated polypeptide according to claim **3** or **4**, or a vector according to claim **4** or **5**, and (ii) means to administer the isolated nucleic acid molecule, the isolated polypeptide, or the vector.

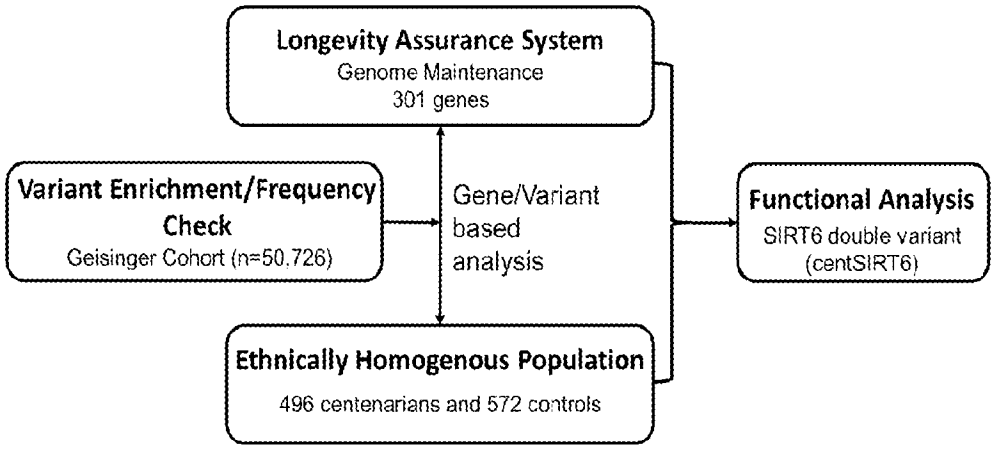
Longevity Assurance System
Genome Maintenance
301 genes
Variant Enrichment/Frequency Check
Geisinger Cohort (n=50.726)
Gene/Variant based analysis
Functional Analysis
SIRT6 double variant (centSIRT6)
Ethnically Homogenous Population
496 centenarians and 572 controls

FIG. 1

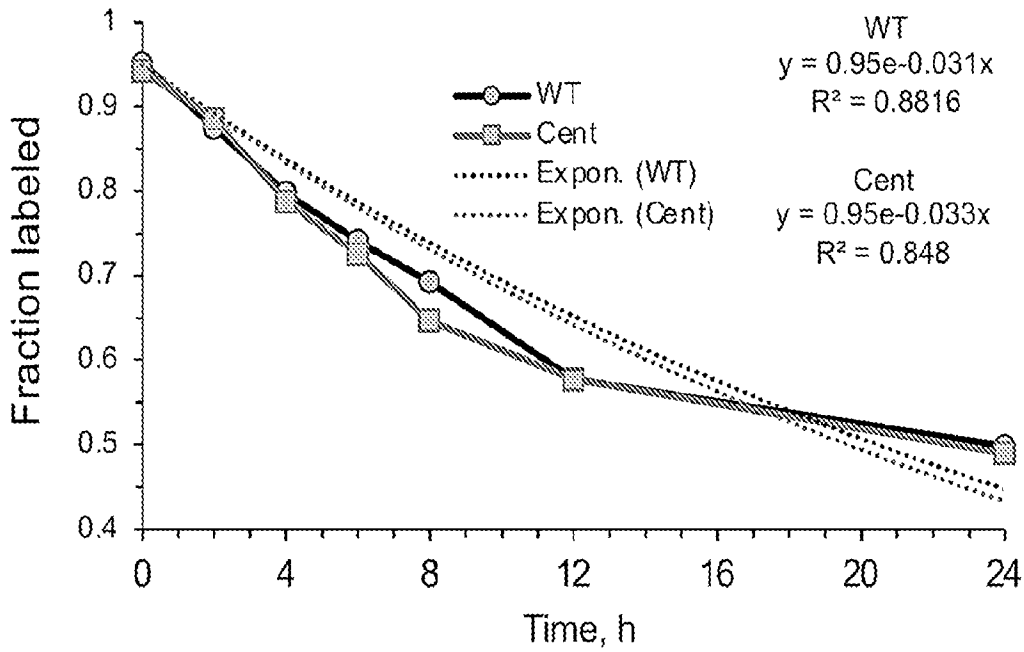
Fraction labeled
1
0.9
0.8
0.7
0.6
0.5
0.4
0
4
8
12
16
20
24
Time, h
WT
Cent
Expon. (WT)
Expon. (Cent)
WT
y = 0.95e-0.031x
R² = 0.8816
Cent
y = 0.95e-0.033x
R² = 0.848

FIG 2

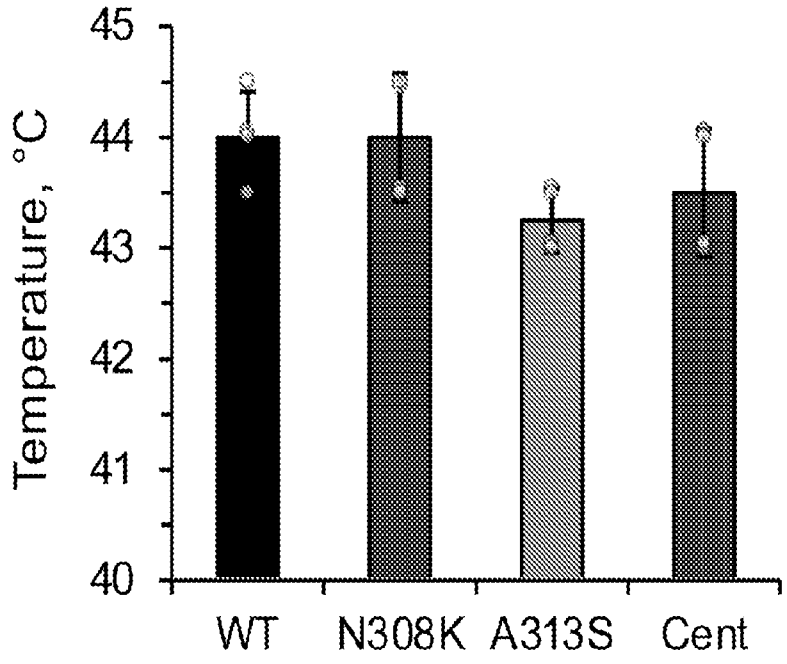
Temperature, °C
45
44
43
42
41
40
WT
N308K
A313S
Cent

FIG. 3

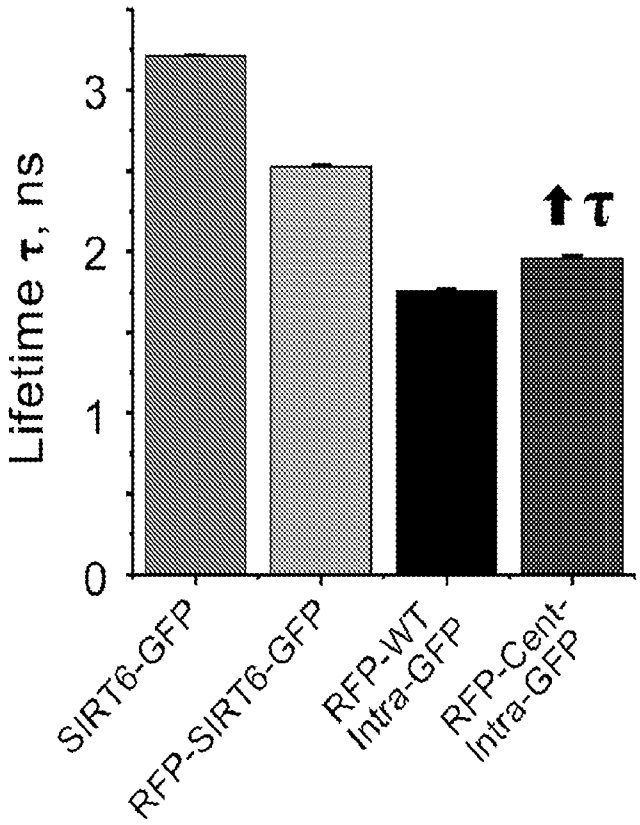
Lifetime τ, ns
3
2
1
0
τ
SIRT6-GFP
RFP-SIRT6-GFP
RFP-WT Intra-GFP
RFP-Cent-Intra-GFP

FIG. 4A

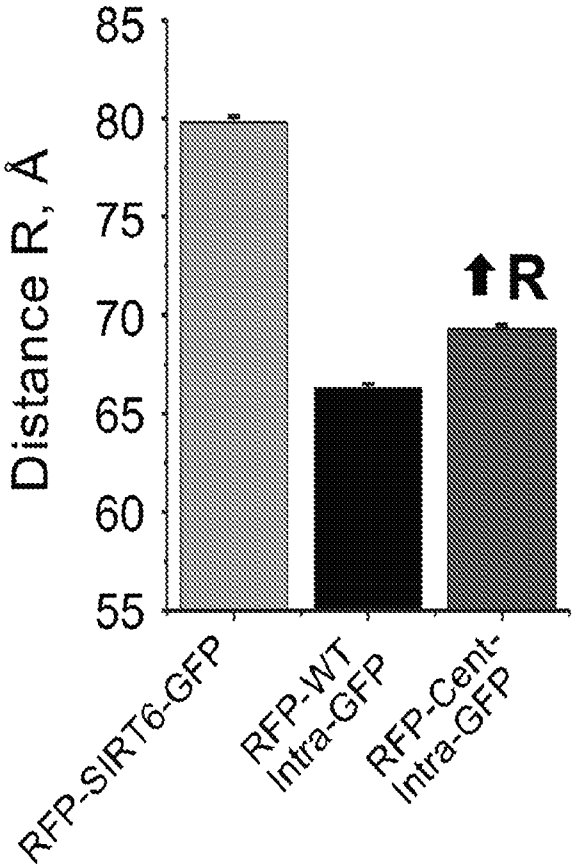
Distance R, Å
85
80
75
70
65
60
55
R
RFP-SIRT6-GFP
RFP-WT Intra-GFP
RFP-Cent-Intra-GFP

FIG. 4B

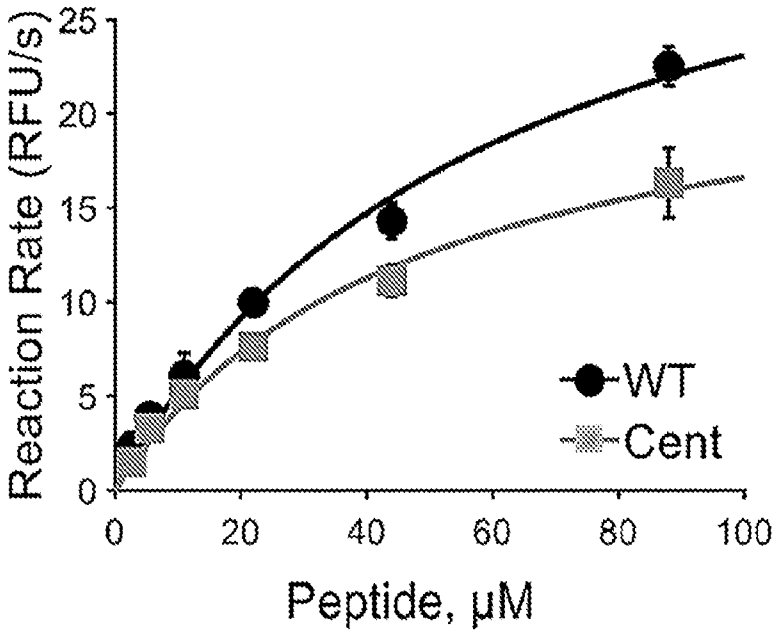
Reaction Rate (RFU/s)
25
20
15
10
5
0
0
20
40
60
80
100
Peptide, μM
WT
Cent

FIG. 5A

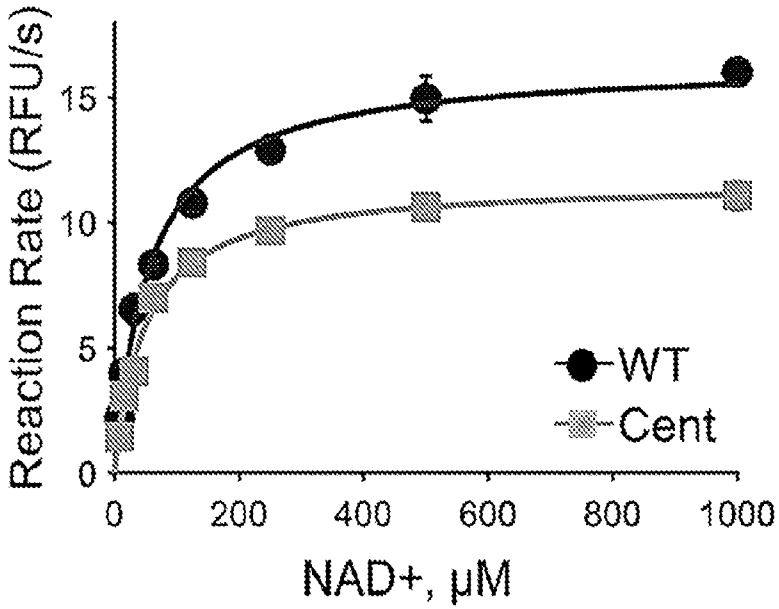
Reaction Rate (RFU/s)
0
5
10
15
0
200
400
600
800
1000
NAD+, μM
WT
Cent

FIG. 5B

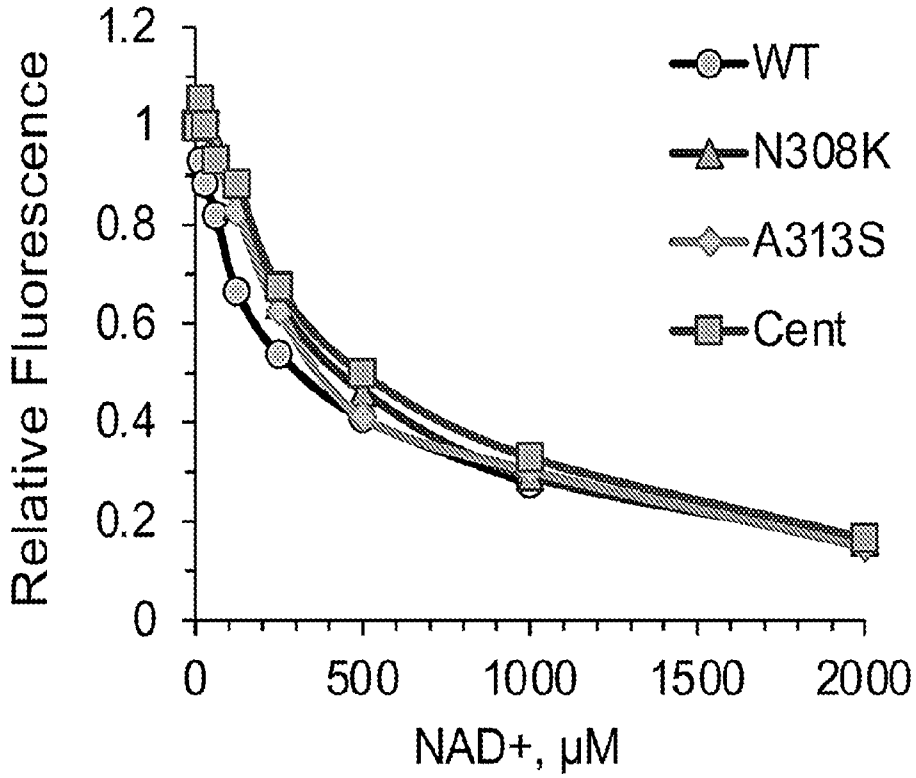
Relative Fluorescence
0
0.2
0.4
0.6
0.8
1
1.2
0
500
1000
1500
2000
NAD+, μM
WT
N308K
A313S
Cent

FIG. 6

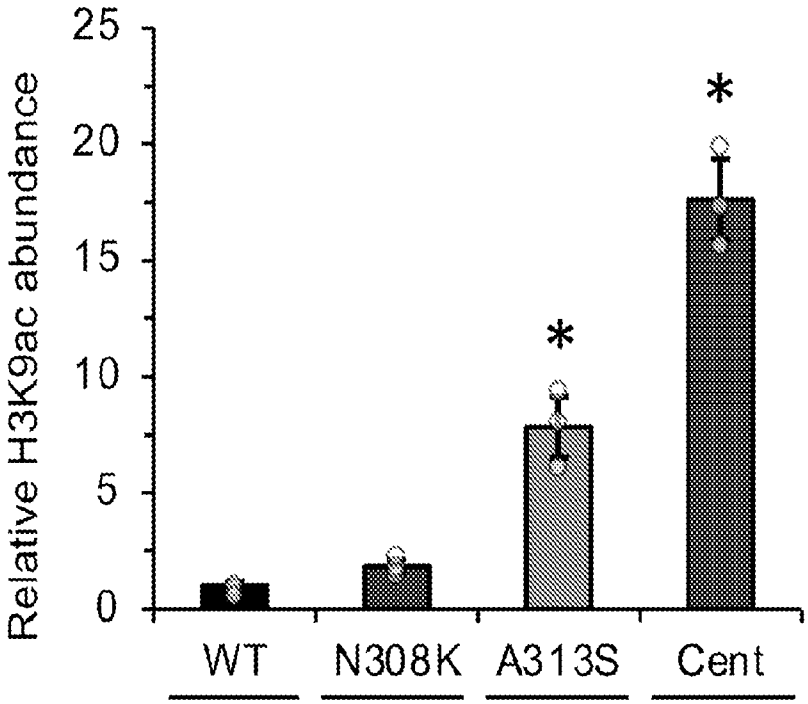
Relative H3K9ac abundance
25
20
15
10
5
0
*
*
*
WT
N308K
A313S
Cent

FIG. 7A

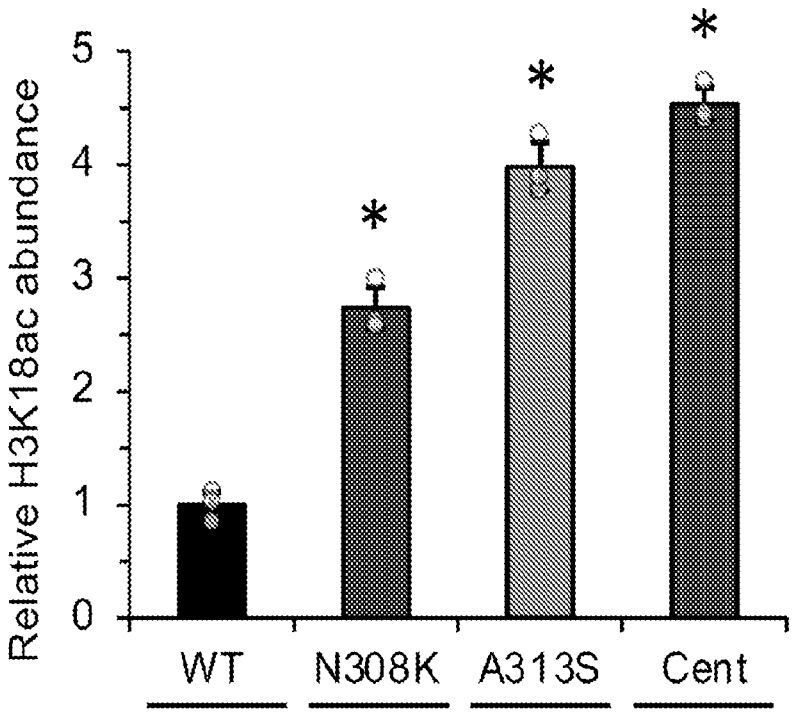
Relative H3K18ac abundance
5
4
3
2
1
0
*
*
*
WT
N308K
A313S
Cent

FIG. 7B

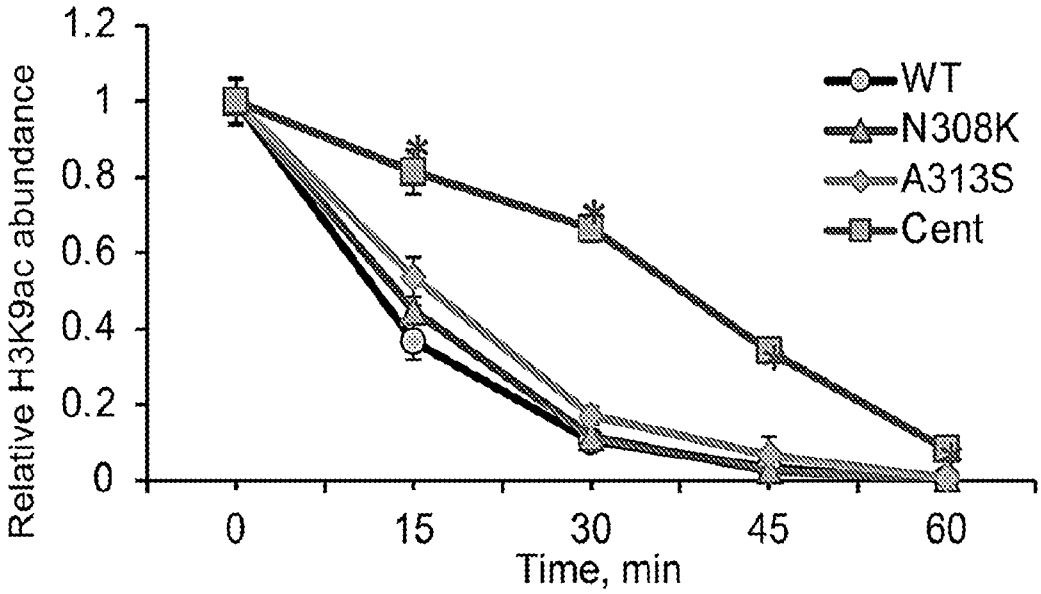
Relative H3K9ac abundance
1.2
1
0.8
0.6
0.4
0.2
0
0
15
30
45
60
Time, min
WT
N308K
A313S
Cent

FIG. 8A

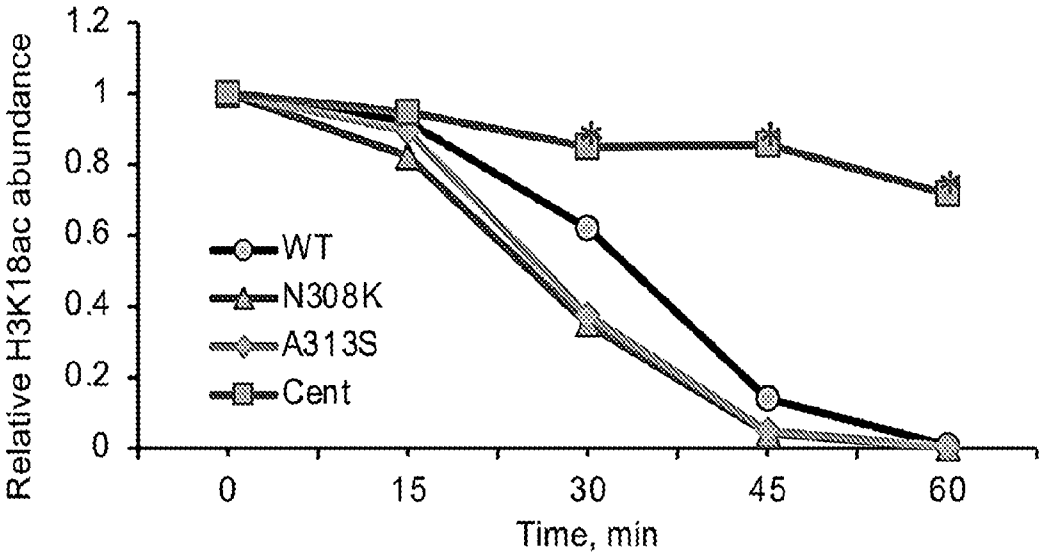
Relative H3K18ac abundance
1.2
1
0.8
0.6
0.4
0.2
0
0
15
30
45
60
Time, min
WT
N308K
A313S
Cent

FIG. 8B

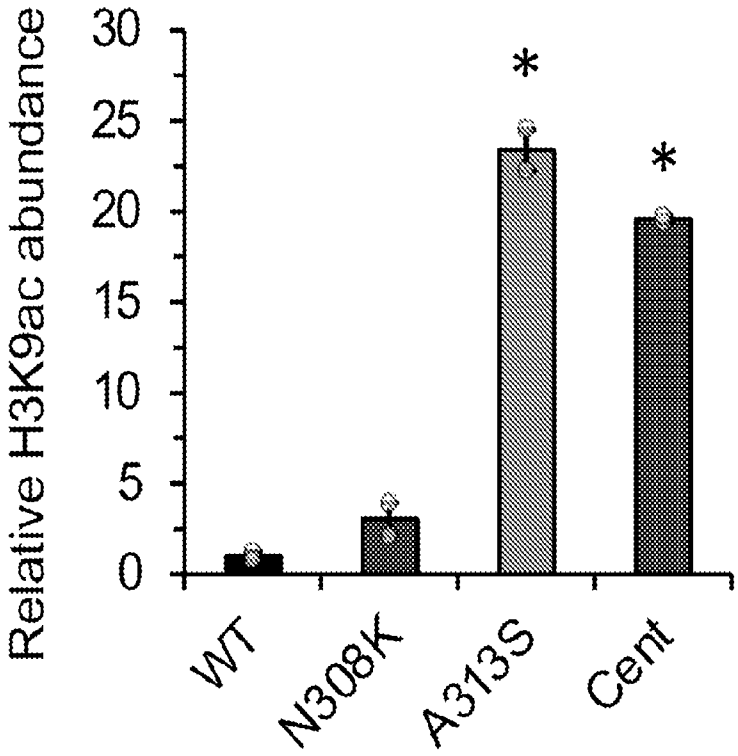
Relative H3K9ac abundance
30
25
20
15
10
5
0
*
*
WT
N308K
A313S
Cent

FIG. 9A

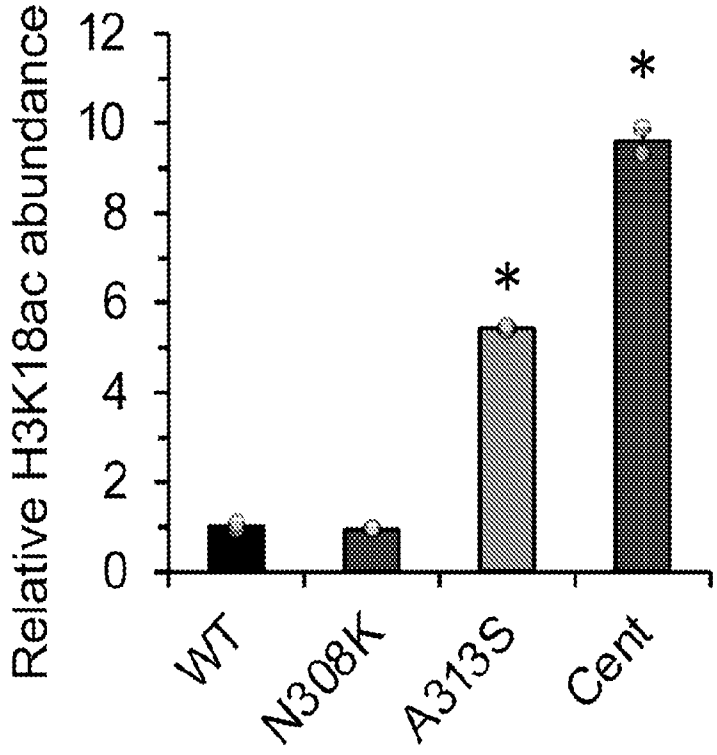
Relative H3K18ac abundance
12
10
8
6
4
2
0
*
*
WT
N308K
A313S
Cent

FIG. 9B

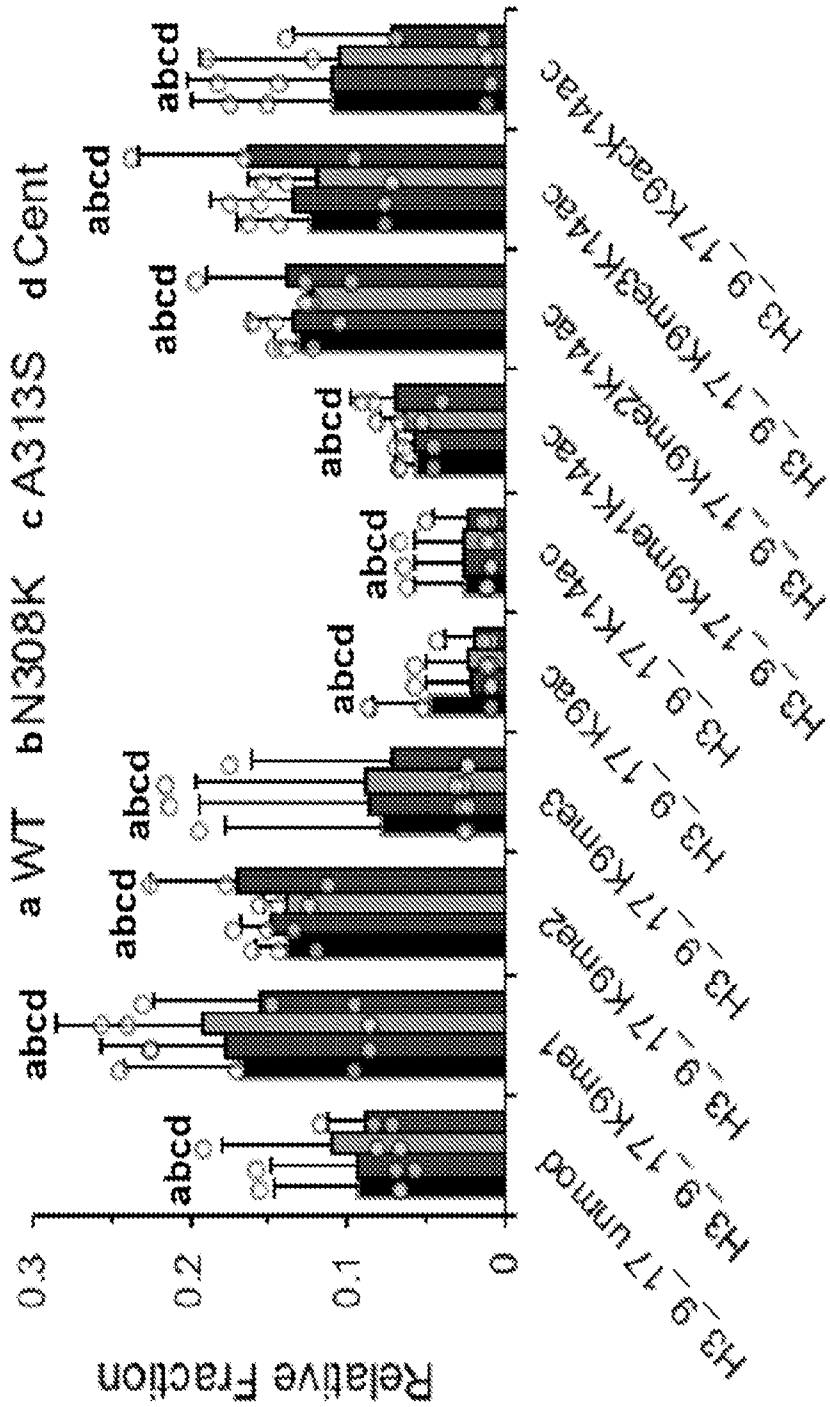
a WT
b N308K
c A313S
d Cent
Relative Fraction
0.3
0.2
0.1
0
abcd
H3_9_17_unmod
H3_9_17_K9me1
H3_9_17_K9me2
H3_9_17_K9me3
H3_9_17_K9ac
H3_9_17_K14ac
H3_9_17_K9me1K14ac
H3_9_17_K9me2K14ac
H3_9_17_K9me3K14ac
H3_9_17_K9acK14ac

FIG. 10

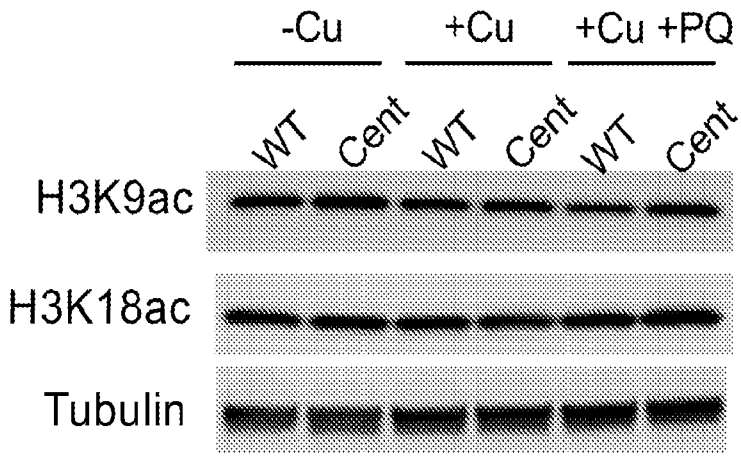
-Cu
+Cu
+Cu +PQ
WT
Cent
WT
Cent
WT
Cent
H3K9ac
H3K18ac
Tubulin

FIG. 11

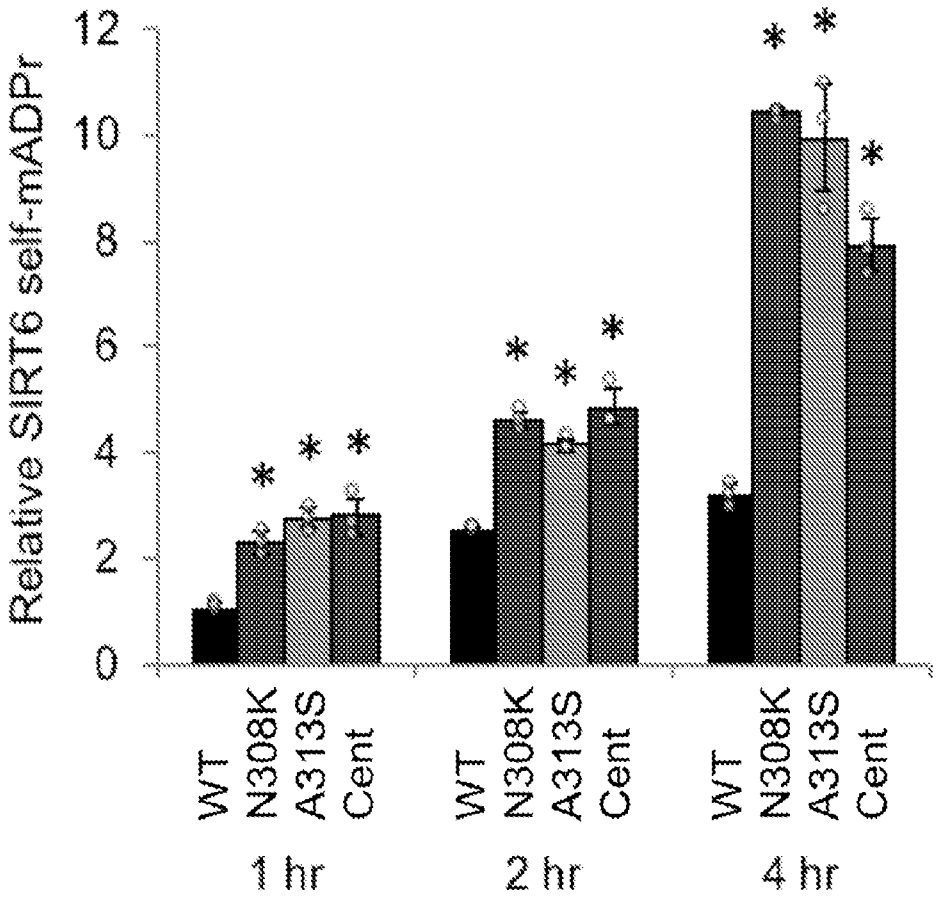
Relative SIRT6 self-mADPr
0
2
4
6
8
10
12
WT
N308K
A313S
Cent
1 hr
2 hr
4 hr

FIG. 12

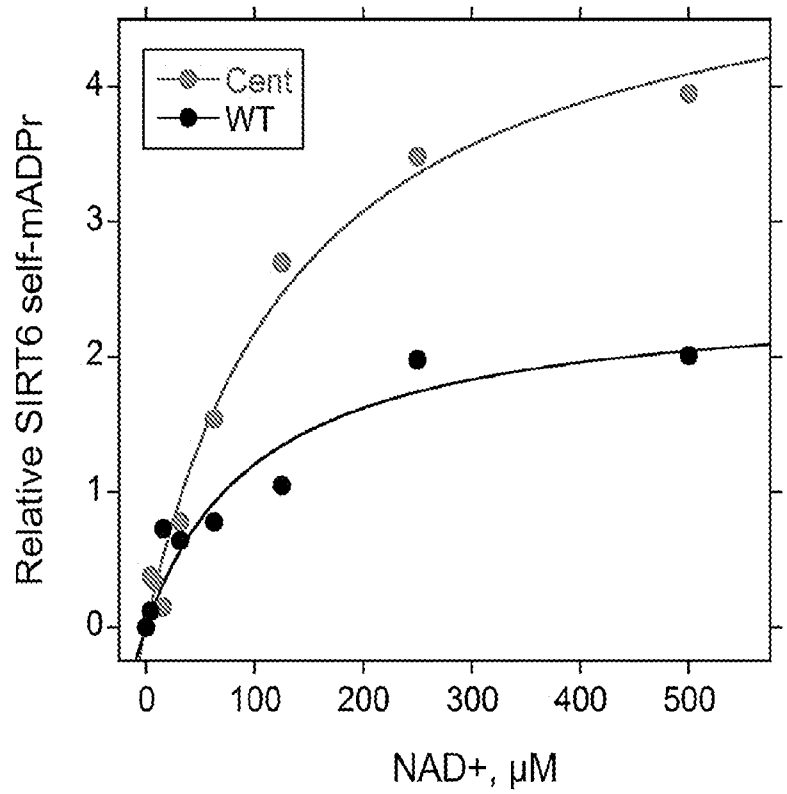
Cent
WT
Relative SIRT6 self-mADPr
0
1
2
3
4
0
100
200
300
400
500
NAD+, μM

FIG. 13

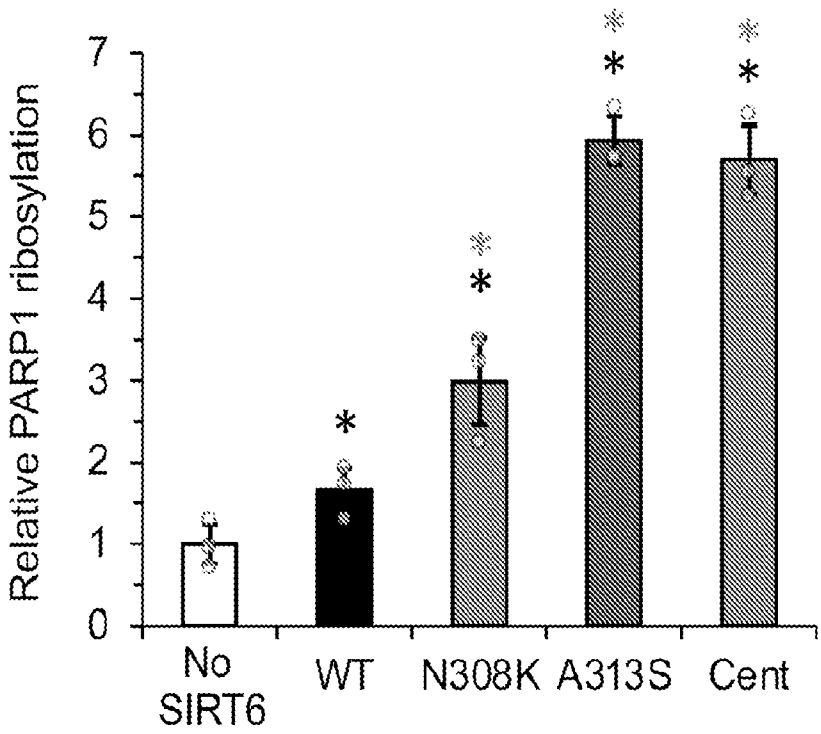
Relative PARP1 ribosylation
0
1
2
3
4
5
6
7
No SIRT6
WT
N308K
A313S
Cent

FIG. 14

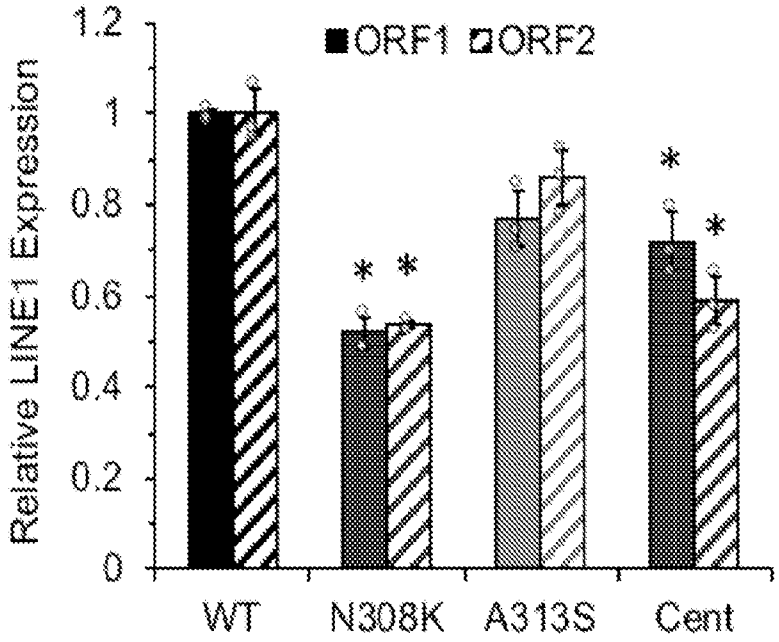

FIG. 15

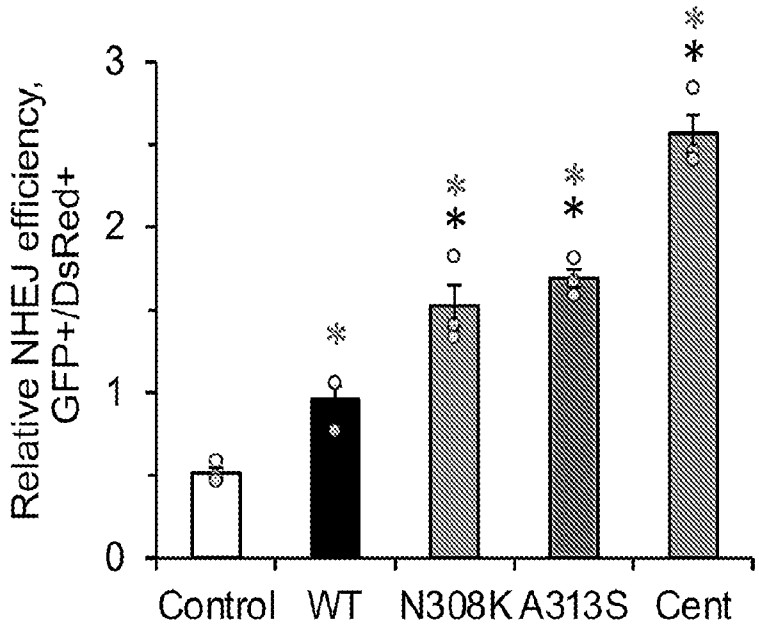

FIG. 16A

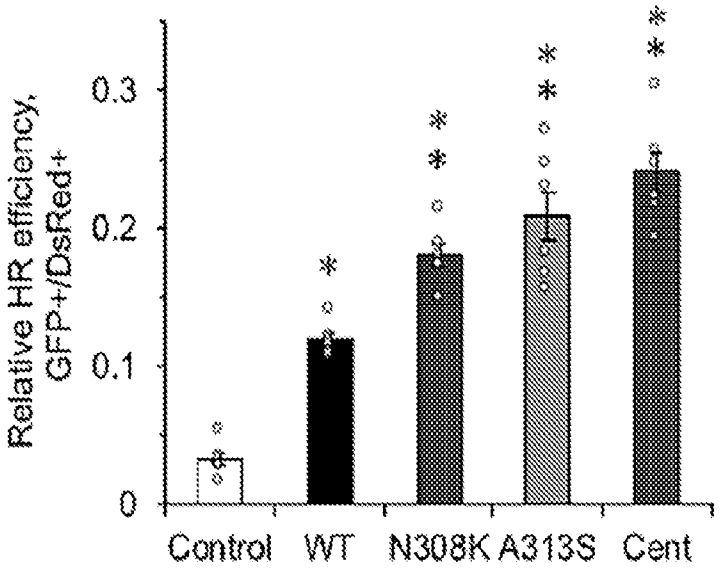

FIG. 16B

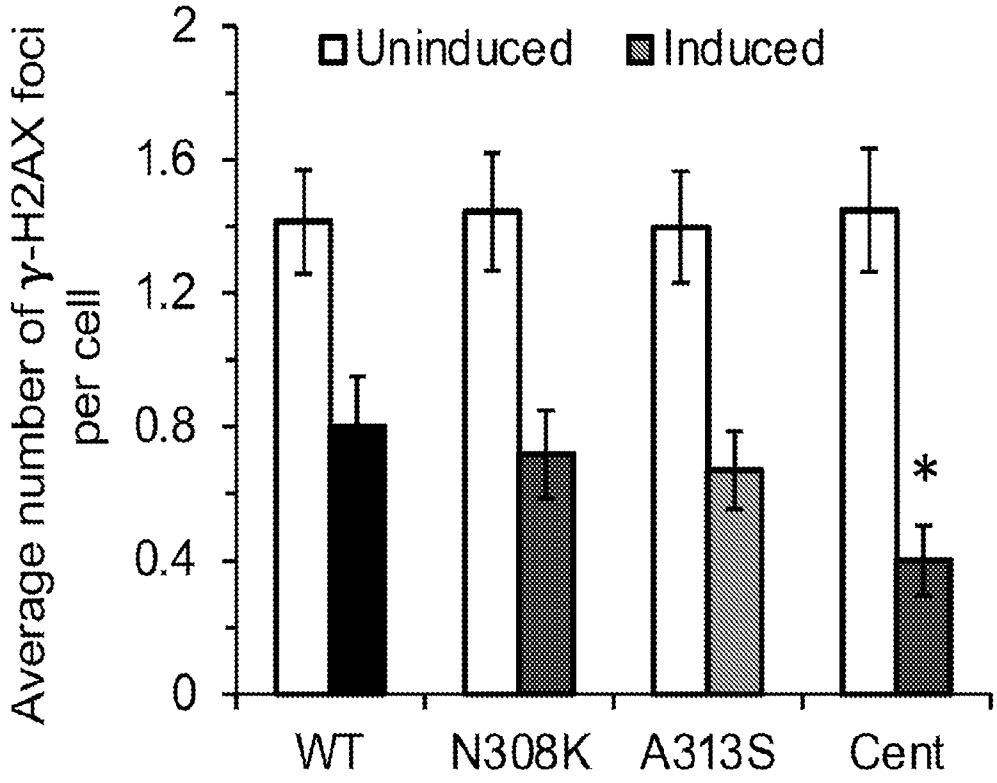

FIG. 17

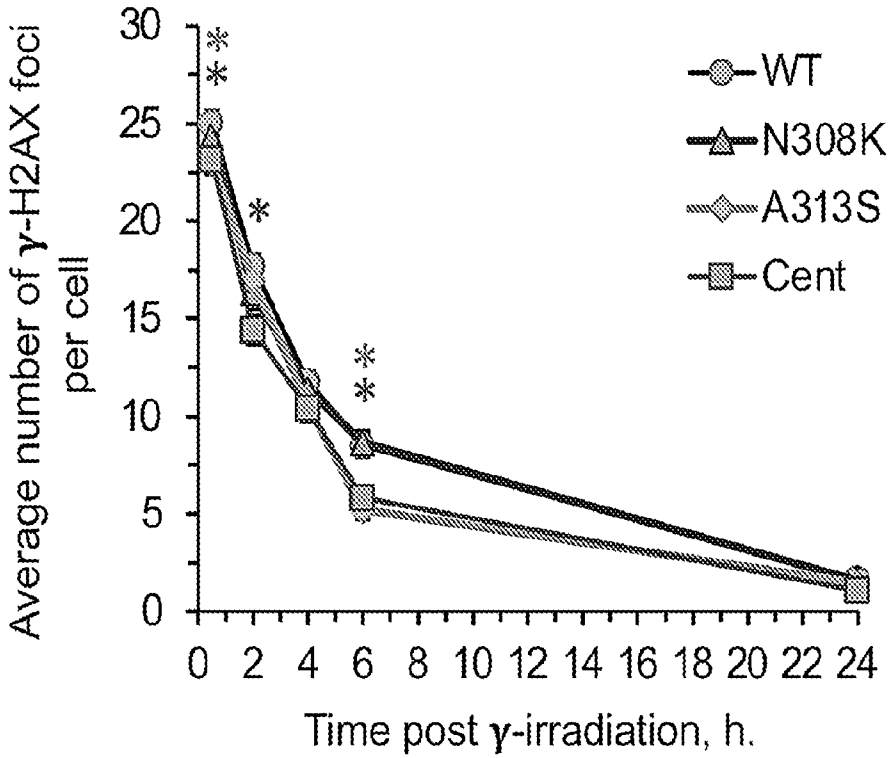

FIG. 18

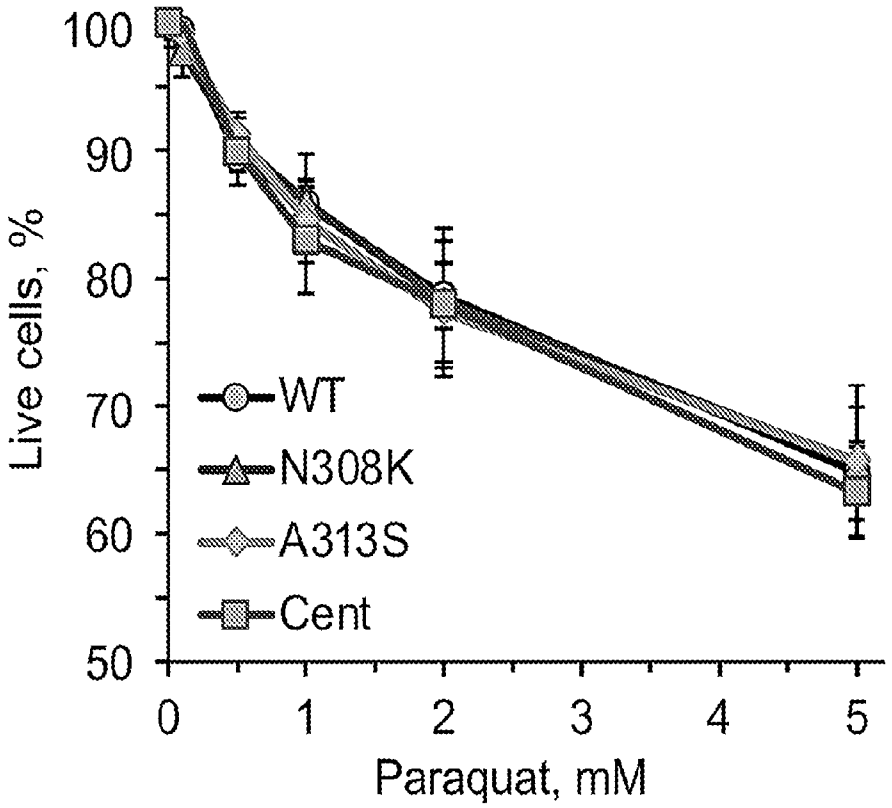

FIG. 19A

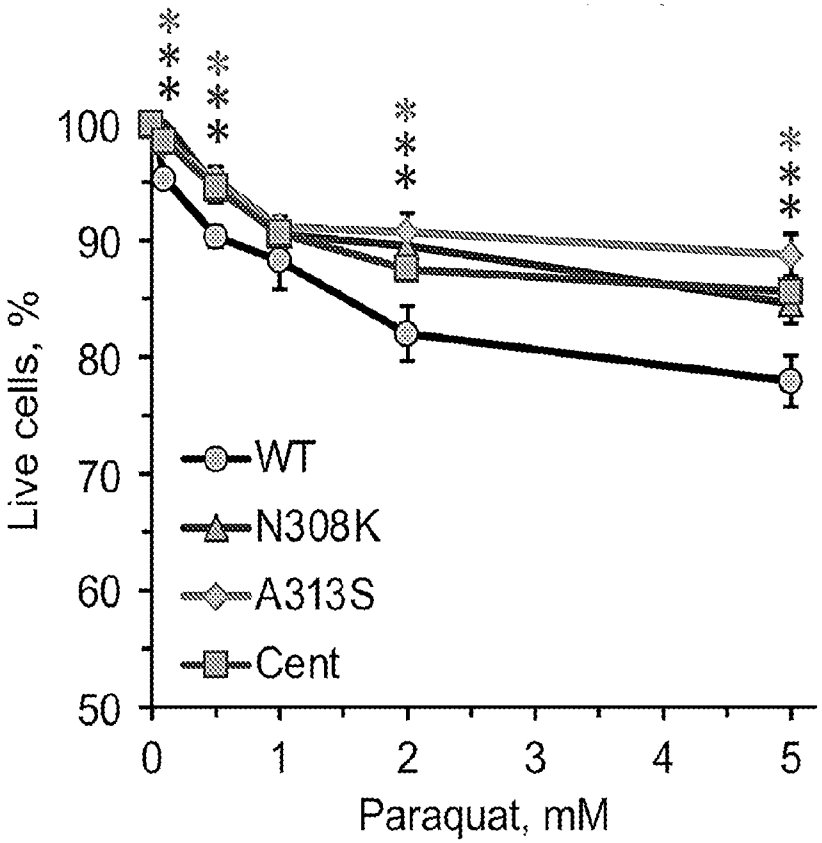

FIG. 19B

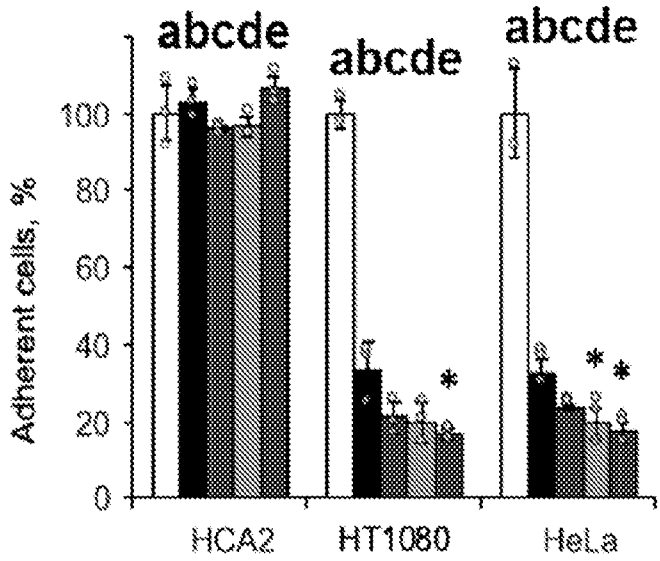

FIG. 20A

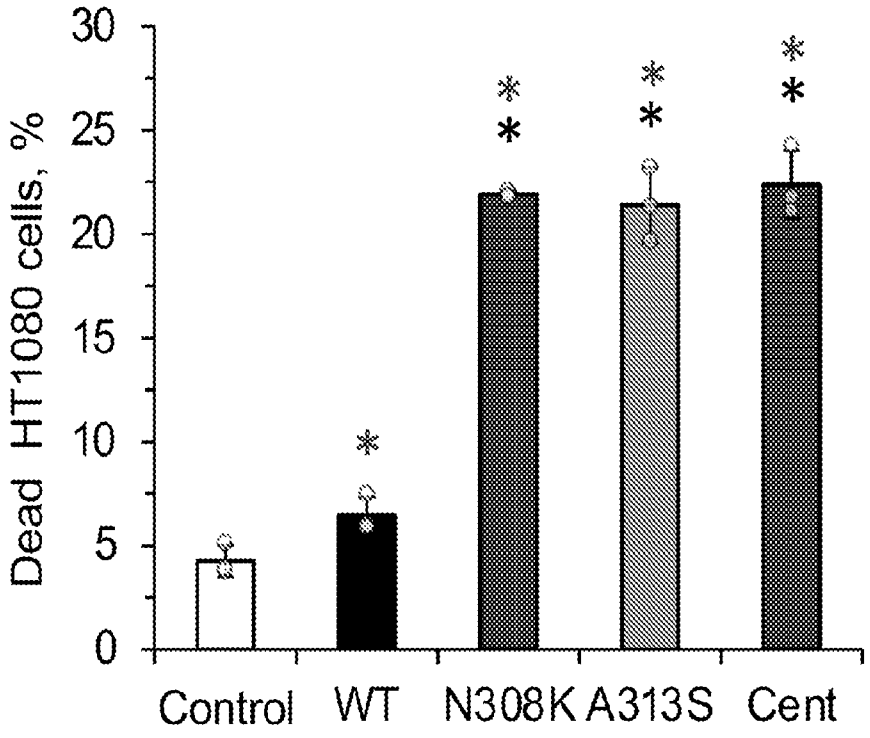

FIG. 20B

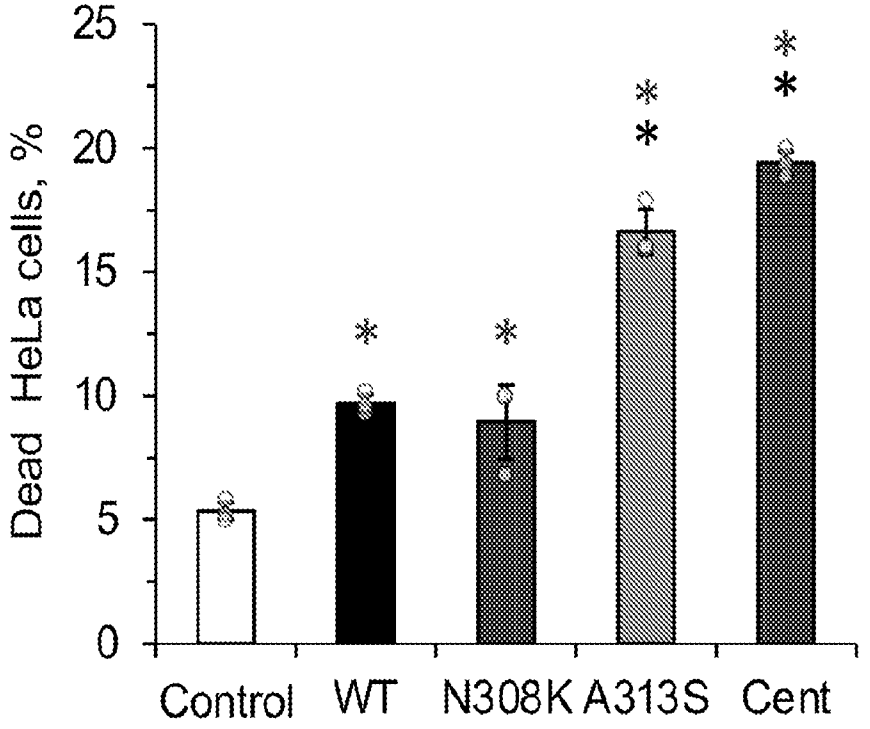

FIG. 20C

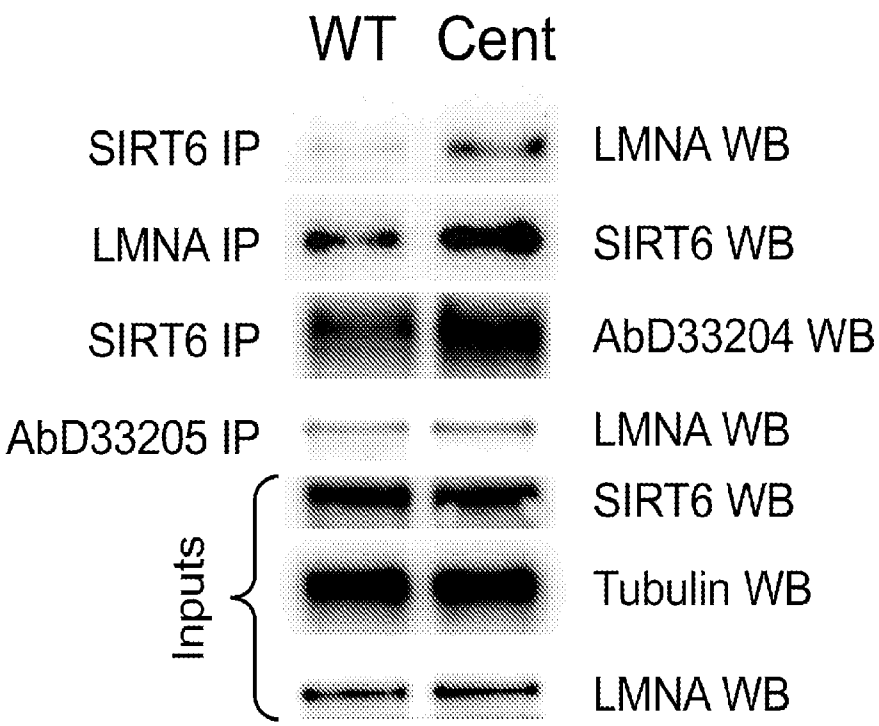
WT Cent
SIRT6 IP
LMNA WB
LMNA IP
SIRT6 WB
SIRT6 IP
AbD33204 WB
AbD33205 IP
LMNA WB
Inputs
SIRT6 WB
Tubulin WB
LMNA WB

FIG. 21

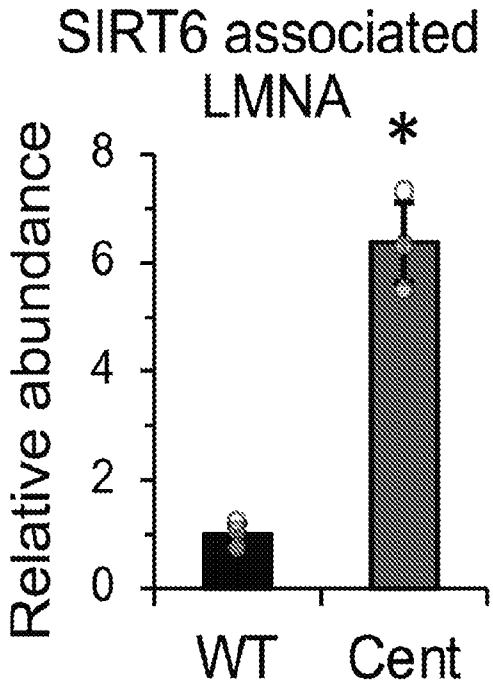
SIRT6 associated LMNA
Relative abundance
0
2
4
6
8
*
WT
Cent

FIG. 22

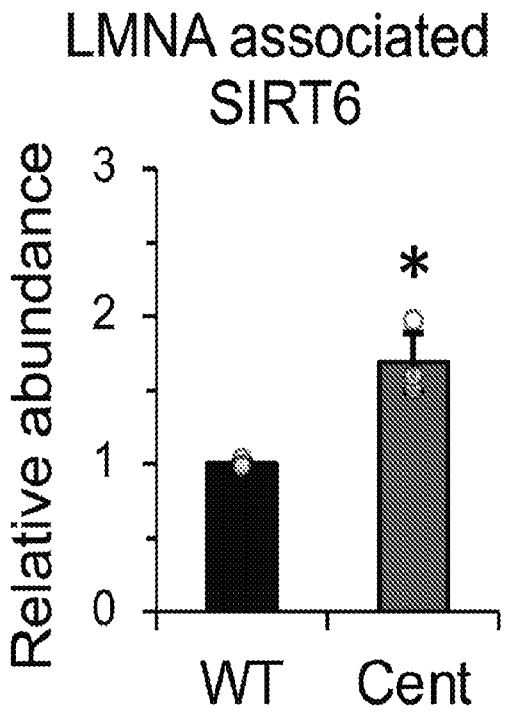
LMNA associated SIRT6
Relative abundance
3
2
1
0
*
WT
Cent

FIG. 23

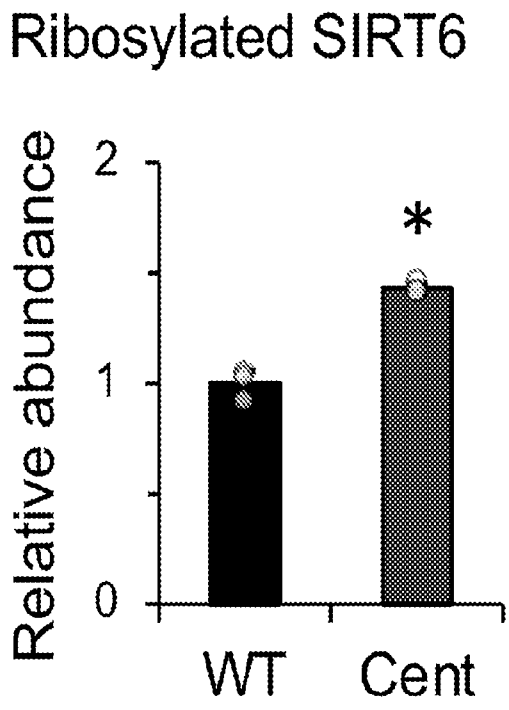
Ribosylated SIRT6
Relative abundance
2
1
0
*
WT
Cent

FIG. 24

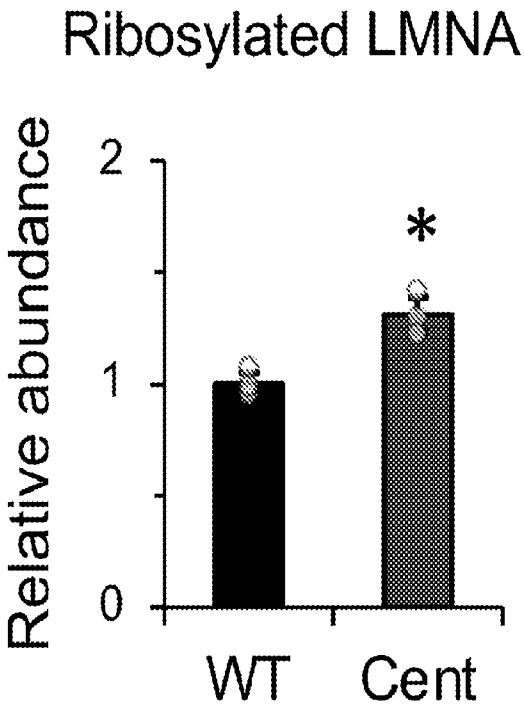

FIG. 25

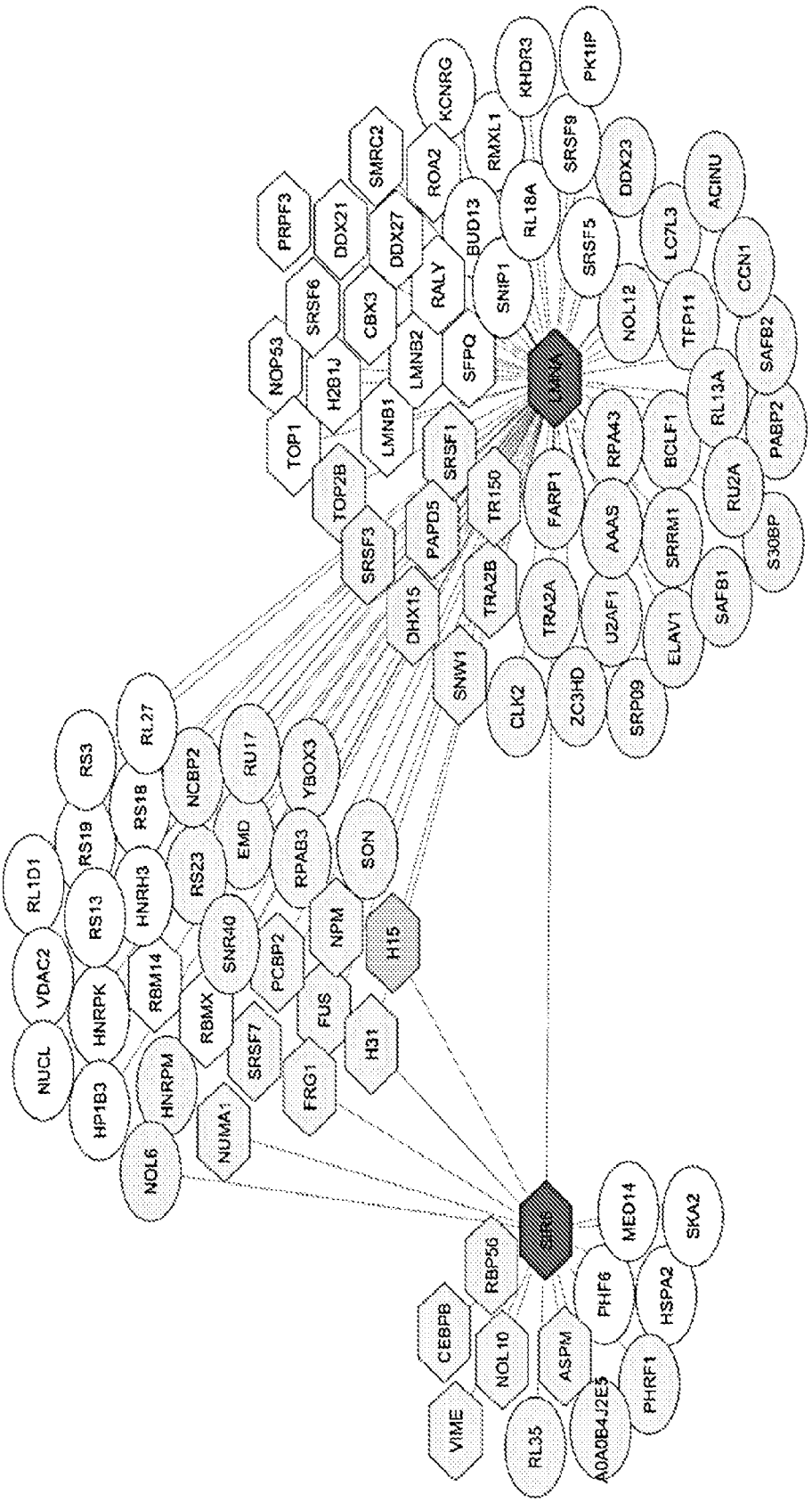
KHDR3
PK1IP
KCNRG
RMXL1
SRSF9
ROA2
DDX23
SMRC2
RL18A
ACINU
PRPF3
DDX21
DDX27
BUD13
SRSF5
LC7L3
CCN1
SRSF6
CBX3
RALY
SNIP1
NOL12
TFP11
SAFB2
NOP53
H2B1J
LMNB2
SFPQ
RL13A
PABP2
TOP1
LMNB1
SRSF1
TR150
FARP1
RPA43
BCLF1
RU2A
S30BP
TOP2B
PAPD5
AAAS
SRRM1
SAF81
SRSF3
DHX15
TRA2B
TRA2A
U2AF1
ELAV1
SNW1
CLK2
ZC3HD
SRP09
RL27
RS3
RS18
NCBP2
RU17
YBOX3
RS19
RL1D1
RS23
EMD
RPAB3
SON
HNRH3
VDAC2
RS13
SNR40
PCBP2
NPM
H15
HNRPK
RBM14
RBMX
FUS
H31
NUCL
HP1B3
HNRPM
SRSF7
FRG1
NUMA1
NCL6
MED14
SKA2
RBP56
PHF6
HSPA2
CEBPB
NOL10
ASPM
PHRF1
VIME
RL35
A0A0B4J2E5

FIG. 26

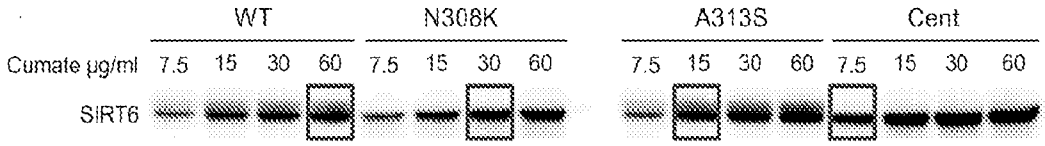

FIG. 27A

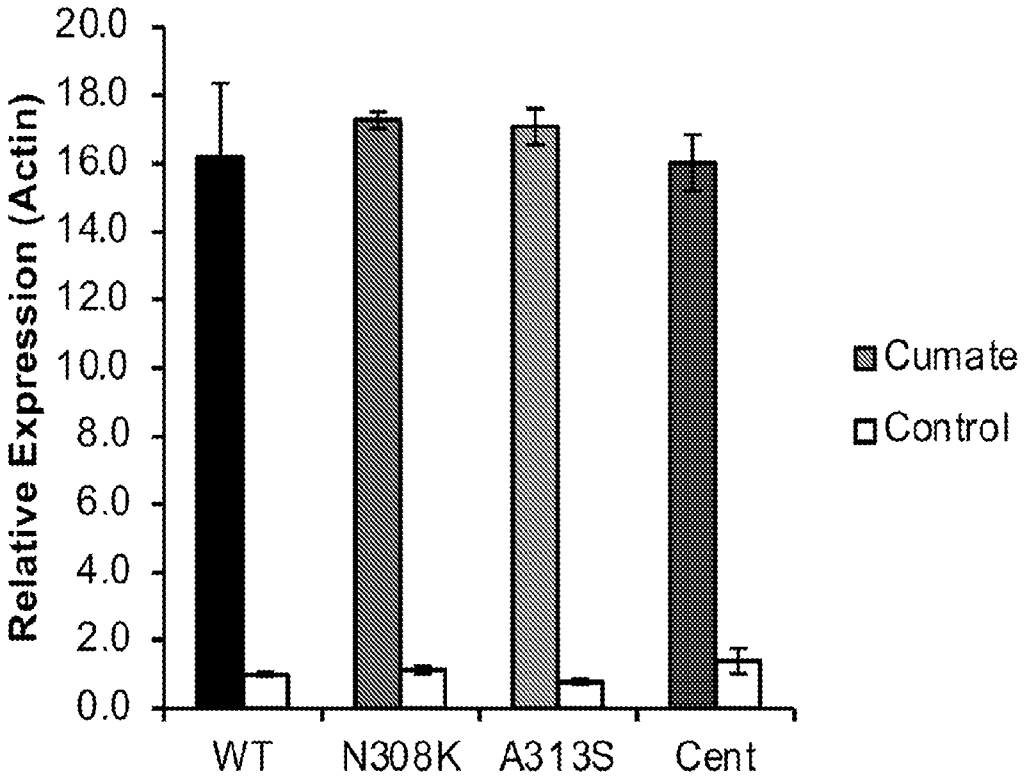

FIG. 27B

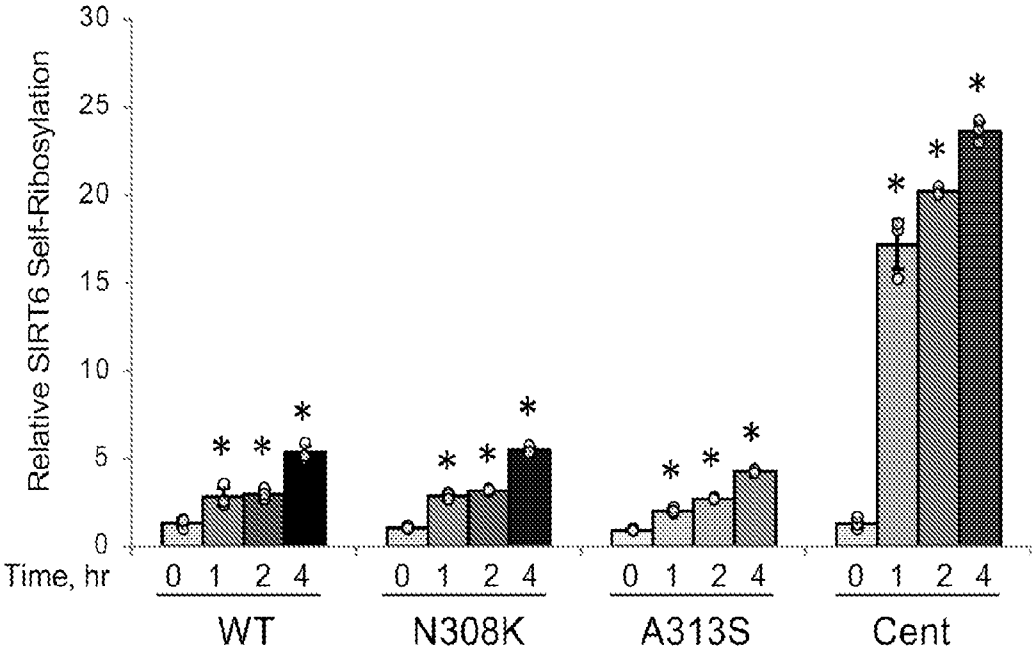

FIG. 28

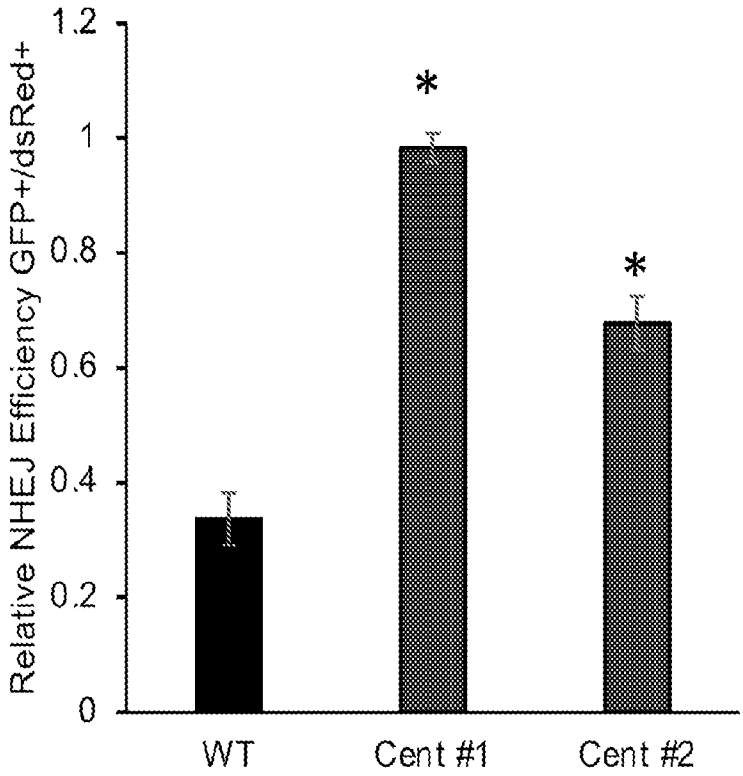

FIG. 29A

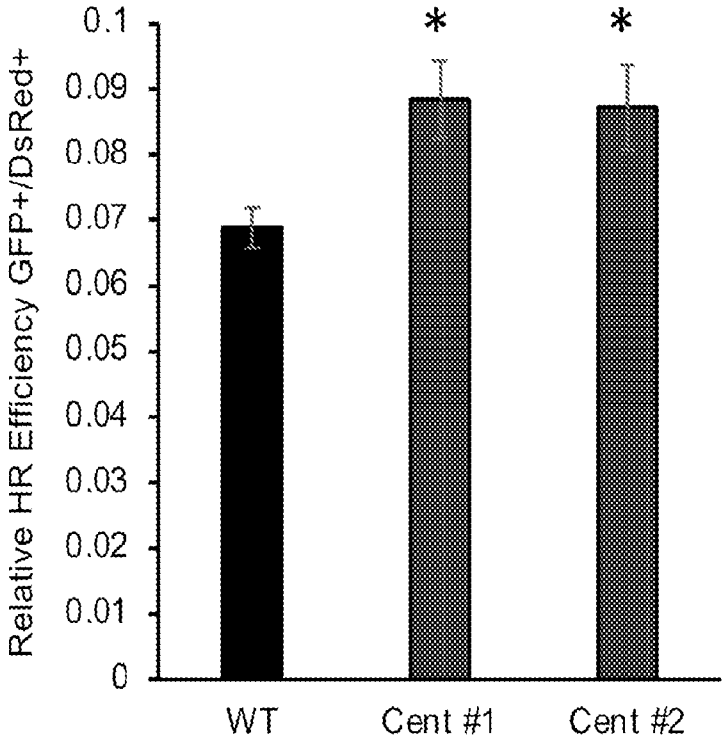

FIG. 29B

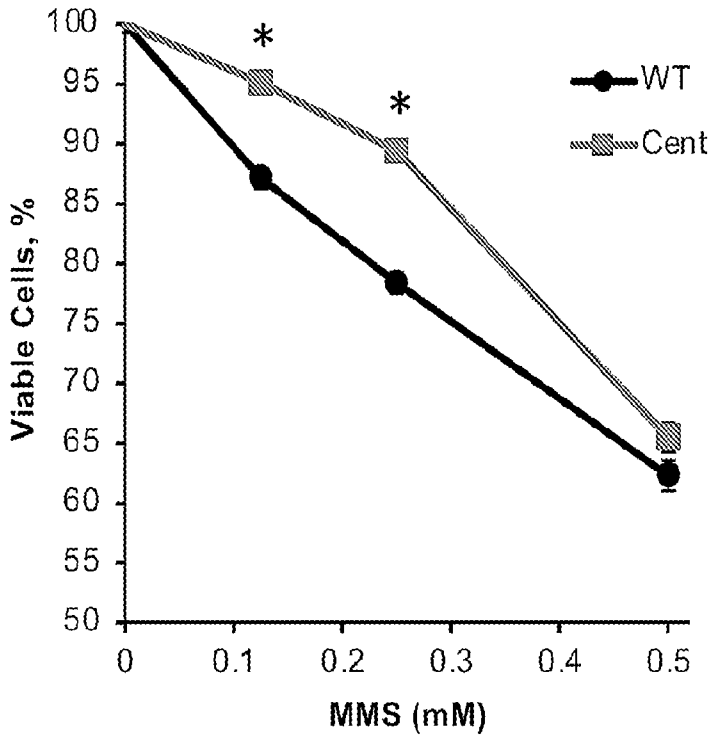

FIG. 29C

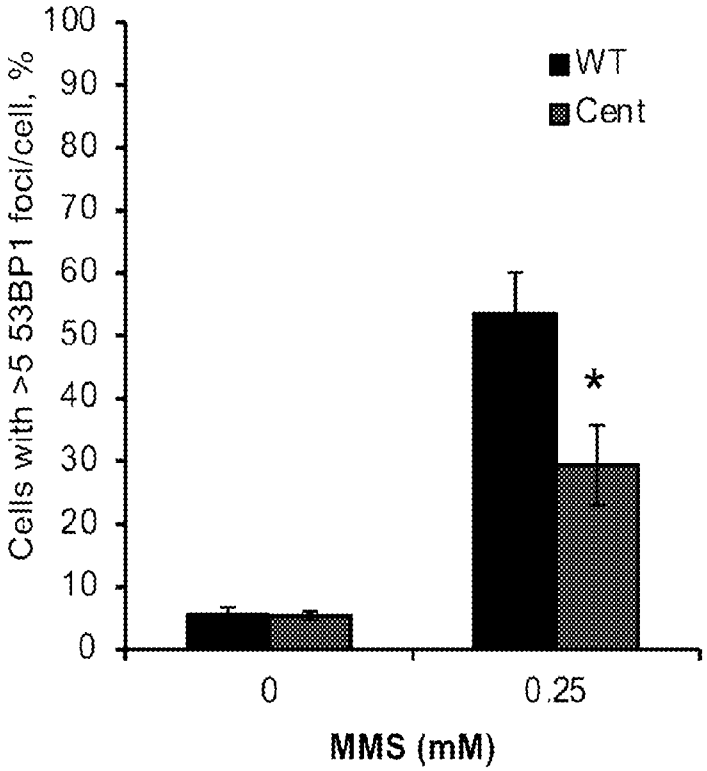

FIG. 29D

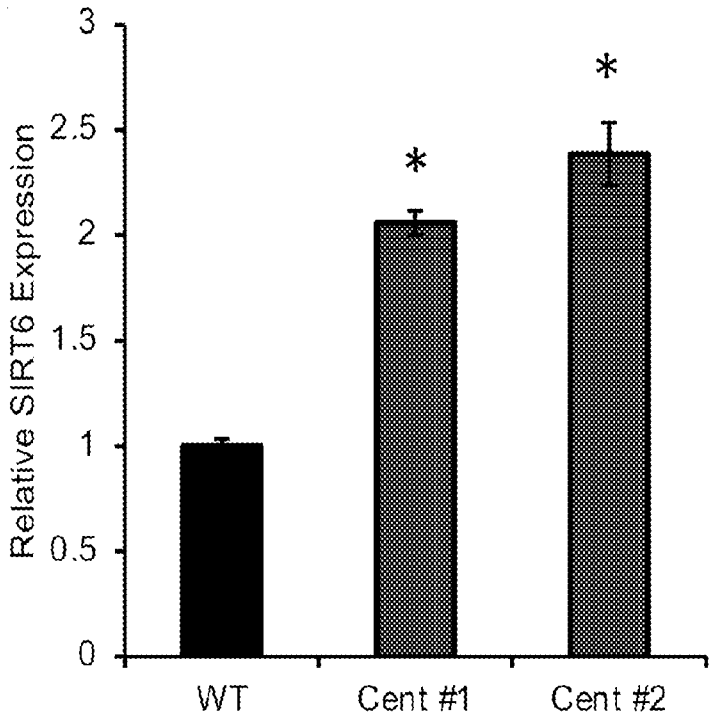

FIG. 29E

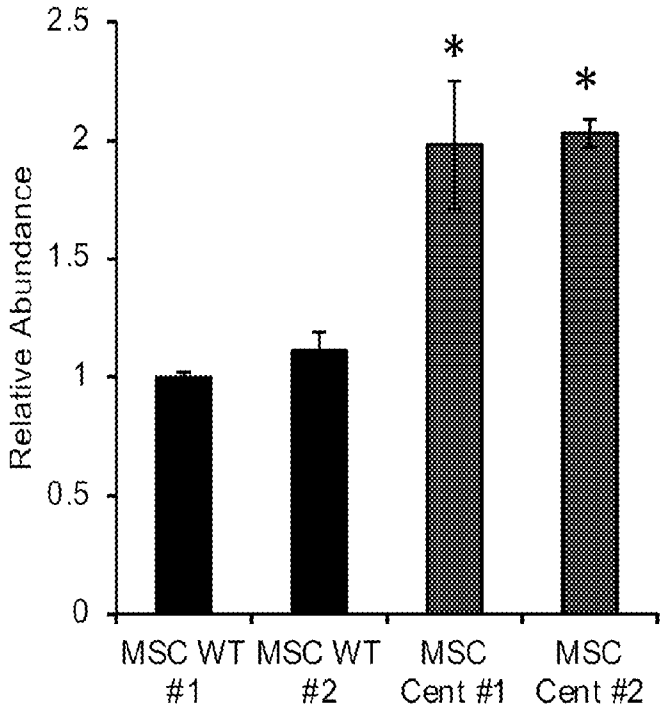

FIG. 29F

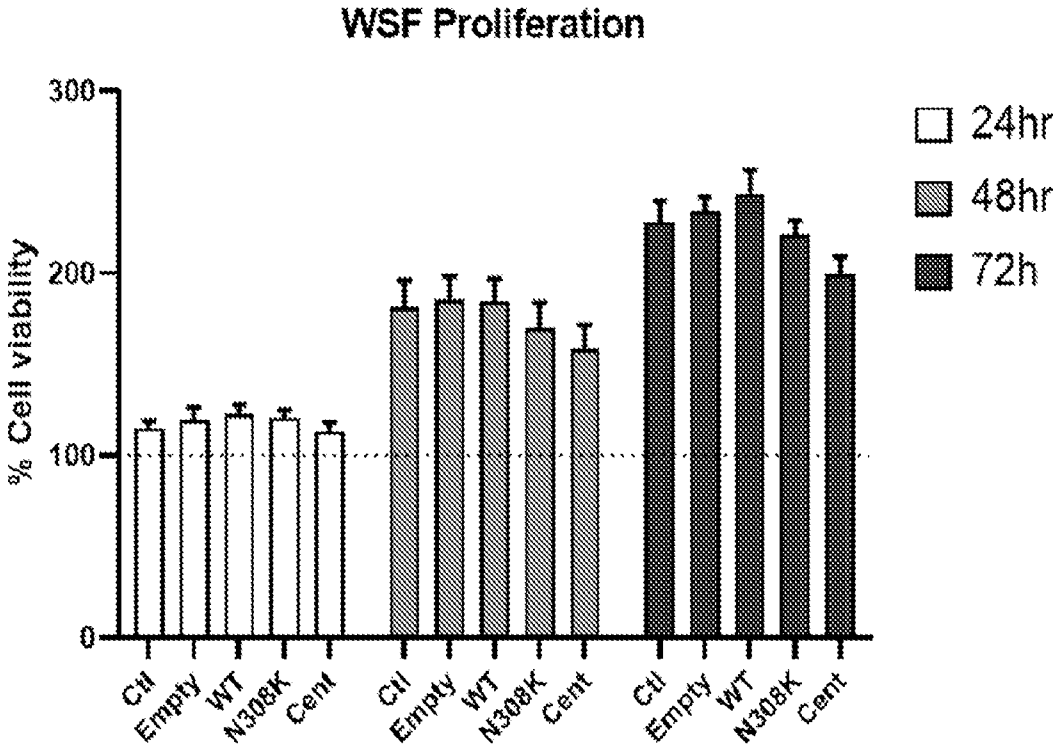

FIG. 30A

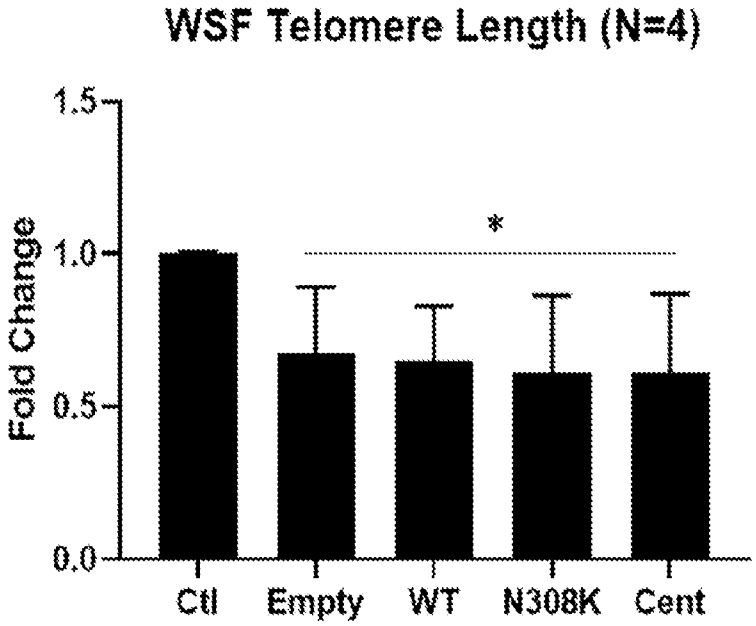

FIG. 30B

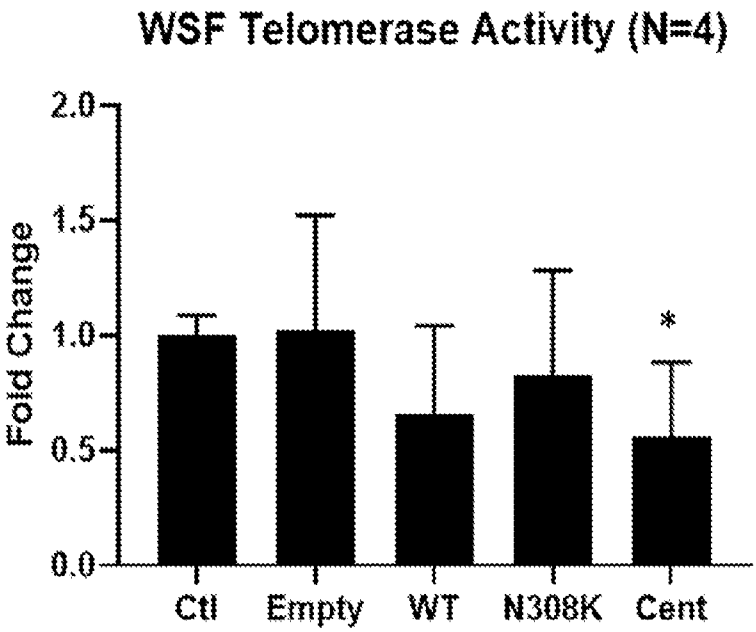

FIG. 30C

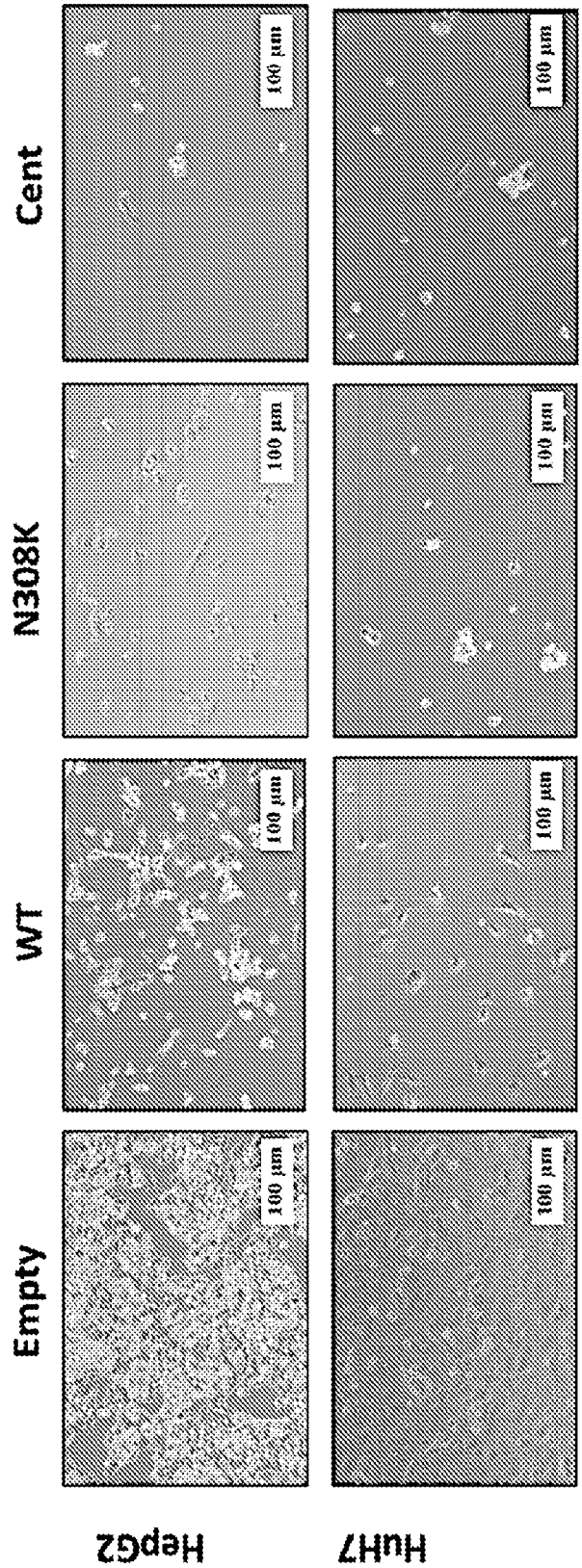

FIG. 31A

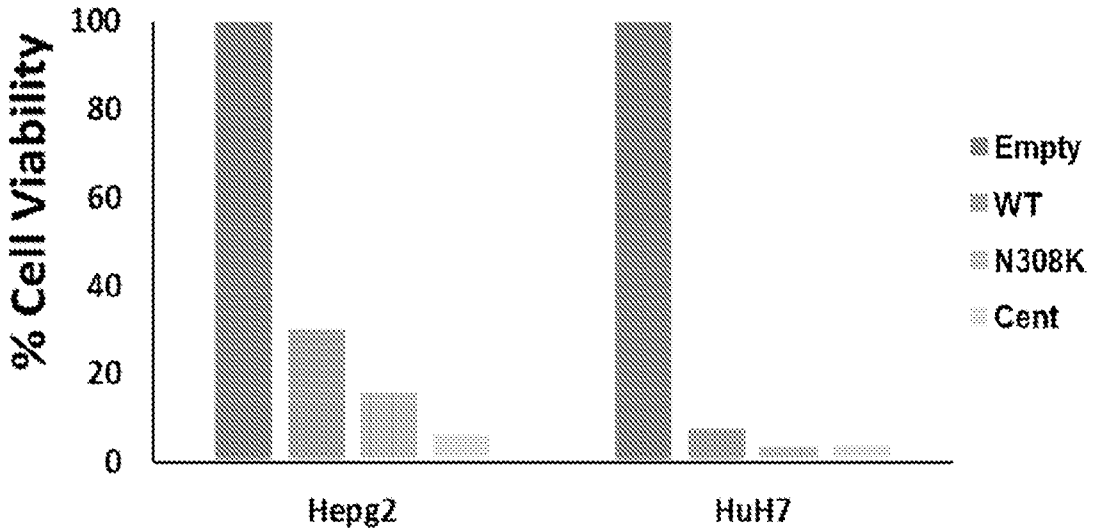

FIG. 31B

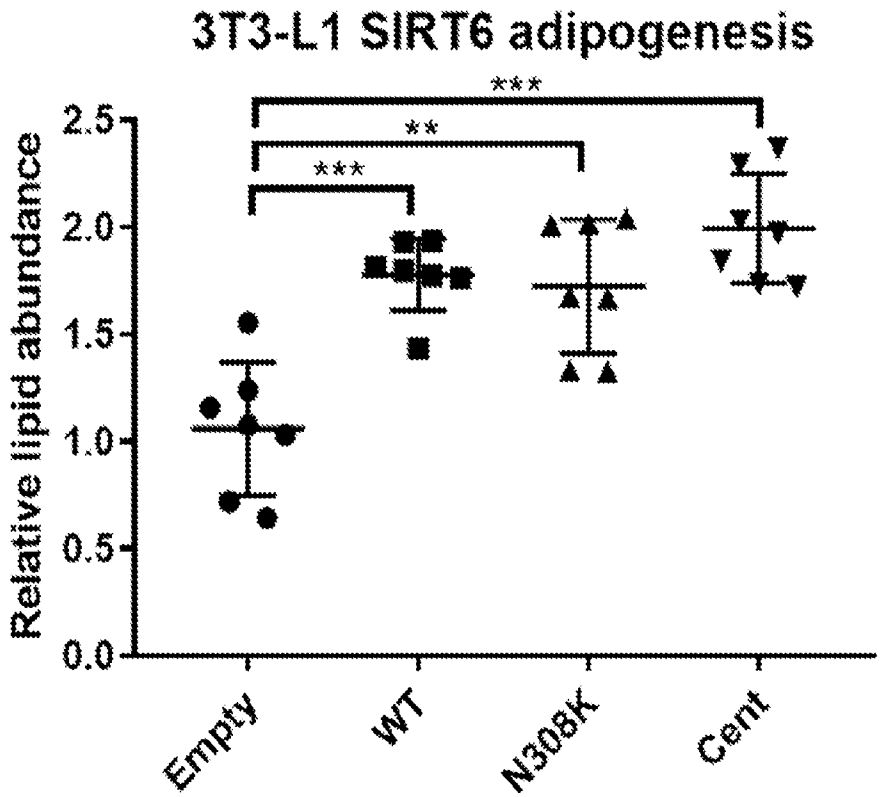

FIG. 32

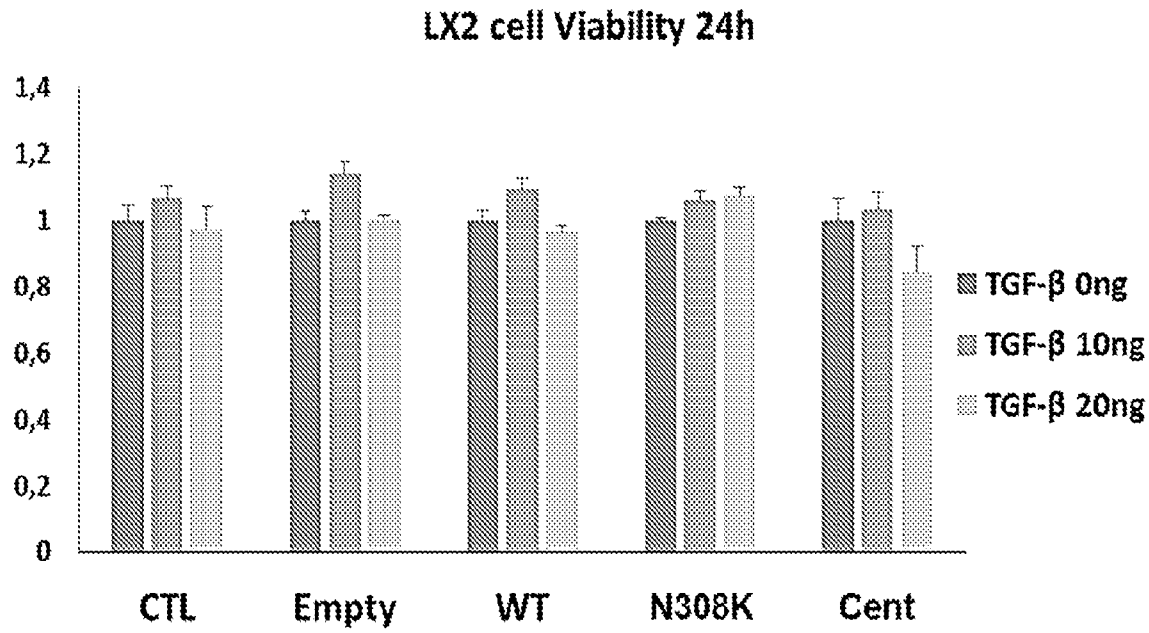

FIG. 33A

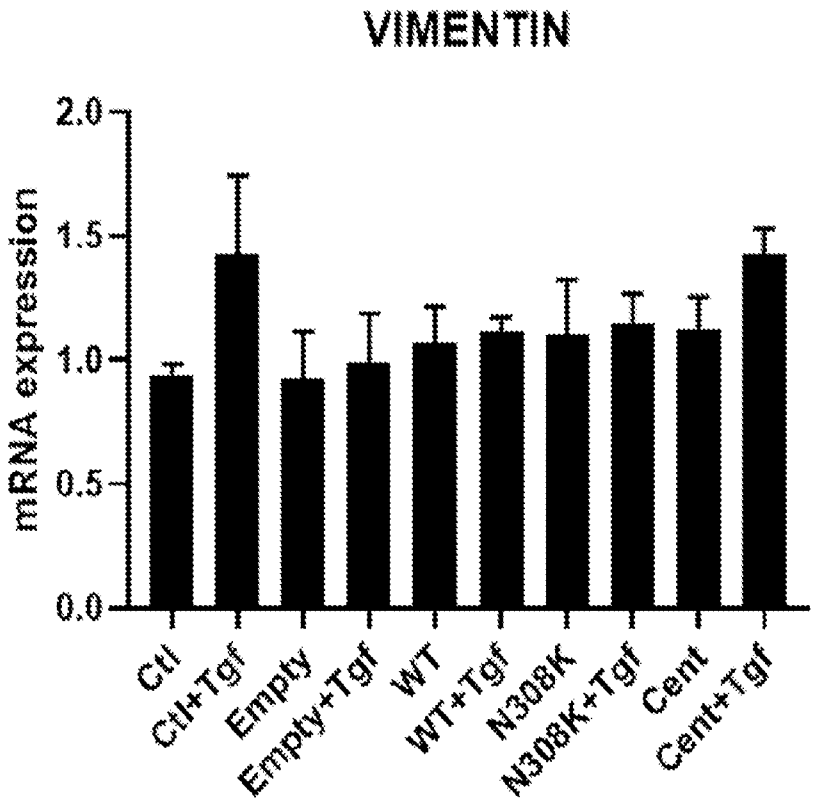

FIG. 34B

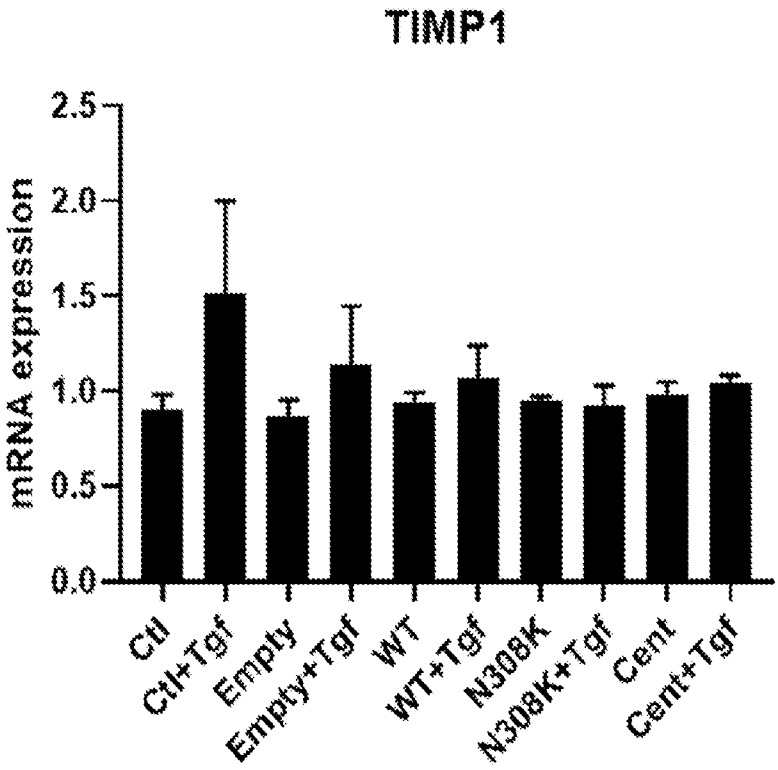

FIG. 34C

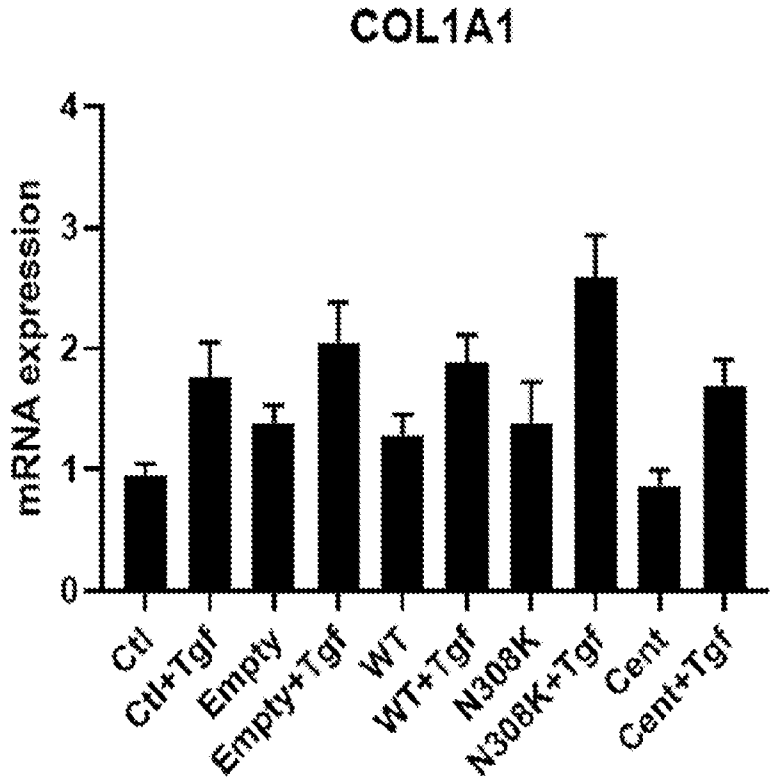

FIG. 34D

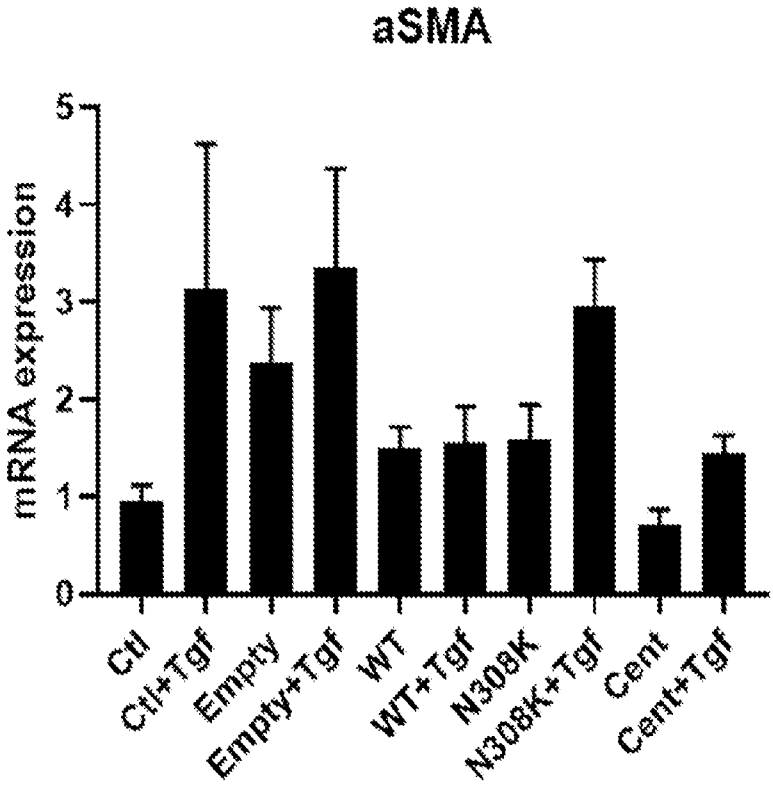

FIG. 34E

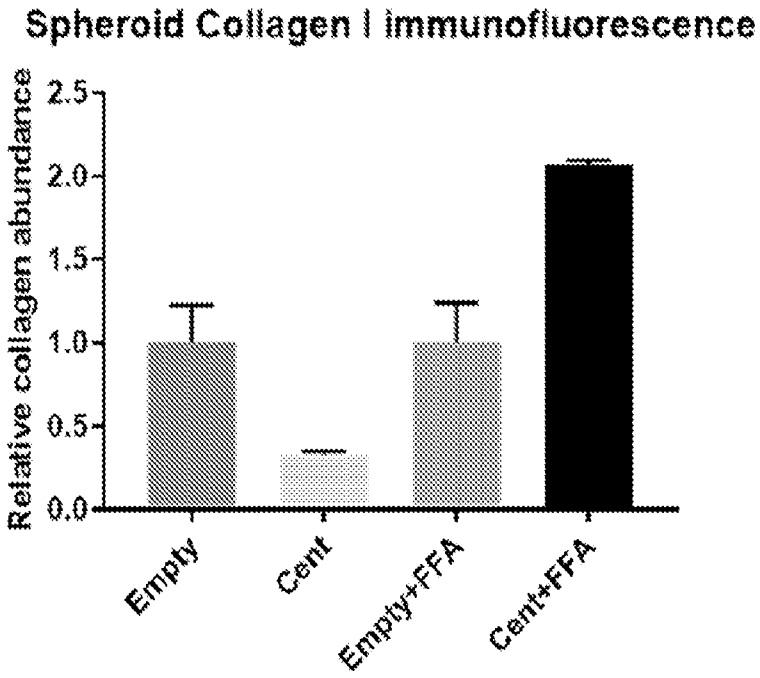

FIG. 35A

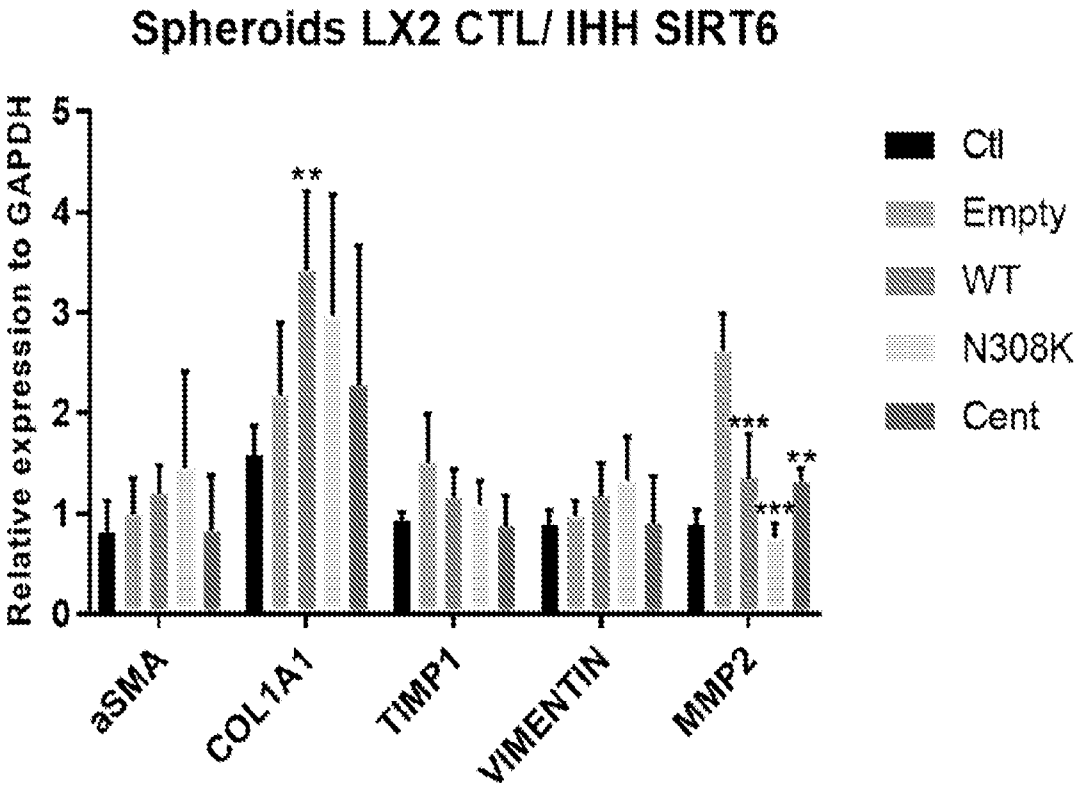

FIG. 35B

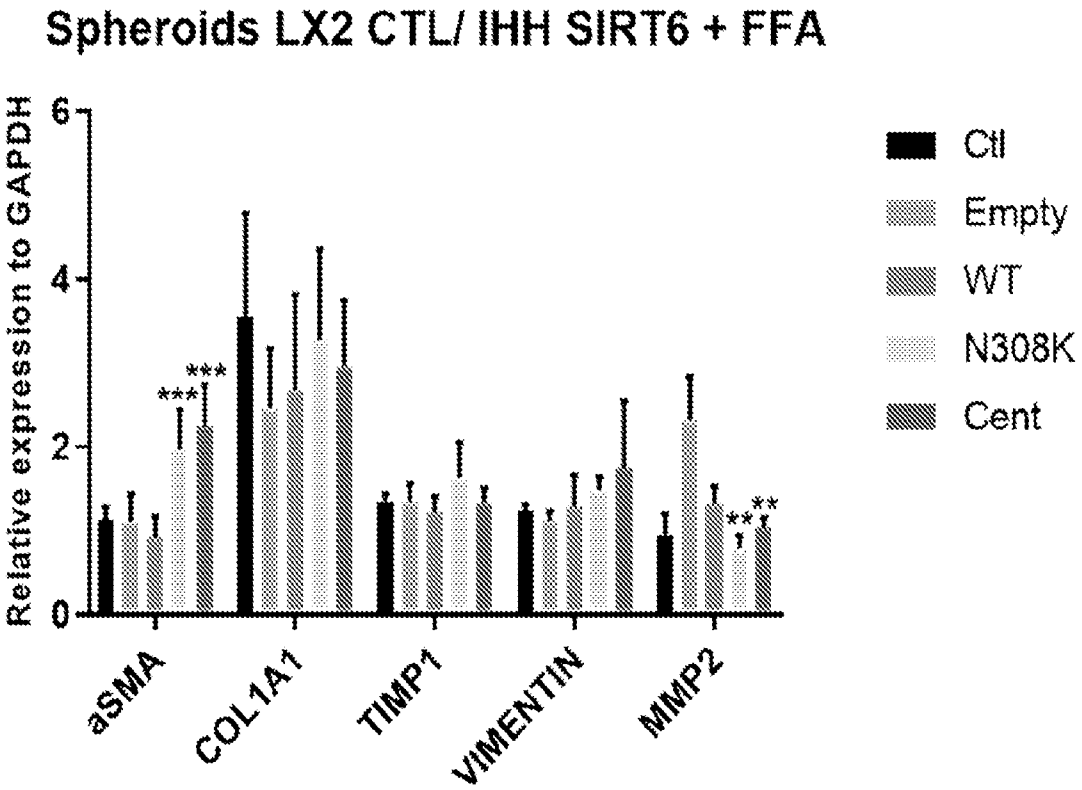

FIG. 35C