



- (51) **International Patent Classification:**
C07K 14/47 (2006.01) *A61K 48/00* (2006.01)
- (21) **International Application Number:**
PCT/US2016/034821
- (22) **International Filing Date:**
27 May 2016 (27.05.2016)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/168,289 29 May 2015 (29.05.2015) US
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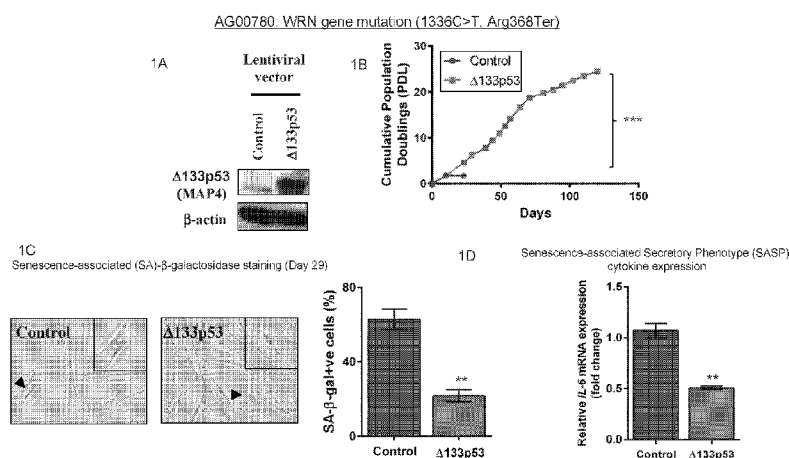
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- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,

[Continued on next page]

- (54) **Title:** EXTENSION OF REPLICATIVE LIFESPAN IN DISEASES OF PREMATURE AGING USING P53 ISOFORMS

Figure 1A-1D



- (57) **Abstract:** The present invention is directed to therapies that modulate naturally occurring p53 isoforms for the treatment of diseases of premature aging.



TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

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

EXTENSION OF REPLICATIVE LIFESPAN IN DISEASES OF PREMATURE AGING USING P53 ISOFORMS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. Provisional Patent Application No. 62/168,289, filed May 29, 2015, which application is herein incorporated by reference for all purposes.

BACKGROUND OF THE INVENTION

10 [0002] Replicative senescence, a state of irreversible growth arrest, can be triggered by multiple mechanisms including telomere shortening, oncogene activation, and DNA damage. These mechanisms limit excessive or aberrant cellular proliferation and put senescence in the crossroads of protecting individuals from cancer progression and contributing to organismal aging.

15 [0003] p53 is a protein of having an apparent molecular weight of about 53 kDa on SDS PAGE that functions as a transcription factor that, among other functions, regulates the cell cycle and acts as a tumor suppressor. In normal cells, p53 is generally held in an inactive form, bound to the protein MDM2 (HDM2 in humans), which prevents p53 activity and promotes p53 degradation by acting as a ubiquitin ligase. Active p53 is induced in response
20 to various cancer-causing agents such as UV radiation, oncogenes, and some DNA-damaging drugs. DNA damage is sensed by 'checkpoints' in a cell's cycle, and causes proteins such as ATM, CHK1 and CHK2 to phosphorylate p53 at sites that are close to or within the MDM2-binding region and p300-binding region of the protein. Oncogenes also stimulate p53 activation, mediated by the protein p14ARF. Some oncogenes can also stimulate the
25 transcription of proteins which bind to MDM2 and inhibit its activity. Once activated, p53 activates expression of several genes including one encoding for p21, a cell cycle inhibitor. p21 binds to G1-S-phase and S-phase cyclin CDK complexes inhibiting their activity. *See, e.g., Mills, Genes & Development*, 19: 2091-2099 (2005) for a review.

[0004] The p53 signaling network plays an important role in regulating senescence. In
30 addition to full-length p53 (p53-FL), the p53 gene (tumor protein p53 gene TP53) encodes at least 13 natural isoforms due to alternative splicing and alternative promoter usage. It has

previously been shown that a natural isoform of p53, $\Delta 133p53$, preferentially inhibits p53-target genes involved in cellular senescence in normal cells (see, e.g., WO 2009/064590).

[0005] Werner syndrome (WS) and Hutchinson-Gilford progeria syndrome (HGPS) are diseases of premature aging in which patients show aging-associated disorders early in life.

5 WS is an autosomal syndrome caused by mutations in the DNA-repair essential WRN helicase gene that result in premature aging in young adults. HGPS is a laminopathy caused by mutations in the LMNA gene resulting in post-translational processing defects that lead to an abnormal nuclear envelope structure and causes Progeria in children. Fibroblasts derived from individuals with WS or HGPS have decreased cellular proliferation and increased
10 senescence in culture, as well as altered DNA damage responses. However, the role of p53 isoforms in the abnormal cellular aging processes that occur in these prematurely aging cells has not been established. There are currently no therapies for inhibiting or delaying the premature aging phenotypes in WS and HGPS patient. Thus, there is a need to identify therapeutic target candidates to develop therapies to treat these disorders.

15 BRIEF SUMMARY OF CERTAIN ASPECTS OF THE DISCLOSURE

[0006] In certain aspects, this disclosure is based, in part, on the inventors' discovery that the p53 isoforms $\Delta 133p53$ and p53 β play a role in abnormal aging processes in cells of patients that have a pre-mature aging syndrome such as WS or HGPS. Thus, in one aspect, the invention provides a method of extending the replicative lifespan of a cell of a subject that
20 has a disease of premature aging, the method comprising contacting the cell with an agent that increases the function or expression of $\Delta 133p53$, thereby inhibiting cell senescence and extending the replicative lifespan of the cell. In some embodiments, the disease of premature aging is Werner syndrome or Hutchinson-Gilford progeria syndrome. In some embodiments, the agent comprises a polynucleotide sequence encoding $\Delta 133p53$, e.g., the agent may
25 comprise an expression cassette comprising a polynucleotide sequence encoding $\Delta 133p53$. In some embodiments, the cell is a stem cell. In some embodiments, the cell is a cardiac progenitor cell, an endothelial progenitor cell, or a bone marrow-derived progenitor cell. In some embodiments, the cell is contacted with the agent *ex vivo*. In some embodiments, the cells are then expanded and administered to the patient.

30 [0007] In another aspect, the invention provides a method of extending the replicative lifespan of a cell of a subject that has a disease of premature aging, the method comprising contacting the cell with an agent that inhibits the function or expression of miR-34a, thereby

inhibiting cell senescence and extending the replicative lifespan of the cell. In some embodiments, the disease of premature aging may be Werner syndrome or Hutchinson-Gilford progeria syndrome. In some embodiments, the agent comprises a polynucleotide sequence that inhibits miR-34a, *e.g.*, an expression cassette comprising a polynucleotide sequence that inhibits miR-34a. In some embodiments, the cell is a stem cell. In some
5 embodiments, the cell is a cardiac progenitor cell, an endothelial progenitor cell, or a bone marrow-derived progenitor cell. In some embodiments, the cell is contacted with the agent *ex vivo*. In some embodiments, the cells are then expanded and administered to the patient.

[0008] In a further aspect, the invention provides a method of treating a disease of
10 premature aging, the method comprising administering a cell modified in accordance with any method described herein to increase $\Delta 133p53$ expression or activity, or to inhibit miR-34a activity, to a patient that has a disease of premature aging. In some embodiments, the disease of premature aging is Werner syndrome or Hutchinson-Gilford progeria syndrome.

[0009] In another aspect, the invention provides a method of extending the replicative
15 lifespan of a cell of a subject that has a disease of premature aging, the method comprising contacting the cell with an agent that inhibits the function or expression of p53 β , thereby inhibiting cell senescence and extending the replicative lifespan of the cell. In some embodiments, the disease of premature aging is Werner syndrome. Alternatively, the disease of premature aging is Hutchinson-Gilford progeria syndrome. In some embodiments, the
20 agent comprises a polynucleotide sequence that inhibits p53 β , or comprises an expression cassette comprising a polynucleotide sequence that inhibits p53 β . In some embodiments, the cell, is a stem cell. In some embodiments, cell is a cardiac progenitor cell or an endothelial progenitor cell. In some embodiments, the cell is a bone marrow-derived progenitor cell. The cell may be contacted with the agent *in vivo*. In some embodiments, the cell is contacted
25 with the agent *ex vivo*.

[0010] In a further aspect, the invention thus provides a method of treating a disease of
premature aging, the method comprising administering a cell modified *ex vivo* to increase
expression of $\Delta 133p53$ or decrease expression of p53 β as described in herein to a patient that
has a disease of premature aging. The disease of premature aging may be Werner syndrome
30 or Hutchinson-Gilford progeria syndrome.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0011] Figure 1A-1D provides illustrative data showing that $\Delta 133p53$ protects Werner syndrome fibroblasts (AG00780) from senescence and extends their replicative lifespan. (1A) Expression of $\Delta 133p53$ isoform in AG00780 fibroblasts derived from a patient with
 5 Werner Syndrome (WS) resulted in: (1B) an increased lifespan and (1C, 1D) diminished premature senescence as shown by (1C) decreased SA- β -gal positive staining and (1D) lower expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine. Data are mean $\pm$ s.d. from three independent experiments. ** $P < 0.01$; *** $P < 0.001$
- [0012] Figure 2A-2D provides illustrative data showing that $\Delta 133p53$ protects WS
 10 fibroblasts (AG03141) from senescence and extends their replicative lifespan. (2A) Expression of $\Delta 133p53$ isoform in AG03141 derived from a patient with Werner Syndrome (WS) resulted in: (2B) increased lifespan; and (2C, 2D) diminished premature senescence as shown by (2C) decreased SA- β -gal positive cells and (2D) lower expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine. Data are mean $\pm$ s.d. from three
 15 independent experiments. ** $P < 0.01$; *** $P < 0.001$
- [0013] Figure 3A-3C provides illustrative data showing that $\Delta 133p53$ protects Werner Syndrome (WS) fibroblasts (AG12797) from senescence and extends their replicative lifespan. (3A) Expression of $\Delta 133p53$ isoform in AG12797 derived from a patient with Werner Syndrome (WS) resulted in: (3B) increased lifespan and (3C) diminished premature
 20 senescence as shown by decreased SA- β -gal staining in $\Delta 133p53$ -expressing cells. Data are mean $\pm$ s.d. from three independent experiments. *** $P < 0.001$
- [0014] Figure 4A-4B provides illustrative data showing that expression of $\Delta 133p53$ stabilizes full-length p53 (FL-p53), but inhibits the senescent-associated p53-target gene p21^{WAF1} in WS fibroblasts. Expression of $\Delta 133p53$ in cells derived from WS patients
 25 (AG00780, AG03141 and AG12797) resulted in (4A) accumulated full-length p53 (FL-p53) at the protein level and (4B) inhibition of p21^{WAF1} expression at the mRNA level. Data are mean $\pm$ s.d. from three independent experiments. *** $P < 0.001$
- [0015] Figure 5A-5B provides illustrative data showing that $\Delta 133p53$ is downregulated during passage of Hutchinson-Gilford Progeria Syndrome (HGPS) fibroblasts (AG11513).
 30 (5A) AG11513 proliferated for a limited number of population doublings (PDLs) in culture before they reached growth arrest. (5B) $\Delta 133p53$ protein levels were downregulated in late-

passage (passage 14) compared to early-passage (passage 7) AG11513 fibroblasts derived from a patient with Hutchinson-Gilford Progeria Syndrome (HGPS).

[0016] Figure 6A-6D provides illustrative data showing that $\Delta 133p53$ protects HGPS fibroblasts (AG11513) from senescence and extends their replicative lifespan. (6A)

5 Expression of $\Delta 133p53$ isoform in AG11513 derived from a patient with Hutchinson-Gilford Progeria Syndrome (HGPS) resulted in: (6B) increased lifespan and (6C, 6D) diminished premature senescence as shown by (6C) decreased SA- β -gal staining and (6D) lower expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine. Data are mean $\pm$ s.d. from three independent experiments. ** $P < 0.01$; *** $P < 0.001$

10 [0017] Figure 7A-7D provides illustrative data showing that $\Delta 133p53$ protects HGPS fibroblasts (AG01972) from senescence and extends their replicative lifespan. (7A) Expression of $\Delta 133p53$ isoform in AG01972 derived from a patient with Hutchinson-Gilford Progeria Syndrome (HGPS) resulted in: (7B) increased lifespan; and (7C, 7D) diminished premature senescence as shown by (7C) decreased SA- β -gal staining and (7D) lower
15 expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine. Data are mean $\pm$ s.d. from three independent experiments. ** $P < 0.01$; *** $P < 0.001$

[0018] Figure 8A-8C provides illustrative data showing that $\Delta 133p53$ dominant-negatively inhibits the senescence-associated p53 targets p21^{WAF1} and miR-34a, and stabilizes full-length p53 (FL-p53) and phosphorylated p53 at serine 15 (pS15-p53) in AG11513 (HGPS)
20 fibroblasts. Expression of $\Delta 133p53$ in AG11513 (HGPS) fibroblasts resulted in: (8A, 8B) inhibition of the senescence-associated p53 targets (8A) p21^{WAF1} and (8B) miR-34a compared to control-transduced cells as shown by quantitative RT-PCR; and (8C) accumulation of full-length p53 and phosphorylated p53 at serine 15 (pS15-p53) as shown by western blot using specific antibodies. 8A and 8B: control, left bar in each set; $\Delta 133p53$,
25 right bar in each set. Data are mean $\pm$ s.d. from three independent experiments. ** $P < 0.01$; *** $P < 0.001$

[0019] Figure 9A-9B provides illustrative data showing that expression of the DNA-repair gene RAD51 is upregulated in $\Delta 133p53$ -expressing HGPS cells. Expression of $\Delta 133p53$ in HGPS fibroblasts (AG11513 and AG01972) resulted in increased expression of the DNA
30 repair factor RAD51 (9A) at the mRNA level as shown by quantitative RT-PCR and (9B) at the protein level as shown by western blot. 9A: control, left bar in each set; $\Delta 133p53$, right bar in each set. Data are mean $\pm$ s.d. from three independent experiments. * $P < 0.05$

[0020] Figure 10A-10C provides illustrative data showing that expression of $\Delta 133p53$ ameliorates spontaneous DNA damage by increasing recruitment of RAD51 to γ H2AX-positive foci in HGPS fibroblasts. Cells were co-immunostained with antibodies against γ H2AX and RAD51. (10A) Representative images are shown. Scale bar= 10 μ m. (10B) Quantification of γ H2AX-positive DSBs was performed using the software ImagePremier Pro to detect and count the number of foci per cell. At least 100 cells were counted per experiment. (10C) RAD51 recruitment to γ H2AX-positive DSBs was quantified. γ H2AX-positive foci were counted and co-localization with RAD51 was scored as RAD51-positive (RAD51+ve) or RAD51-negative (RAD51-ve). Results are shown as percentage (%) of γ H2AX-positive foci. The area inside the columns represents RAD51-positive foci (grey) or RAD51-negative (white). Data are mean $\pm$ s.d. from three independent experiments. ** $P < 0.01$.

[0021] Figure 11 A-11D provide illustrative data showing that p53 β expression is associated with senescence of HGPS fibroblasts. (11A, 11B) AG11513 (HGPS) fibroblasts were analyzed at early passage (passage 10, P10) or senescence (passage 19, P19). (11A) Immunoblot to detect p53 β using the polyclonal rabbit serum TLQi9. (11B) Quantitative real time PCR (qRT-PCR) to analyze p53 β mRNA levels in the indicated cells. (11C, 11D) Overexpression of p53 β or a control vector in proliferative HGPS fibroblasts accelerated the onset of senescence in HGPS fibroblasts. (11C) Immunoblot to confirm overexpression of p53 β . (11D) Representative images and quantification of SA- β -gal staining. * $P < 0.05$; ** $P < 0.01$.

[0022] Figure 12 A-12E provides illustrative data showing that SRSF3 regulates p53 β expression during senescence of HGPS. (12A) AG11513 (HGPS) fibroblasts were analyzed at early passage (passage 10, P10) or senescence (passage 19, P19). Immunoblotting was used to detect SRSF3 expression. (12B-12E) Proliferative HGPS cells were transfected with one of two siRNAs targetting SRSF3 or a control siRNA. Cells were analyzed 72 hours after transfection. (12B) Immunoblotting was used to detect the indicated proteins in transfected cells. (12C, 12D) Quantitative real time PCR (qRT-PCR) of (12C) p53 β and (12D) the senescence-associated FLp53-target p21 mRNA expression. β -actin or B2M were used for normalization. (12E) Senescent cells were detected using SA- β -gal assay. Representative images and quantification of SA- β -gal staining are shown. A minimum of 100 cells were counted per assay. ** $P < 0.01$; *** $P < 0.001$.

DETAILED DESCRIPTION OF THE INVENTION

Terminology

[0023] The term “p53” refers generally to a protein of a molecular weight of about 53kDa on SDS PAGE that functions as a transcription factor and plays a role in cell cycle control and DNA damage repair as well as other essential pathways in cell growth. The protein and nucleic sequences of the p53 protein from a variety of organisms from humans to *Drosophila* are known and are available in public databases, such as in accession numbers, NM_000546, NP_000537, NM_011640, and NP_035770, for the human and mouse sequences. Mammalian p53 sequences are highly conserved between species. Mouse and human p53 proteins are 85% identical. In humans, p53 is encoded by the TP53 gene located on the short arm of chromosome 17 (17p13.1). A p53 protein in the context of this invention includes allelic variants and other functional variants and orthologs. In some embodiments, variants have at least 85%, at least 90%, or at least 95%, or greater, amino acid sequence identity across their whole sequence compared to a naturally occurring p53 family member, such as the polypeptide sequence listed under accession number NP_000537 (human p53). Generally, the same species of protein will be used with the species of cells being manipulated.

[0024] The term “Δ133p53” refers generally to the isoform of p53 that arises from initiation of transcription of the p53 gene from an alternative promoter in intron 4 of human p53, which results in an N-terminally truncated p53 protein translated from codon 133. A Δ133p53 isoform for use in this invention comprises the following p53 protein domains: the majority of the DNA binding domain, the NLS, and the C-terminal oligomerization domain (see Bourdon, *Brit. J. Cancer*, 97: 277-282 (2007)). An illustrative human Δ133p53 protein sequence is listed under accession number ABA29755.1.

[0025] The term “p53β” refers generally to the isoform of p53 that arises from alternative splicing of intron 9 to provide a p53 isoform comprising the following p53 protein domains: TAD1, TAD2, prD, the DNA binding domain, the NLS, and the C-terminal sequence DQTSFQKENC (see Bourdon, *Brit. J. Cancer*, 97: 277-282 (2007)).

[0026] The term “cell senescence” refers generally to the phenomenon where normal diploid differentiated cells lose the ability to divide after undergoing a finite number of cell divisions characteristic of a particular type of cell.

[0027] The term “replicative lifespan” refers generally to the finite number of cell divisions undergone by a particular cell type before undergoing cell senescence and losing the ability to further divide.

[0028] The term “extending replicative lifespan” refers generally to the continuation of cell division in a normal diploid cell beyond the finite number of cell divisions at which cell senescence would occur.

[0029] The term “inhibiting” as used, for example in the context of “inhibiting senescence” of a cell, refers generally to conditions or agents which reduce, decrease, hinder, down-regulate, or otherwise decrease cell senescence compared to a corresponding cell that is not treated with the agent.

[0030] As used herein, the term “expression” or “increasing expression” of $\Delta 133p53$ in the context of this invention often refers to introducing a $\Delta 133p53$ nucleic acid into a cell. In some embodiments, the level of expression of $\Delta 133p53$ may be increased using another agent that upregulates expression. An “increase” in $\Delta 133p53$ expression is generally determined relative to control cells to which an agent, *e.g.*, a nucleic acid encoding $\Delta 133p53$ or other agent that increases levels of $\Delta 133p53$, has not been added. Expression is typically considered to be increased when levels of $\Delta 133p53$ protein, or RNA, are increased by at least 20%, typically by at least 50%, or 100% or more. In some embodiments, expression levels may be two-fold higher than the control, or five-fold higher than the control, or greater than five-fold higher than the control. Expression of $\Delta 133p53$ can also be evaluated by functional analyses, such as measuring cellular senescence, *e.g.*, using a Senescence-Associated (SA)- β -Galactosidase assay to determine cellular senescence levels, or evaluating expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine.

[0031] As used herein, the term “decreasing” or “inhibiting” expression of $p53\beta$ in the context of this invention refers to decreasing the level of $p53\beta$ or activity in a cell. In some embodiments, the level of expression of $p53\beta$ may be decreased using a polynucleotide inhibitor of $p53\beta$. In some embodiments, another agent that down regulates expression is employed. A “decrease” in $p53\beta$ expression is generally determined relative to control cells to which an agent, *e.g.*, a nucleic acid encoding a polynucleotide inhibitor of $p53\beta$, has not been added. Expression is typically considered to be decreased when levels of $p53\beta$ protein or RNA, are decreased by at least 10% or at least 20%, typically by at least 50%, or 75% or

more. In some embodiments, expression levels may be two-fold lower than the control, or five-fold lower than the control, or greater than five-fold lower than the control. As noted above, in some embodiments, inhibited expression of p53 β can be assessed using functional analyses, such as measuring cellular senescence, *e.g.*, using a Senescence- Associated (SA)-
5 β -Galactosidase assay to determine cellular senescence levels, or evaluating expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine.

[0032] A polynucleotide sequence is "heterologous to" a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified by human action from its original form. For example, a promoter operably linked to a heterologous coding
10 sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is different from any naturally occurring allelic variants.

[0033] The term "exogenous" refers to a substance present in a cell or organism other than its native source. For example, the terms "exogenous nucleic acid" or "exogenous protein"
15 refer to a nucleic acid or protein that has been introduced by a process involving the hand of man into a biological system such as a cell or organism in which it is not normally found or in which it is found in lower amounts. A substance will be considered exogenous if it is introduced into a cell or an ancestor of the cell that inherits the substance. The term "endogenous" refers to a substance that is native to the biological system.

20 [0034] The term "identity" as used herein refers to the extent to which the sequence of two or more nucleic acids or polypeptides is the same. The percent identity between a sequence of interest and a second sequence over a window of evaluation, *e.g.*, over the length of the sequence of interest, may be computed by aligning the sequences, determining the number of residues (nucleotides or amino acids) within the window of evaluation that are opposite an
25 identical residue allowing the introduction of gaps to maximize identity, dividing by the total number of residues of the sequence of interest or the second sequence (whichever is greater) that fall within the window, and multiplying by 100. When computing the number of identical residues needed to achieve a particular percent identity, fractions are to be rounded to the nearest whole number. Percent identity can be calculated with the use of a variety of
30 computer programs. For example, computer programs such as BLAST2, BLASTN, BLASTP, Gapped BLAST, etc., generate alignments and provide percent identity between sequences of interest. The algorithm of Karlin and Altschul (Karlin and Altschul, Proc. Natl.

Acad. Sci. USA 87:22264-22268, 1990) modified as in Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993 is incorporated into the NBLAST and XBLAST programs of Altschul et al. (Altschul, et al., J. Mol. Biol. 215:403-410, 1990). To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (Altschul, et al. Nucleic Acids Res. 25: 3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs may be used. A PAM250 or BLOSUM62 matrix may be used. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI). See the Web site having URL world-wide web address of: "ncbi.nlm.nih.gov" for these programs.

10 In a specific embodiment, percent identity is calculated using BLAST2 with default parameters as provided by the NCBI.

[0035] The term "isolated" or "partially purified" as used herein refers, in the case of a nucleic acid or polypeptide, to a nucleic acid or polypeptide separated from at least one other component (e.g., nucleic acid or polypeptide) that is present with the nucleic acid or polypeptide as found in its natural source and/or that would be present with the nucleic acid or polypeptide when expressed by a cell, or secreted in the case of secreted polypeptides. A chemically synthesized nucleic acid or polypeptide or one synthesized using in vitro transcription/translation is considered "isolated".

15

[0036] The term "isolated cell" as used herein refers to a cell that has been removed from an organism in which it was originally found or a descendant of such a cell. An "isolated" cell may be cultured *in vitro* in the presence of other cells. Optionally, the cell is later introduced into a second organism or re-introduced into the organism from which it (or the cell from which it is descended) was isolated.

20

[0037] The term "vector" refers to a carrier DNA molecule into which a DNA sequence can be inserted for introduction into a host cell. In some embodiments, vectors of use in the invention are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". Thus, an "expression vector" is a specialized vector that contains the necessary regulatory regions needed for expression of a gene of interest in a host cell. In some embodiments the gene of interest is operably linked to another sequence in the vector, e.g., a promoter. Vectors include non-viral vectors such as plasmids and viral vectors.

25

30

[0038] The term "operably linked" refers to a functional linkage between a first nucleic acid sequence and a second nucleic acid sequence, such that the first and second nucleic acid sequences are transcribed into a single nucleic acid sequence. Operably linked nucleic acid sequences need not be physically adjacent to each other. The term "operably linked" also
5 refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a transcribable nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the transcribable sequence.

[0039] The term "viral vectors" refers to the use of viruses, or virus-associated vectors as
10 carriers of a nucleic acid construct into a cell. Constructs may be integrated and packaged into non-replicating, defective viral genomes like Adenovirus, Adeno-associated virus (AAV), or Herpes simplex virus (HSV) or others, including retroviral and lentiviral vectors, for infection or transduction into cells. The vector may or may not be incorporated into the cell's genome. The constructs may include viral sequences for transfection, if desired.
15 Alternatively, the construct may be incorporated into vectors capable of episomal replication, e.g., EPV and EBV vectors.

[0040] In the context of this invention, "transfection" is used to refer to any method of introducing nucleic acid molecules into a cell including both viral and non-viral techniques. The term as used here thus includes techniques such as transduction with a virus.

[0041] The terms "regulatory sequence" and "promoter" are used interchangeably herein, and refer to nucleic acid sequences, such as initiation signals, enhancers, and promoters, which induce or control transcription of protein coding sequences with which they are operatively linked. In some examples, transcription of a recombinant gene is under the control of a promoter sequence (or other transcriptional regulatory sequence) which controls
20 the expression of the recombinant gene in a cell- type in which expression is intended. It will also be understood that the recombinant gene can be under the control of transcriptional regulatory sequences which are the same or which are different from those sequences which control transcription of the naturally-occurring form of a protein. In some instances the promoter sequence is recognized by the synthetic machinery of the cell, or introduced
25 synthetic machinery, required for initiating transcription of a specific gene.
30

[0042] As used herein, "proliferating" and "proliferation" refer to an increase in the number of cells in a population (growth) by means of cell division. Cell proliferation is generally

understood to result from the coordinated activation of multiple signal transduction pathways in response to the environment, including growth factors and other mitogens. Cell proliferation may also be promoted by release from the actions of intra- or extracellular signals and mechanisms that block or negatively affect cell proliferation.

5 [0043] The terms "nucleic acid" and "polynucleotide" are used interchangeably herein to refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and
10 which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0044] Unless otherwise indicated, a particular nucleic acid sequence also encompasses
15 conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J.*
20 *Biol. Chem.* 260:2605-2608 (1985); Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)).

[0045] Amino acids can be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, can be referred to by their commonly accepted single-letter codes.

25 [0046] "Conservatively modified variants" as used herein applies to amino acid sequences. One of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a
30 chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of

the invention. The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

Overview of aspects of the disclosure

[0047] The disclosure is based, in part on the discovery that the naturally occurring p53 isoform $\Delta 133p53$ extends the replicative lifespan of cells in patients who have diseases of premature aging. Premature aging disease or conditions are those in which aspects of the aging process, e.g., external signs of old age, is greatly accelerated. Children affected with a disease of premature aging develop external signs of old age, including baldness, hunched posture, and dry, inelastic, and wrinkled skin. However, in contrast to normal aging, the ovaries or testes are inactive, resulting in sterility. Affected children are also unusually short. There are several premature aging syndromes. In Hutchinson-Gilford syndrome and Werner syndrome, the central nervous system and therefore the ability to do many daily activities are largely unaffected unless a stroke occurs. Thus, although premature aging diseases mimic some aspects of normal aging, the aging processes also exhibit marked differences.

[0048] In some embodiments, the invention thus provides methods of extending replicative life-span in cells of patients that have a pre-mature aging syndrome, e.g., WS or HGPS, by increasing the level or activity of $\Delta 133p53$ in cells of such patients. Increasing the level or activity of $\Delta 133p53$ may be achieved using any number of methods, including, but not limited to, introducing a polynucleotide encoding $\Delta 133p53$ into cells, e.g., an expression vector that expresses $\Delta 133p53$, and/or using an agent that upregulates $\Delta 133p53$ expression.

[0049] In some embodiments, cells from a patient may be modified *ex vivo* and then re-introduced into the patient. In some embodiments, cells that are modified *ex vivo* for reintroduction into the patient are stem cells or progenitor cells, such as cardiac progenitor cells, bone marrow-derived stem cells, or endothelial progenitor cells.

[0050] In some embodiments, the patient may be treated with an agent that up-regulates $\Delta 133p53$ expression and/or activity. In some embodiments, such an agent targets a cellular autophagy degradation pathway.

[0051] In further embodiments, the invention provides methods of identifying agents that increase $\Delta 133p53$ levels and/or activity in cells of patients having a disease of pre-mature aging, *e.g.*, WS or HGPS, where the method comprises screening candidate small molecule compounds for the ability up-regulate $\Delta 133p53$ expression or activity in WS and/or HGPS cells.

[0052] The disclosure is additionally based, in part on the discovery that expression of the naturally occurring p53 isoform p53 β is associated with senescence in cells of patients who have a premature aging disease.

[0053] In some embodiments, the disclosure thus provides methods of extending replicative life-span in cells of patients that have a pre-mature aging syndrome, *e.g.*, WS or HGPS, by decreasing the level or activity of p53 β in cells of such patients. Decreasing the level or activity of p53 β may be achieved using any number of methods, including, but not limited to, introducing a polynucleotide that inhibits expression of p53 β into cells and/or using an agent that downregulates p53 β expression.

[0054] In some embodiments, cells from a patient may be modified *ex vivo* to inhibit expression of p53 β and then re-introduced into the patient. In some embodiments, cells that are modified *ex vivo* for reintroduction into the patient are stem cells or progenitor cells, such as cardiac progenitor cells, bone marrow-derived stem cells, or endothelial progenitor cells.

[0055] In some embodiments, the patient may be treated with an agent that down-regulates p53 β expression and/or activity.

[0056] In further embodiments, the invention provides methods of identifying agents that decrease p53 β levels and/or activity in cells of patients having a disease of pre-mature aging, *e.g.*, WS or HGPS, where the method comprises screening candidate small molecule compounds for the ability down-regulate p53 β expression or activity in WS and/or HGPS cells.

Methods for expressing $\Delta 133p53$

[0057] Nucleic acid sequences encoding $\Delta 133p53$ and related nucleic acid sequence homologues can be cloned from cDNA libraries or are typically isolated using amplification techniques with oligonucleotide primers.

[0058] In typical embodiments, the cloning of $\Delta 133p53$, or other p53 isoforms, or desired genes, can employ the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (see U.S. Patents 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain
5 reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of $\Delta 133p53$ directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Genes obtained via an amplification reaction can be purified and cloned into an appropriate vector.

[0059] The nucleic acids encoding $\Delta 133p53$ are typically cloned into intermediate vectors
10 before transformation into eukaryotic cells for replication and/or expression. These intermediate vectors are typically prokaryote vectors, *e.g.*, plasmids, or shuttle vectors. The isolated nucleic acids encoding $\Delta 133p53$ or other p53 isoforms may also encode interspecies homologues, alleles and polymorphic variants of the illustrative $\Delta 133p53$ described herein.

[0060] Expression of $\Delta 133p53$ (or of other proteins that it may be desirable to express as
15 described herein) is performed using a variety of techniques. Basic texts disclosing general methods that can be employed in this invention include Green and Sambrook (2012) *Molecular Cloning: A laboratory manual* 4th ed. Cold Spring Harbor Laboratory Press; and *Current Protocols in Molecular Biology* and supplements through supplement 110, 2015) John Wiley and Sons.

20 *Expression vectors*

[0061] Any number of vectors may be employed, including plasmid and viral vectors, to introduce a nucleic acid encoding $\Delta 133p53$ into cells.

[0062] In some embodiments, a nucleic acid construct encoding $\Delta 133p53$ is a plasmid-based vector (*e.g.*, “naked” DNA).

[0063] In some embodiments a nucleic acid construct encoding $\Delta 133p53$ is contained
25 within a viral vector and administered as a virus. Viral delivery systems include adenovirus vectors, adeno-associated viral vectors, herpes simplex viral vectors, retroviral vectors, pox viral vectors, lentiviral vectors, alphavirus vectors, poliovirus vectors, and other positive and negative stranded RNA viruses, viroids, and virusoids, or portions thereof. One of skill in the
30 arts understands that any number of methods of constructing and using such vectors can be employed.

[0064] For example, recombinant viruses in the pox family of viruses can be used for delivering the nucleic acid molecules encoding $\Delta 133p53$. These include vaccinia viruses and avian poxviruses, such as the fowlpox and canarypox viruses. Illustrative methods for generating these viruses using genetic recombination can be found, *e.g.* in WO 91/12882; 5 WO 89/03429; WO 92/03545; and n US Patent No. 5,863,542. Representative examples of recombinant pox viruses include ALVAC, TROVAC, and NYVAC.

[0065] A number of adenovirus vectors, including, for example, Ad2, Ad5, and Ad7, can also be used to deliver a nucleic acid that encodes $\Delta 133p53$ (Haj-Ahmad and Graham, J. Virol. (1986) 57:267-274; Bett et al., J. Virol. (1993) 67:5911-5921; Mittereder et al., Human 10 Gene Therapy (1994) 5:717-729; Seth et al., J. Virol. (1994) 68:933-940; Barr et al., Gene Therapy (1994) 1:51-58; Berkner, K. L. BioTechniques (1988) 6:616-629; and Rich et al., Human Gene Therapy (1993) 4:461-476). Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. These vectors can be readily constructed. See, *e.g.*, U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication 15 Nos. WO 92/01070 (published 23 Jan. 1992) and WO 93/03769 (published 4 Mar. 1993); Lebkowski et al., Molec. Cell. Biol. (1988) 8:3988-3996; Vincent et al., Vaccines 90 (1990) (Cold Spring Harbor Laboratory Press); Carter, B. J. Current Opinion in Biotechnology (1992) 3:533-539; Muzyczka, N. Current Topics in Microbiol. and Immunol. (1992) 158:97-129; Kotin, R. M. Human Gene Therapy (1994) 5:793-801; Shelling and Smith, Gene 20 Therapy (1994) 1:165-169; and Zhou et al., J. Exp. Med. (1994) 179:1867-1875.

[0066] Retroviruses also provide a platform for gene delivery systems. A number of retroviral systems have been described (U.S. Pat. No. 5,219,740; Miller and Rosman, BioTechniques (1989) 7:980-990; Miller, A. D., Human Gene Therapy (1990) 1:5-14; Scarpa et al., Virology (1991) 180:849-852; Burns et al., Proc. Natl. Acad. Sci. USA (1993) 25 90:8033-8037; and Boris-Lawrie and Temin, Cur. Opin. Genet. Develop. (1993) 3:102-109. Additional gene delivery systems include lentiviral vectors that employ lentiviral vector backbones. Thus, in some embodiments, a nucleic acid encoding $\Delta 133p53$ may be introduced into cells from a patient having a disease of pre-mature aging using a lentiviral vector.

30 [0067] Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, can also be used as viral vectors to deliver a nucleic acid encoding $\Delta 133p53$. For a description of illustrative

Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., J. Virol. (1996) 70:508-519; and International Publication Nos. WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr., T. W., et al., U.S. Pat. No. 5,843,723, issued Dec. 1, 1998, and Dubensky, Jr., T. W., U.S. Pat. No. 5,789,245, issued Aug. 4, 1998).

- 5 **[0068]** As appreciated by one of skill in the art, the techniques used to obtain $\Delta 133p53$ nucleic acids and introduce them into cells may also be used for other nucleic acids as described herein that are employed to extend replicative lifespan of cells from patients with diseases of premature aging.

Agents that up-regulate $\Delta 133p53$

- 10 **[0069]** In some embodiments, an agent that up-regulates $\Delta 133p53$, *i.e.*, that increases the level and/or activity of $\Delta 133p53$ in a cell, is administered to a subject that has a disease of premature aging, *e.g.*, WS or HGPS. Such agents are often small organic molecules. As used herein, a “small organic molecule” refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 daltons and less than about 2500
15 daltons, preferably less than about 2000 daltons, preferably between about 100 to about 1000 daltons, more preferably between about 200 to about 500 daltons.

- [0070]** In some embodiments, the agent may be an agent that targets selective autophagy, the mechanism by which $\Delta 133p53$ is degraded in cells of a patient that has a premature aging syndrome, *e.g.*, WS or HGPS, to reduce the selective $\Delta 133p53$ autophagy, thereby increasing
20 cellular $\Delta 133p53$ levels.

- [0071]** In some embodiments, an agent that up-regulates $\Delta 133p53$ in cells from subjects that have a premature aging disease, *e.g.*, WS or HGPS, may be an agent that increases the level or activity of a protein that interacts with $\Delta 133p53$, such as STUB1 (STIP1 homology and U-Box-containing protein 1, also referred to as CHIP, carboxy terminus of Hsp70-
25 interacting protein). STUB1 has been mapped to 16p33 on human chromosome 16. Protein and nucleic acid sequences for human CHIP are known, as are structural features of the protein that are important to activity. For example, it has been shown that STUB1 has intrinsic E3 ubiquitin ligase activity and promotes ubiquitylation in an *in vitro* ubiquitylation assay with recombinant proteins (Jiang *et al.*, J. Biol. Chem. 276:42938-42944, 2001). This
30 activity was dependent on the C-terminal U box.

Inhibition of endogenous p53 β expression

[0072] In some aspects of the disclosure, it is desirable to disrupt endogenous expression of p53 β . Inhibitory nucleic acids to p53 β such as siRNA, shRNA, ribozymes, or antisense molecules, can be synthesized and introduced into cells using methods known in the art.

5 Molecules can be synthesized chemically or enzymatically *in vitro* (Micura, *Agnes Chem. Int. Ed. Emgl.* 41: 2265–9 (2002); Paddison *et al.*, *Proc. Natl. Acad. Sci. USA*, 99: 1443–8 (2002)) or endogenously expressed inside the cells in the form of shRNAs (Yu *et al.*, *Proc. Natl. Acad. Sci. USA*, 99: 6047–52 (2002); McManus *et al.*, *RNA* 8, 842–50 (2002)). Plasmid-based expression systems using RNA polymerase III U6 or H1, or RNA polymerase II U1,
10 small nuclear RNA promoters, have been used for endogenous expression of shRNAs (Brummelkamp *et al.*, *Science*, 296: 550–3 (2002); Sui *et al.*, *Proc. Natl. Acad. Sci. USA*, 99: 5515–20 (2002); Novarino *et al.*, *J. Neurosci.*, 24: 5322–30 (2004)). Synthetic siRNAs can be delivered by electroporation or by using lipophilic agents (McManus *et al.*, *RNA* 8, 842–50 (2002); Kishida *et al.*, *J. Gene Med.*, 6: 105–10 (2004)). Alternatively, plasmid systems
15 can be used to stably express small hairpin RNAs for the suppression of target genes (Dykxhoorn *et al.*, *Nat. Rev. Mol. Biol.*, 4: 457–67 (2003)). Various viral delivery systems have been developed to deliver shRNA-expressing cassettes into cells that are difficult to transfect (Brummelkamp *et al.*, *Cancer Cell*, 2: 243–7 (2002); Robinson *et al.*, *Nat. Genet.*, 33: 401–6 (2003)). Furthermore, siRNAs can also be delivered into live animals. (Hasuwa *et al.*, *FEBS Lett.*, 532, 227–30 (2002); Carmell *et al.*, *Nat. Struct. Biol.*, 10: 91–2 (2003);
20 Kobayashi *et al.*, *J. Pharmacol. Exp. Ther.*, 308: 688–93 (2004)).

[0073] Methods for the design of siRNA or shRNA target sequences have been described in the art. Among the factors to be considered include: siRNA target sequences should be specific to the gene of interest and have ~20–50% GC content (Henshel *et al.*, *Nucl. Acids*
25 *Res.*, 32: 113–20 (2004); G/C at the 5' end of the sense strand; A/U at the 5' end of the antisense strand; at least 5 A/U residues in the first 7 bases of the 5' terminal of the antisense strand; and no runs of more than 9 G/C residues (Ui-Tei *et al.*, *Nucl. Acids Res.*, 3: 936–48 (2004)). Additionally, primer design rules specific to the RNA polymerase will apply. For example, for RNA polymerase III, the polymerase that transcribes from the U6 promoter, the
30 preferred target sequence is 5'-GN18-3'. Runs of 4 or more Ts (or As on the other strand) will serve as terminator sequences for RNA polymerase III and should be avoided. In addition, regions with a run of any single base should be avoided (Czauderna *et al.*, *Nucl. Acids Res.*, 31: 2705–16 (2003)). It has also been generally recommended that the mRNA

target site be at least 50–200 bases downstream of the start codon (Sui *et al.*, *Proc. Natl. Acad. Sci. USA*, 99: 5515–20 (2002); Elbashir *et al.*, *Methods*, 26: 199–213 (2002); Duxbury and Whang, *J. Surg. Res.*, 117: 339–44 (2004) to avoid regions in which regulatory proteins might bind. Additionally, a number of computer programs are available to aid in the design of suitable siRNA and shRNAs for use in the practice of this invention.

[0074] Ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy target mRNAs, particularly through the use of hammerhead ribozymes. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. Preferably, the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art.

[0075] Gene targeting ribozymes necessarily contain a hybridizing region complementary to two regions, each of at least 5 and preferably each 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleotides in length of a target mRNA, *e.g.*, a p53 β mRNA. In addition, ribozymes possess highly specific endoribonuclease activity, which autocatalytically cleaves the target sense mRNA.

[0076] With regard to antisense, siRNA or ribozyme oligonucleotides, phosphorothioate oligonucleotides can be used. Modifications of the phosphodiester linkage as well as of the heterocycle or the sugar may provide an increase in efficiency. Phosphorothioate is used to modify the phosphodiester linkage. An N3'-P5' phosphoramidate linkage has been described as stabilizing oligonucleotides to nucleases and increasing the binding to RNA. Peptide nucleic acid (PNA) linkage is a complete replacement of the ribose and phosphodiester backbone and is stable to nucleases, increases the binding affinity to RNA, and does not allow cleavage by RNase H. Its basic structure is also amenable to modifications that may allow its optimization as an antisense component. With respect to modifications of the heterocycle, certain heterocycle modifications have proven to augment antisense effects without interfering with RNase H activity. An example of such modification is C-5 thiazole modification. Finally, modification of the sugar may also be considered. 2'-O-propyl and 2'-methoxyethoxy ribose modifications stabilize oligonucleotides to nucleases in cell culture and *in vivo*.

[0077] One of skill understands that inhibitory nucleic acids that target p53 β can be introduced into cells using principles and methods as described above for the introducing of Δ 133p53 nucleic acids into cells.

[0078] Inhibitory oligonucleotides can be delivered to a cell by direct transfection or
5 transfection and expression via an expression vector. Appropriate expression vectors include mammalian expression vectors and viral vectors, *e.g.*, the illustrative vectors described for delivery of Δ 133p53 polynucleotides, into which has been cloned an inhibitory
oligonucleotide with the appropriate regulatory sequences including a promoter to result in
expression of the RNA in a host cell. Suitable promoters can be constitutive or development-
10 specific promoters. In some embodiments in which polynucleotides that inhibit p53 β are delivered into the cell, mammalian expression vector can be employed. For example, mammalian expression vectors suitable for siRNA expression are commercially available, for example, from Ambion (*e.g.*, pSilencer vectors), Austin, TX; Promega (*e.g.*, GeneClip, siSTRIKE, SiLentGene), Madison, WI; Invitrogen, Carlsbad, CA; InvivoGen, San Diego,
15 CA; and Imgenex, San Diego, CA. In some embodiments, inhibitory RNA sequences are delivered into cells via a viral expression vector. Viral vectors suitable for delivering such molecules to cells include those described above, *e.g.*, adenoviral vectors, adeno-associated vectors, and retroviral vectors (including lentiviral vectors).

Agents that down-regulate p53 β

[0079] In some embodiments, an agent that down-regulates p53 β , *i.e.*, that decreases the
20 level and/or activity of p53 β in a cell, is administered to a subject that has a disease of premature aging, *e.g.*, WS or HGPS. Such agents are often small organic molecules. As used herein, a “small organic molecule” refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 daltons and less than about 2500
25 daltons, preferably less than about 2000 daltons, preferably between about 100 to about 1000 daltons, more preferably between about 200 to about 500 daltons.

[0080] In some embodiments, cells can be treated with an agent that increases SRSF3
expression in cells of patients with disease of premature aging to inhibit endogenous p53 β . SRSF3, previously named as SRp20, is the smallest member of the highly conserved
30 serine/arginine-rich splicing factor family and modulates the splicing of numerous genes. It has been shown that down regulation of SRSF3 elevates p53 β levels (Tang *et al.*, *Oncogene*

32:2792-2798, 2013). In some embodiments, increasing expression of SRSF3 can be achieved by introducing an expression construct that expresses SRSF3, preferably human SRSF3, into cells of patients having a disease of premature aging.

Additional agents to extend the replicative lifespan

5 [0081] In some embodiments, an agent that inhibits the function or expression of miR-34a may be used to extend the replicative lifespan of cells from a subject that have a premature aging disease, *e.g.*, WS or HGPS. An exemplary agent useful for this purpose is an antisense oligonucleotide that specifically inactivates the microRNA miR-34a (anti-miR34a).

[0082] MicroRNAs (miRNAs) are endogenous short, *e.g.*, about 21 nucleotides in length,
10 RNA molecules that regulate gene expression post-transcriptionally by interacting with protein-encoding messenger RNAs. miRNAs are similar to short interfering RNAs (siRNAs) in size and function; however, unlike siRNAs, miRNAs are endogenous transcripts encoded by the genome that function to regulate endogenous gene expression. miRNAs possess antisense activity that negatively regulates the expression of genes with sequences that are
15 complementary to specific miRNAs. The term “miR-34a” refers to the mature miRNA having the sequence UGGCAGUGUCUUAGCUGGUUGU.

[0083] Examples of nucleic acids that specifically inactivate miR-34a include antisense oligonucleotides. The antisense oligonucleotides may be ribonucleotides or deoxyribonucleotides. In some embodiments, the oligonucleotides may comprise peptide
20 nucleic acids. In some embodiments, the oligonucleotide comprises one or more modified nucleotides, such as 2'-O-methyl-sugar modifications. In some embodiments, such inactivating oligonucleotides comprise only modified nucleotides. The oligonucleotides may also contain one or more modifications to the phosphodiester linkage, *e.g.*, may contain one or more phosphorothioate linkages to provide a partial or complete phosphorothioate
25 backbone. Other modifications to enhance stability and improve efficacy may also be employed (see, *e.g.*, U.S. Patent No. 6,838,283). In some embodiments, the antagonist oligonucleotide may be linked to a cholesterol moiety at its 3' end for use *in vivo*. Antagonizing oligonucleotides for inhibiting miR-34a may be about 14 to about 50 nucleotides in length, about 14 to about 30 nucleotides in length, and 14 to about 25
30 nucleotides in length. In some embodiments, an oligonucleotide may be partially complementary, *i.e.*, the oligonucleotide need to be 100% complementary to the target nucleotide. Typically, the oligonucleotide is at least 75%, 80%, 85%, or 90%, or greater,

e.g., 95%, or 100%, complementary to the target sequence, *e.g.*, to the mature miR-34a sequence.

[0084] In some embodiments, an inhibitory nucleic acid used for inhibiting miR-34a may be an inhibitory RNA molecule, such as a ribozyme, siRNAs, or shRNA. The region of the inhibitory RNA that has sequence identity to mature miR-34a typically has 100% identity to the mature sequence, but may also have less than 100% identity, *e.g.*, at least 75%, 80%, 85%, 90%, or 95%, identity to the mature miR-34a sequence. Any number of methods of generating such inhibitory RNA molecules can be used. As explained above in the context of antisense RNAs, the oligonucleotides may contain one or more modifications of the phosphodiester linkage as well as of the heterocycle or the sugar to provide an increase in efficiency.

[0085] As explained above regarding delivery of p53 β inhibitory polynucleotides, inhibitory oligonucleotides can be delivered to a cell by direct transfection or transfection and expression via an expression vector. Appropriate expression vectors include mammalian expression vectors and viral vectors, *e.g.*, the illustrative vectors described for delivery of Δ 133p53 polynucleotides, into which has been cloned an inhibitory oligonucleotide with the appropriate regulatory sequences including a promoter to result in expression of the RNA in a host cell. Suitable promoters can be constitutive or development-specific promoters. In some embodiments in which polynucleotides that inhibit miR-34a are delivered into the cell, mammalian expression vector can be employed. For example, mammalian expression vectors suitable for siRNA expression are commercially available, for example, from Ambion (*e.g.*, pSilencer vectors), Austin, TX; Promega (*e.g.*, GeneClip, siSTRIKE, SiLentGene), Madison, WI; Invitrogen, Carlsbad, CA; InvivoGen, San Diego, CA; and Imgenex, San Diego, CA. In some embodiments, inhibitory RNA sequences are delivered into cells via a viral expression vector. Viral vectors suitable for delivering such molecules to cells include those described above, *e.g.*, adenoviral vectors, adeno-associated vectors, and retroviral vectors (including lentiviral vectors).

Assays to test activity of 133p53 or p53 β

[0086] Agents can be tested for the ability to up-regulate Δ 133p53 or down-regulate p53 β using any measure of Δ 133p53 or p53 β RNA or protein expression or protein activity. In some embodiments, other endpoints may be measured to assess the functional effect of a candidate agent. "Functional effects" include *in vitro*, *in vivo*, and *ex vivo* activities. For

example, any of a number of methods for the determination and measurement of cell senescence or cell proliferation can be used. In some embodiments, direct measurements of cell proliferation by counting cells may be employed. Other markers of cellular proliferation, *e.g.*, cell markers that are expressed in proliferating cells, such PCNA, or a marker for
5 cellular metabolism such as MTT (*see, e.g.*, Hughes, D., *Cell proliferation and apoptosis*, Taylor & Francis Ltd, UK (2003)), may also be used to assess cell proliferation and replicative lifespan.

[0087] A number of markers for cell senescence may be used to monitor senescence in the practice of this invention. A common marker is senescence-associated- β -galactoside (Dimri,
10 G. P. *et al.*, *Proc. Natl. Acad. Sci. USA* 92:9363 (1995)), although others markers of cell senescence may also be employed. For example senescence can be measured by direct measurement of telomere length by in situ hybridization or by measurement of age-dependent cellular accumulation of lipofuscin in cells (Coates, *J. Pathol.*, 196: 371-3 (2002)). Other markers of senescence include SAHF senescence-associated heterochromatin foci (SAHF)
15 (*see, e.g.*, *Methods Mol Biol.* 965:185–196, 2013).

[0088] Up-regulation of $\Delta 133p53$ is achieved when the measured value relative to the control (untreated with agents to increase $\Delta 133p53$ level or activity) is at least 110%, typically at least 120% or at least 150%, or at least 200-at least 500% (*i.e.*, at least two to at least five fold higher relative to the control) in those assays that measure $\Delta 133p53$ protein or
20 RNA levels, or that measure an endpoint that is increased when $\Delta 133p53$ is increased, *e.g.*, replicative lifespan. For assays that measure endpoint markers that are decreased upon increased activity or levels of $\Delta 133p53$, *e.g.*, markers of cellular senescence, up-regulation of $\Delta 133p53$ is achieved when the activity value relative to the control is decreased by at least 10% or at least 20%, typically at least 50%, 100%, or 200% or more.

25 [0089] Similarly, an agent that down-regulates $p53\beta$ may also be assessed for functional activity using cell senescence assays, assays for replicative lifespan, or other endpoints as described herein to identify agents that decrease $p53\beta$. Down-regulation of $p53\beta$ is achieved when the measured value relative to the control (untreated with an agent that decreases the level or activity of $p53\beta$) is typically reduced by at least 10%, more often at least 20%, 50%,
30 75%, 80%, or greater, in those assays that measure $p53\beta$ protein or RNA levels, or that measure an endpoint that is increased when $p53\beta$ is inhibited, *e.g.*, replicative lifespan. For assays that measure endpoint markers that are increased upon down-regulation of $p53\beta$, *e.g.*,

replicative lifespan, inhibition of p53 β is achieved when the value relative to the control is increased by at least 110%, typically at least 120% or at least 150%, or at least 200-at least 500%.

[0090] Similarly, an agent that inhibits miR-34a may also be assessed for functional activity using cell senescence assays, assays for replicative lifespan, or other endpoints as described herein to identify agents that decrease miR-34a.

[0091] The term “test compound” or “drug candidate” or grammatical equivalents as used herein describes any molecule, either naturally occurring or synthetic, *e.g.*, protein, oligopeptide (*e.g.*, from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, peptide, circular peptide, lipid, fatty acid, inhibitory RNAs, polynucleotide, oligonucleotide, etc., to be tested for the capacity to directly or indirectly up-regulate $\Delta 133p53$. The test compound can be in the form of a library of test compounds, such as a combinatorial or randomized library that provides a sufficient range of diversity. Test compounds are optionally linked to a fusion partner, *e.g.*, targeting compounds, rescue compounds, dimerization compounds, stabilizing compounds, addressable compounds, and other functional moieties. Conventionally, new chemical entities with useful properties are generated by identifying a test compound with some desirable property or activity, *e.g.*, increasing $\Delta 133$ levels and/or activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput screening (HTS) methods are employed for such an analysis. Such assay formats are well-known in the art.

Administration of agents

[0092] Any number of cell types may be targeted in a patient that has a disease of premature aging. For example, in some embodiments, stem cells of a patient that has a disease of premature aging, *e.g.*, WS or HGPS, may be treated with an agent or otherwise modified to overexpress $\Delta 133p53$. In some embodiments, the cells are cardiac stem or progenitor cells or endothelial progenitor cells. In some embodiments, bone marrow-derived stem/progenitor cells may be treated or otherwise modified to overexpress $\Delta 133p53$.

[0093] In some embodiments of the disclosure, stem cells of a patient that has a disease of premature aging, *e.g.*, WS or HGPS, may be treated with an agent or otherwise modified to

decrease expression of p53 β . In some embodiments, the cells are cardiac stem or progenitor cells or endothelial progenitor cells. In some embodiments, bone marrow-derived stem/progenitor cells may be treated or otherwise modified to decrease expression of p53 β .

Vector Delivery and Cell Transformation

5 [0094] Any suitable method for nucleic acid delivery for transformation of a cell or a tissue of a subject that has a disease of premature aging, e.g., WS or HGPS, can be used in the current invention. Such methods include, but are not limited to, direct delivery of nucleic acids, DNA or RNA, such as by *ex vivo* transfection (Wilson et al., *Science*, 244:1344-1346, 1989, Nabel and Baltimore, *Nature* 326:711-713, 1987), optionally with Eugene6 (Roche) or
10 Lipofectamine (Invitrogen), by injection (U.S. Pat. Nos. 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection and gene gun injection (Harland and Weintraub, *J. Cell Biol.*, 101:1094-1099, 1985; U.S. Pat. No. 5,789,215, incorporated herein by reference); by electroporation (U.S. Pat. No. 5,384,253, incorporated herein by reference);
15 Tur-Kaspa et al., *Mol. Cell Biol.*, 6:716-718, 1986; Potter et al., *Proc. Nat'l Acad. Sci. USA*, 81:7161-7165, 1984); by calcium phosphate precipitation (Graham and Van Der Eb, *Virology*, 52:456-467, 1973; Chen and Okayama, *Mol. Cell Biol.*, 7(8):2745-2752, 1987; Rippe et al., *Mol. Cell Biol.*, 10:689-695, 1990); by using DEAE-dextran followed by polyethylene glycol (Gopal, *Mol. Cell Biol.*, 5:1188-1190, 1985); by direct sonic loading
20 (Fechheimer et al., *Proc. Nat'l Acad. Sci. USA*, 84:8463-8467, 1987); by liposome mediated transfection (Nicolau and Sene, *Biochim. Biophys. Acta*, 721:185-190, 1982; Fraley et al., *Proc. Nat'l Acad. Sci. USA*, 76:3348-3352, 1979; Nicolau et al., *Methods Enzymol.*, 149:157-176, 1987; Wong et al., *Gene*, 10:87-94, 1980; Kaneda et al., *Science*, 243:375-378, 1989; Kato et al., *J Biol. Chem.*, 266:3361-3364, 1991) and receptor-mediated transfection (Wu and
25 Wu, *Biochemistry*, 27:887-892, 1988; Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987); each incorporated herein by reference). Further, any combination of such methods may be employed. As explained above, nucleic acids may also be delivered using viral vector systems in which the nucleic acids are delivered in viral particles that infect the desired cells.

[0095] For transfection, a composition comprising one or more nucleic acid molecules
30 (within or without vectors) can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid

molecules are described, for example, in Gilmore, *et al.*, *Curr Drug Delivery* (2006) 3:147-5 and Patil, *et al.*, *AAPS Journal* (2005) 7:E61-E77, each of which are incorporated herein by reference. Delivery of inhibitory RNA molecules is also described in several U.S. Patent Publications, including for example, 2006/0019912; 2006/0014289; 2005/0239687; 5 2005/0222064; and 2004/0204377, the disclosures of each of which are hereby incorporated herein by reference. Nucleic acid molecules can be administered to cells by a variety of methods, including, but not restricted to, encapsulation in liposomes, by iontophoresis, by electroporation, or by incorporation into other vehicles, including biodegradable polymers, hydrogels, cyclodextrins (*see, for example* Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 10 1068-1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (*see for example* U.S. Pat. No. 6,447,796 and US Patent Application Publication No. 2002/130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). In another 15 embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives.

[0096] Examples of liposomal transfection reagents of use with this invention include, for 20 example: CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,N₁,N₂,N₃,N₄-tetramethyl-N,N₁,N₂,N₃,N₄-tetrapalmit-γ-spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-tri-methyl-ammoniummethylsulfate) (Boehringer Mannheim); Lipofectamine, 3:1 (M/M) liposome 25 formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL); and (5) siPORT (Ambion); HiPerfect (Qiagen); X-treme GENE (Roche); RNAicARRIER (Epoch Biolabs) and TransPass (New England Biolabs).

[0097] Nucleic acids for administration to a subject are formulated for pharmaceutical 30 administration. While any suitable carrier known may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, including intranasal, intradermal, subcutaneous or intramuscular injection or electroporation, the carrier preferably comprises water, saline, and optionally an alcohol, a fat, a polymer, a wax, one or more stabilizing amino acids or a

buffer. General formulation technologies are known to those of skill in the art (see, for example, Remington: The Science and Practice of Pharmacy (20th edition), Gennaro, ed., 2000, Lippincott Williams & Wilkins; Injectable Dispersed Systems: Formulation, Processing And Performance, Burgess, ed., 2005, CRC Press; and Pharmaceutical
5 Formulation Development of Peptides and Proteins, Frkjr et al., eds., 2000, Taylor & Francis).

[0098] Nucleic acid compositions, *e.g.*, polynucleotides encoding $\Delta 133p53$, can be administered once or multiple times. Multiple administrations can be administered, for example, bi-weekly, weekly, bi-monthly, monthly, or more or less often, as needed, for a
10 time period sufficient to achieve the desired response.

[0099] The nucleic acid constructs in accordance with the invention are administered to a mammalian host. The mammalian host usually is a human or a primate. In some embodiments, the mammalian host can be a domestic animal, for example, canine, feline, lagomorpha, rodentia, rattus, hamster, murine. In other embodiment, the mammalian host is
15 an agricultural animal, for example, bovine, ovine, porcine, equine, etc.

[0100] Compositions comprising nucleic acids that encode $\Delta 133p53$, or a nucleic acid that inhibits miR-34a, or a nucleic acid that inhibits $p53\beta$ can be formulated in accordance with standard techniques well known to those skilled in the pharmaceutical art. Such compositions can be administered in dosages and by techniques well known to those skilled
20 in the medical arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient, and the route of administration.

[0101] In therapeutic applications, the nucleic acids may be administered in an amount sufficient to elicit a therapeutic effect that at least partially arrests or slows one or more symptoms and/or complications of a disease of pre-mature aging, *e.g.*, WS or HGPS. An
25 amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition of the vaccine regimen administered, the manner of administration, the stage and severity of the disease, the general state of health of the patient, and the judgment of the prescribing physician.

[0102] The amounts and formulations depend on various factors, including delivery
30 methods. In embodiments that employ a naked nucleic acid composition, the dose of a naked nucleic acid composition is from about 1.0 ng to about 10 mg for a patient. Subcutaneous or

intramuscular doses for naked nucleic acid (typically DNA encoding a fusion protein) may range from 0.1 ug to 100 ug for a subject. For example, naked DNA or polynucleotide in an aqueous carrier can be injected into tissue, *e.g.*, intramuscularly or intradermally, in amounts of from 10 μ l per site to about 1 ml per site. The concentration of polynucleotide in the formulation is usually from about 0.1 μ g/ml to about 5 mg/ml. In some embodiments, the DNA may be administered in ng amounts, for example at a level of 1 to 100 ng. For example, the dose is 0.1 μ g, 0.5 μ g, 1 μ g, 1.5 μ g, 2 μ g, 3 μ g, 4 μ g, 5 μ g, 6 μ g, 7 μ g, 8 μ g, 9 μ g, 10 μ g, 15 μ g, 20 μ g, 25 μ g, 30 μ g, 35 μ g, 40 μ g, 45 μ g, 50 μ g, or 60 μ g of nucleic acids per dose. In a specific embodiment, the dose is in the range of 10 ng to 100 mg, or 50 ng to 100 mg, or 100 ng to 100 mg of nucleic acids per dose. In some specific embodiments, the dose is in the range of 10 pg to 100 mg, or 50 pg to 100 mg, or 100 pg to 100 mg, or 100 pg to 100 ng of nucleic acids per dose.

[0103] In some embodiments, a nucleic acid encoding Δ 133p53, or an inhibitory nucleic acid that targets miR-34a, or inhibits p53 β , is delivered using a viral delivery system in which virus particles that comprise the nucleic acid are introduced into the recipient.

[0104] In some embodiments, a nucleic acid encoding Δ 133p53 is introduced into cells of a subject having a disease of premature aging, *e.g.*, WS or HGPS, *ex vivo*. The modified cells may then be cultured, and if desired expanded, and introduced back into the patient. Examples of cells that may be modified *ex vivo* include stem cells, endothelial progenitor cells (*e.g.*, Yoder Human Endothelial Progenitor Cells, *Cold Spring Harb Perspect Med* 2012;2:a006692) and cardiac progenitor cells (*e.g.*, U.S. Patent Application Publication No. 20140274765), or bone marrow-derived stem cells and progenitor cells (see, *e.g.*, Lévesque et al, *Handb Exp Pharmacol.* 180:3-36, 2007; Granick et al., *Bone Marrow Res.* Article ID 165107, 2012).

[0105] For other agents that increase expression of Δ 133p53, the amount of the therapeutic agent that will be effective in the prevention, treatment and/or management of a disease of premature aging such as WS or HGPS can be determined by standard clinical techniques. In vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed will also depend, *e.g.*, on the route of administration, the type of symptoms, and the seriousness of the symptoms, and should be decided according to the judgment of the practitioner and each patient's or subject's circumstances.

- [0106] In some embodiments, a nucleic acid that inhibits p53 β , or a nucleic acid that inhibits miR-34a, is introduced into cells of a subject having a disease of premature aging, e.g., WS or HGPS, *ex vivo*. The modified cells may then be cultured, and if desired expanded, and introduced back into the patient. Examples of cells that may be modified *ex vivo* include stem cells, endothelial progenitor cells (e.g., Yoder Human Endothelial Progenitor Cells, *Cold Spring Harb Perspect Med* 2012;2:a006692) and cardiac progenitor cells (e.g., U.S. Patent Application Publication No. 20140274765), or bone marrow-derived stem cells and progenitor cells (see, e.g., Lévesque et al, *Handb Exp Pharmacol.* 180:3-36, 2007; Granick et al., *Bone Marrow Res.* Article ID 165107, 2012).
- [0107] For other agents that decrease expression of p53 β , or that inhibit miR-34a, the amount of the therapeutic agent that will be effective in the prevention, treatment and/or management of a disease of premature aging such as WS or HGPS can be determined by standard clinical techniques. In vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed will also depend, e.g., on the route of administration, the type of symptoms, and the seriousness of the symptoms, and should be decided according to the judgment of the practitioner and each patient's or subject's circumstances.

EXAMPLES

- [0108] The effects of expression of Δ 133p53 on cellular senescence were evaluated in fibroblasts from a Werner syndrome patient were evaluated. The results (Figure 1A-1D) demonstrated that Δ 133p53 protected Werner syndrome fibroblasts (AG00780) from senescence and extended their replicative lifespan. Similar results were obtained using fibroblasts from two additional Werner Syndrome patients (Figures 2A-2D and 3A-3C).
- [0109] Additional analyses of WS fibroblasts demonstrated that expression of Δ 133p53 stabilized full-length p53 (FL-p53), but inhibited the senescent-associated p53-target gene p21^{WAF1} (Figure 4A-4B).
- [0110] It was also demonstrated that Δ 133p53 was downregulated during passage of Hutchinson-Gilford Progeria Syndrome (HGPS) fibroblasts (AG11513) (Figure 5A-5B). These experiments showed that AG11513 proliferated for a limited number of population doublings (PDLs) in culture before they reached growth arrest. Further, Δ 133p53 protein

levels were downregulated in late-passage (passage 14) compared to early-passage (passage 7) AG11513 fibroblasts.

[0111] Expression of the $\Delta 133p53$ isoform in fibroblasts AG11513 and AG01972 derived from HGPS patients also resulted in increased lifespan and diminished premature senescence, as shown by decreased SA- β -gal staining and lower expression of IL-6, a senescence-associated secretory phenotype (SASP) cytokine (Figures 6A-6D and 7A-7D).

[0112] It was also demonstrated that expression of $\Delta 133p53$ in AG11513 (HGPS) fibroblasts resulted in inhibition of the senescence-associated p53 targets p21^{WAF1} and miR-34a compared to control-transduced cells as shown by quantitative RT-PCR, and in accumulation of full-length p53 and phosphorylated p53 at serine 15 (pS15-p53) as shown by western blot using specific antibodies (Figure 8A-8C).

[0113] Further analysis of $\Delta 133p53$ -expressing HGPS cells showed that expression of the DNA-repair gene RAD51 is upregulated (Figure 9A-9B). Expression of $\Delta 133p53$ in HGPS fibroblasts (AG11513 and AG01972) resulted in increased expression of the DNA repair factor RAD51 at the mRNA level as shown by quantitative RT-PCR and at the protein level as shown by western blot.

[0114] It was additionally demonstrated that expression of $\Delta 133p53$ ameliorates spontaneous DNA damage by increasing recruitment of RAD51 to γ H2AX-positive foci in HGPS fibroblasts (Figure 10A-10C). Fibroblasts derived from an HGPS patient (cell strain AG11513) transduced with the lentivirus constructs indicated in Figure 10A-10C were analyzed at passage 13.

[0115] The expression of p53 β expression was also analyzed. It was shown that p53 β expression was associated with senescence of HGPS fibroblasts (Figure 11A-11D). Additional experiments demonstrated SRSF3 regulated p53 β expression during senescence of HGPS (Figure 12A-12E).

Summary

[0116] These examples provide illustrative data showing that expression of $\Delta 133p53$ is almost undetectable in nearly-senescent WS and HGPS fibroblasts; and that reconstitution of $\Delta 133p53$ expression restored cell proliferation and increased replicative lifespan of WS and HGPS cells. Importantly, $\Delta 133p53$ expression significantly diminished the senescent

phenotypes of WS and HGPS cells, as shown by decreased senescence-associated- β -galactosidase staining, as well as decreased expression of the senescence-associated secretory cytokine IL-6, in $\Delta 133p53$ -expressing cells compared to control cells.

[0117] A zebrafish ortholog of $\Delta 133p53$ was recently reported to have gain-of-function roles in transactivating DNA repair genes in response to DNA damage. Thus, we hypothesized that expression of $\Delta 133p53$ might contribute to DNA repair of chronic DNA damage lesions in HGPS cells. The studies described in this example also showed that the DNA repair gene RAD51 is upregulated at the mRNA and protein level in $\Delta 133p53$ -expressing HGPS cells.

[0118] Overall, these studies show that manipulation of $\Delta 133p53$ expression can control cell proliferation and senescence in premature aging fibroblasts from patients suffering from WS or HGPS. Additionally, these studies demonstrate that p53 β expression is associated with senescence in HGPS fibroblasts.

Methods

[0119] *Cell Culture and transductions.* Fibroblasts derived from Werner Syndrome (WS) or Hutchinson-Gilford Progeria Syndrome (HGPS) were obtained from Coriell Cell Repository. Cells were grown in complete growth DMEM or MEM (Life Technologies) medium supplemented with 10% fetal bovine serum (Gibco), 2 mM glutamine (Life Technologies) and 50 IU/ml penicillin/streptomycin (Invitrogen). Population doubling levels were calculated as $\log_{10}(\text{number of cells counted after expansion}) - \log_{10}(\text{number of cells seeded})/\log_{10}2$.

[0120] *Lentiviral Transductions.* Cells were incubated with lentiviral constructs containing $\Delta 133p53$ cDNA or a vector-control sequence in complete growth media for 24h. Two days after infection, cells were selected using blasticidin (5 $\mu\text{g/ml}$, Life Technologies) for approximately 10 days or until all untransformed cells were dead.

[0121] *SA- β -gal assay.* Cellular senescence was examined using the Senescence-Associated (SA)- β -Galactosidase Staining Kit (Cell Signaling) per the manufacturer's instructions.

[0122] *Real-time qRT-PCR.* RNA samples were prepared using a miReasy micro kit (QIAGEN) according to the manufacturer's instructions. TaqMan Gene Expression Assay

(Applied Biosystems) was used with the following set of probe and primers purchased from Applied Biosystems: *IL-6* (Hs00174131_m1), *p21* (also known as *CDKN1A*, Hs99999142_m1), *miR-34a-5p* (cat. no. 000426) and *RAD51* (Hs00153418_m1). The endogenous control for mRNA expression was *B2M* (β -2-microglobulin, cat. no. 4333766) and for microRNA was RNU66 (cat. no. 001002). Quantitative data analysis was performed using the $\Delta\Delta C_t$ method.

[0123] *Immunoblot Analysis.* Cells were lysed using RIPA buffer (Cell Signaling) with protease and phosphatase inhibitors (Thermo Scientific) and immunoblotted using Primary antibodies used were as follows: MAP4 (1:7,500; a rabbit polyclonal antibody raised with a mixture of peptides MFCQLAKTC and FCQLAKTCP corresponding to the N terminus of human $\Delta 133p53$ protein) for $\Delta 133p53$; DO-1 (1:2,000; Santa Cruz Biotechnology) for p53FL; AC-15 (1:10,000; Sigma-Aldrich) for β -actin; P-p53S15 (1:1,000; Cell Signaling) for phosphorylated p53; RAD51 (1:1,000, Abcam). Horseradish peroxidase-conjugated goat anti-mouse (1:5,000) or anti-rabbit (1:5,000) antibodies (Santa Cruz Biotechnology) were used as secondary antibodies. Signals were detected according to standard procedures using ECL detection (Amersham Biosciences) or SuperSignal West Dura Extended Duration system (Pierce Biotechnology). Quantitative analysis of the immunoblot data was performed using ImageJ 1.42q software (see, website at [http address rsb.info.nih.gov/ij/](http://rsb.info.nih.gov/ij/)).

[0124] *Immunostaining and quantification of double strand breaks (DSBs).* Cells previously plated onto coverslips were washed with PBS, fixed for 5-10 min on ice-cold methanol at -20°C and subsequently washed with PBS. Cells were permeabilized with 0.25% Triton-X for 5 min on ice, washed with PBS and then blocked in 5% bovine serum albumin (BSA) for 1 h at room temperature. Cells were incubated with primary antibodies overnight at 4°C . The primary antibodies used were: anti- γH2AX against phosphorylated H2AX (mouse, monoclonal) and anti-RAD51 (rabbit, polyclonal) at a dilution of 1:2000 and 1:1000, respectively. Cells were washed with PBS before incubation for 1h with the the following secondary antibody: Alexa-568-conjugated anti-mouse and Alexa-488-conjugated anti-rabbit at a dilution of 1:400 (Life Technologies). 4',6-diamidino-2-phenylindole was used to stain the nucleus. Coverslips were mounted on to slides with FluorSave mounting medium (Chemicon). A Zeiss LSM 780 confocal microscope was used to take images. Quantification of γH2AX -positive DSBs was performed using the automated software ImagePremier Pro to detect and count nuclear γH2AX -positive foci. Co-localization of γH2AX -positive DSBs

with RAD51 was quantified and DSBs were scored as RAD51-positive (RAD51+ve) or RAD51-negative (RAD51-ve). At least 100 cells were counted per experiment.

[0125] Additional experiments were performed as described in the Description of the Figures.

5

[0126] The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

[0127] All publications, patents, accession numbers, and patent applications cited in this
10 specification are hereby incorporated herein by reference in their entirety for their disclosures of the subject matter in whose connection they are cited herein.

WHAT IS CLAIMED IS:

- 1 1. A method of extending the replicative lifespan of a cell of a subject
2 that has a disease of premature aging, the method comprising contacting the cell with an
3 agent that activates the function or expression of $\Delta 133p53$, thereby inhibiting cell senescence
4 and extending the replicative lifespan of the cell.
- 1 2. The method of claim 1, wherein the disease of premature aging is
2 Werner syndrome.
- 1 3. The method of claim 1, wherein the disease of premature aging is
2 Hutchinson-Gilford progeria syndrome.
- 1 4. The method of claim 1, 2, or 3, wherein the agent comprises a
2 polynucleotide sequence encoding $\Delta 133p53$.
- 1 5. The method of claim 1, 2, or 3, wherein the agent comprises an
2 expression cassette comprising a polynucleotide sequence encoding $\Delta 133p53$.
- 1 6. The method of any one of claims 1 to 5, wherein the cell is a stem cell.
- 1 7. The method of any one of claims 1 to 5, wherein the cell is a cardiac
2 progenitor cell or an endothelial progenitor cell.
- 1 8. The method of any one of claims 1 to 5, wherein the cell is a bone
2 marrow-derived progenitor cell.
- 1 9. The method of any one of the preceding claims, wherein the cell is
2 contacted with the agent *ex vivo*.
- 1 10. A method of extending the replicative lifespan of a cell of a subject
2 that has a disease of premature aging, the method comprising contacting the cell with an
3 agent that inhibits the function or expression of miR-34a, thereby inhibiting cell senescence
4 and extending the replicative lifespan of the cell.
- 1 11. The method of claim 10, wherein the disease of premature aging is
2 Werner syndrome.

- 1 12. The method of claim 10, wherein the disease of premature aging is
2 Hutchinson-Gilford progeria syndrome.
- 1 13. The method of claim 10, 11, or 12, wherein the agent comprises a
2 polynucleotide sequence that inhibits miR-34a.
- 1 14. The method of claim 10, 11, or 12, wherein the agent comprises an
2 expression cassette comprising a polynucleotide sequence that inhibits miR-34a.
- 1 15. The method of any one of claims 10 to 14, wherein the cell is a stem
2 cell.
- 1 16. The method of any one of claims 10 to 14, wherein the cell is a cardiac
2 progenitor cell or an endothelial progenitor cell.
- 1 17. The method of any one of claims 10 to 14, wherein the cell is a bone
2 marrow-derived progenitor cell.
- 1 18. The method of any one of claims 10 to 17, wherein the cell is
2 contacted with the agent *ex vivo*.
- 1 19. A method of treating a disease of premature aging, the method
2 comprising administering a cell modified by the method of claim 9 or 18 to a patient that has
3 a disease of premature aging.
- 1 20. The method of claim 19, wherein the disease of premature aging is
2 Werner syndrome.
- 1 21. The method of claim 19, wherein the disease of premature aging is
2 Hutchinson-Gilford progeria syndrome.
- 1 22. A method of extending the replicative lifespan of a cell of a subject
2 that has a disease of premature aging, the method comprising contacting the cell with an
3 agent that inhibits the function or expression of p53 β , thereby inhibiting cell senescence and
4 extending the replicative lifespan of the cell.
- 1 23. The method of claim 22, wherein the disease of premature aging is
2 Werner syndrome.

1 24. The method of claim 22, wherein the disease of premature aging is
2 Hutchinson-Gilford progeria syndrome.

1 25. The method of claim 22, 23, or 24, wherein the agent comprises a
2 polynucleotide sequence that inhibits p53 β .

1 26. The method of claim 22, 23, or 24, wherein the agent comprises an
2 expression cassette comprising a polynucleotide sequence that inhibits p53 β .

1 27. The method of any one of claims 22 to 26, wherein the cell is a stem
2 cell.

1 28. The method of any one of claims 22 to 26, wherein the cell is a cardiac
2 progenitor cell or an endothelial progenitor cell.

1 29. The method of any one of claims 22 to 26, wherein the cell is a bone
2 marrow-derived progenitor cell.

1 30. The method of The method of any one of claims 22 to 29, wherein the
2 cell is contacted with the agent *ex vivo*.

1 31. A method of treating a disease of premature aging, the method
2 comprising administering a cell modified by the method of claim 30 to a patient that has a
3 disease of premature aging.

1 32. The method of claim 31, wherein the disease of premature aging is
2 Werner syndrome.

1 33. The method of claim 31, wherein the disease of premature aging is
2 Hutchinson-Gilford progeria syndrome.

1

Figure 1A-1D

AG00780: WRN gene mutation (1336C>T, Arg368Ter)

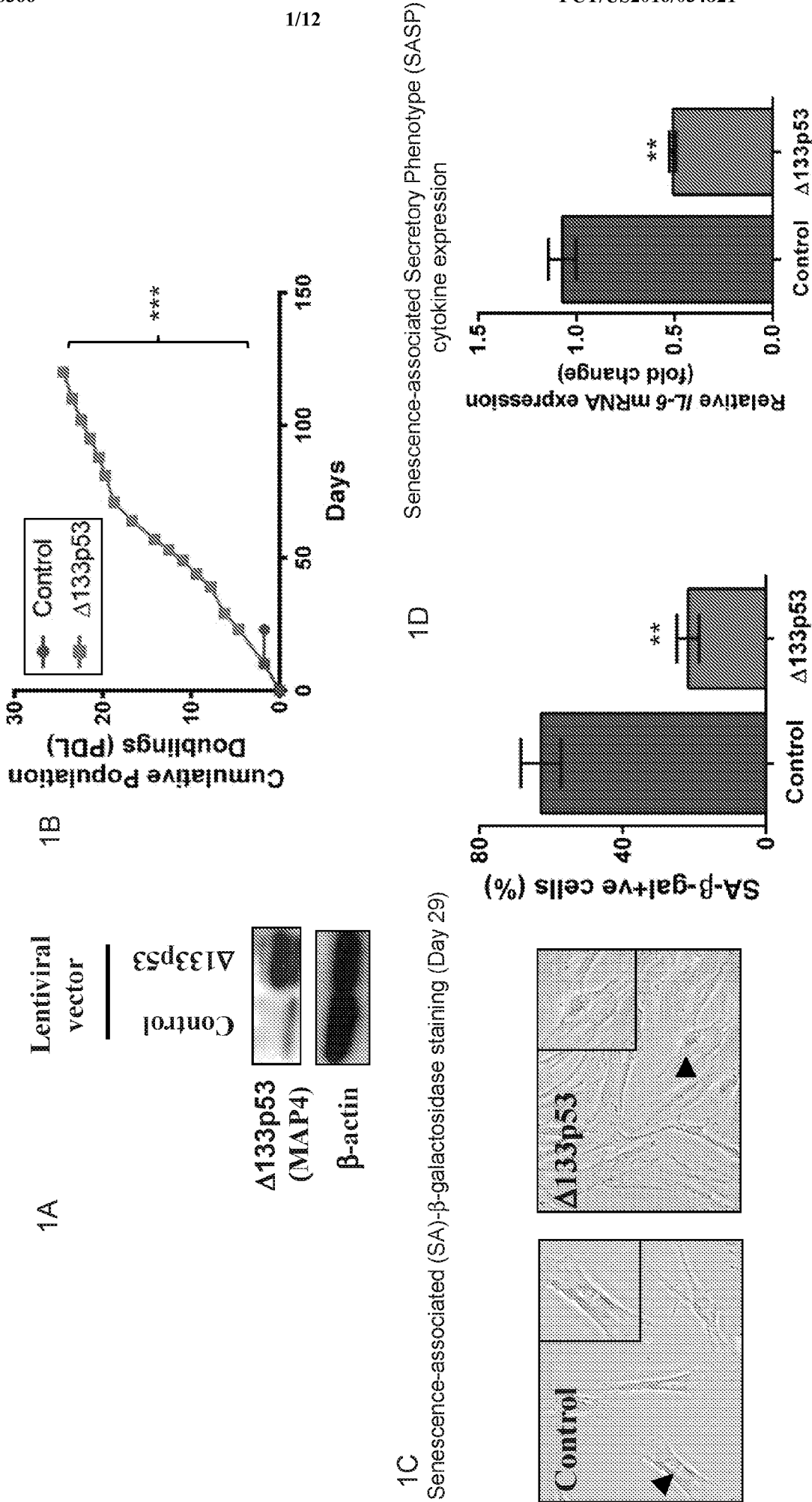


Figure 2A-2D

AG03141: WRN gene mutation (2476C>T, Gln748Ter)

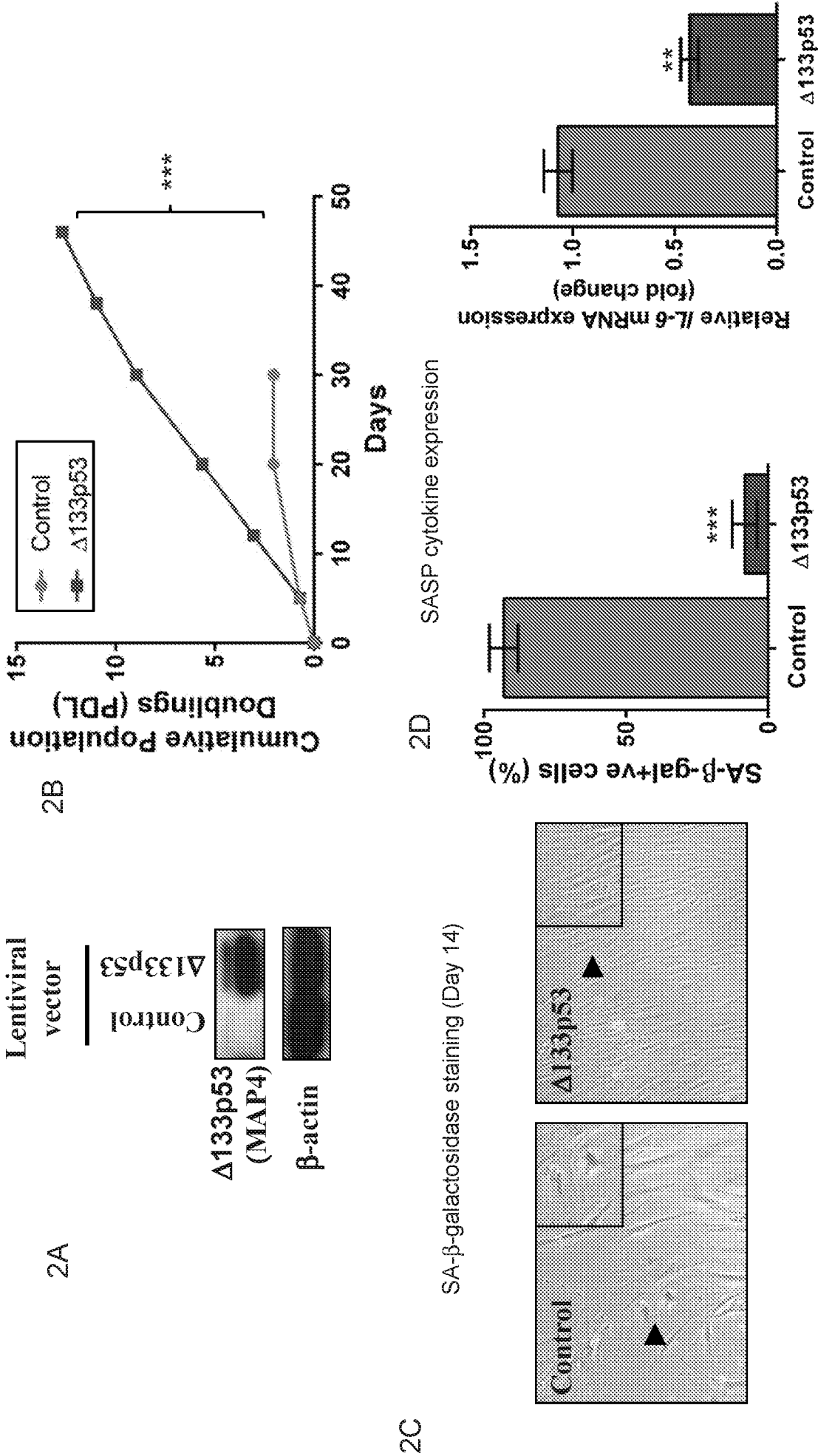
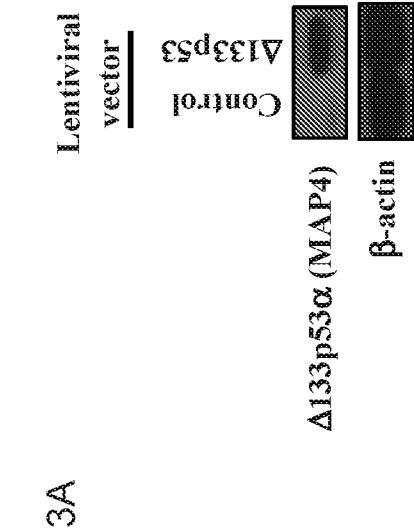
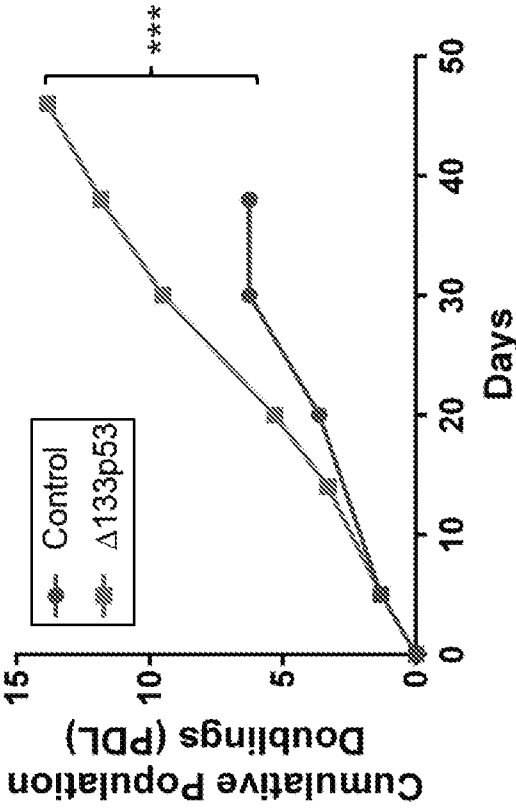


Figure 3A-3C

AG12797: WRN gene mutation (3139G>C)



SA-β-galactosidase staining (Day 30)

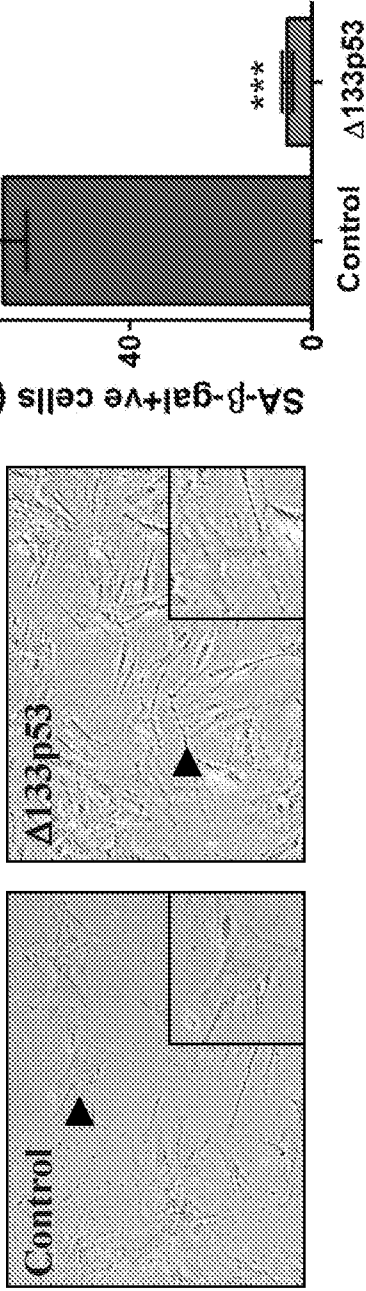
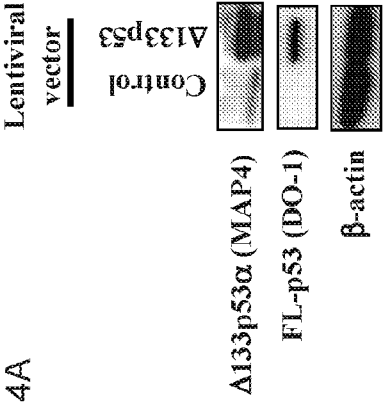
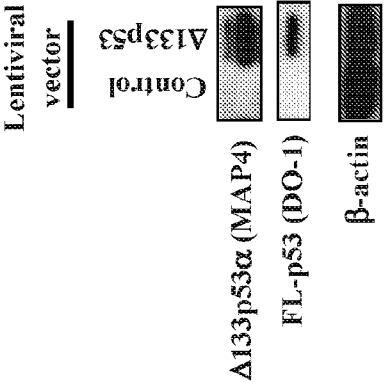


Figure 4A-4B

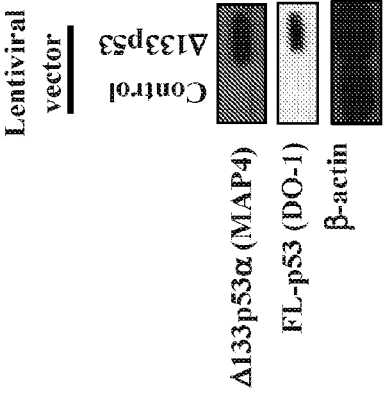
AG00780
(*WRN* mutation, 1336C>T, Arg368Ter)



AG03141
(*WRN* mutation, 2476C>T, Gln748Ter)



AG12797
(*WRN* mutation, 3139G>C)



4/12

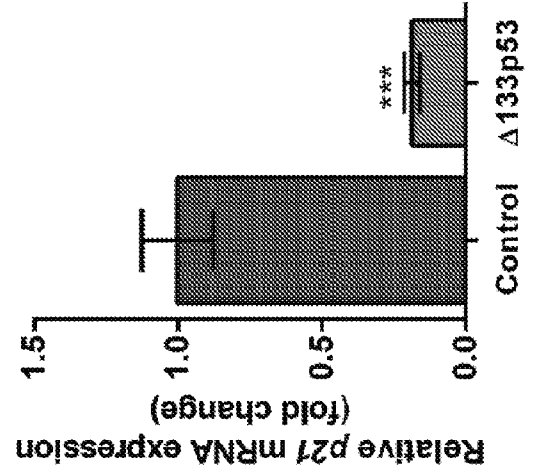
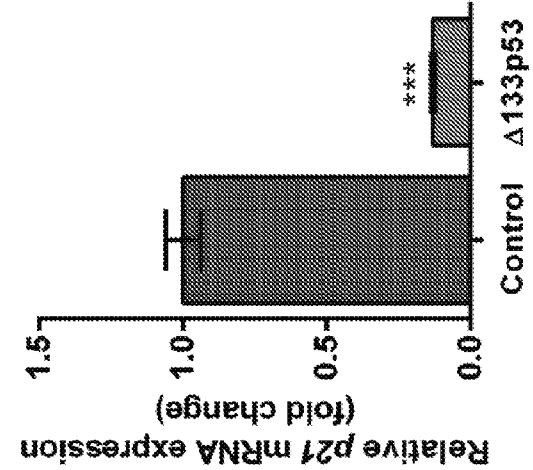
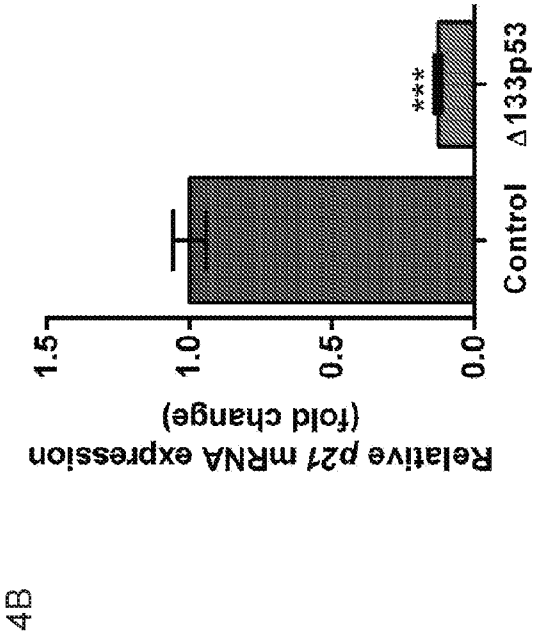


Figure 5A-5B

AG11513: Lamin A gene mutation (2036C>T, Deletion of 50 amino acids)

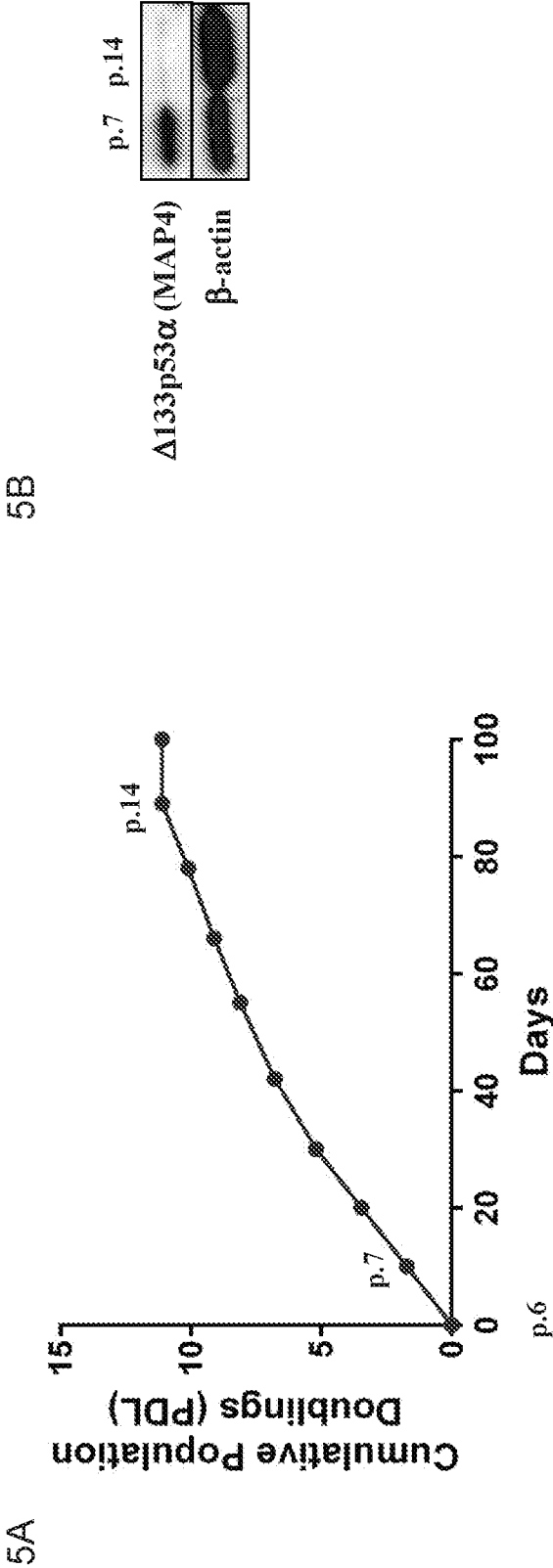
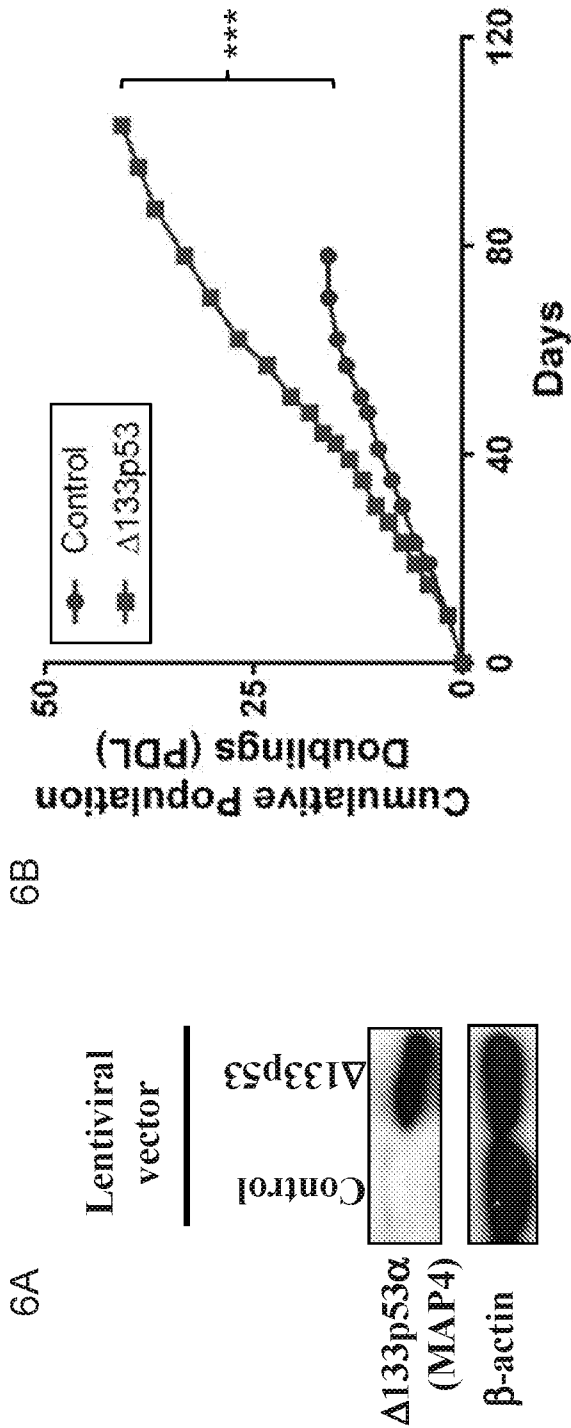


Figure 6A-D

AG11513: Lamin A gene mutation (2036C>T, Deletion of 50 amino acids)



6C SA- β -galactosidase staining (Day 48)

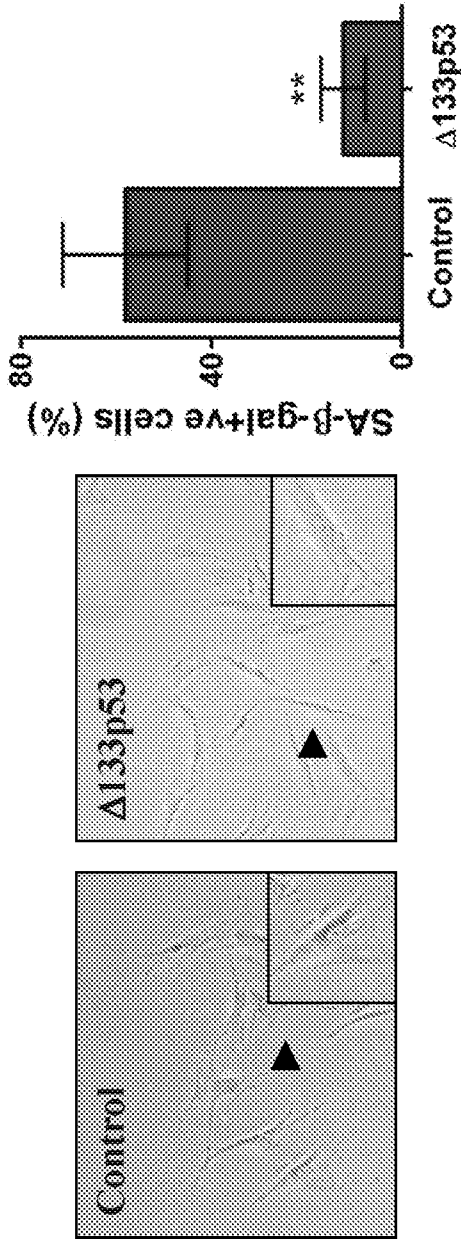


Figure 7A-7D

AG01972: Lamin A gene mutation (2036C>T, Deletion of 50 amino acids)

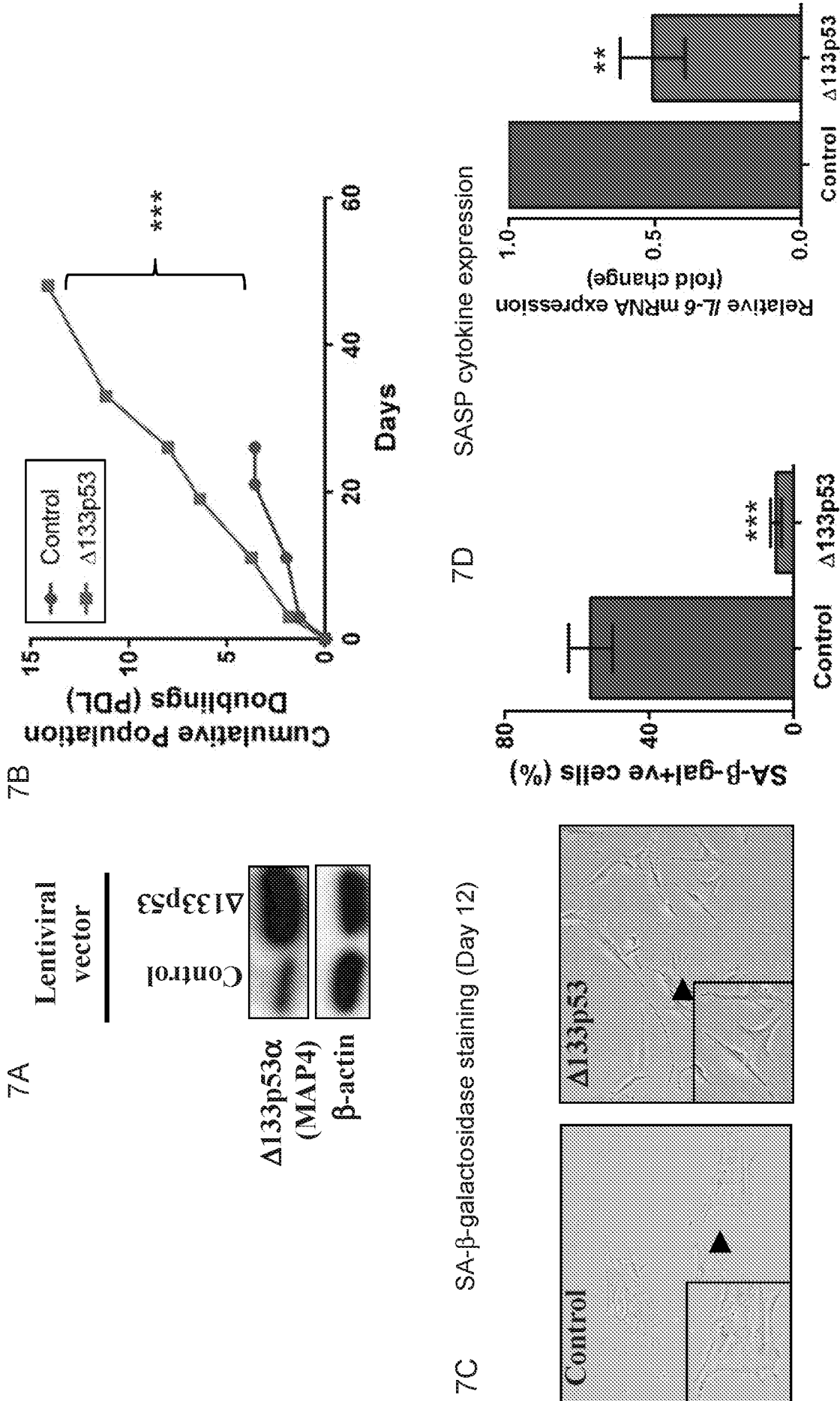


Figure 8A-8C

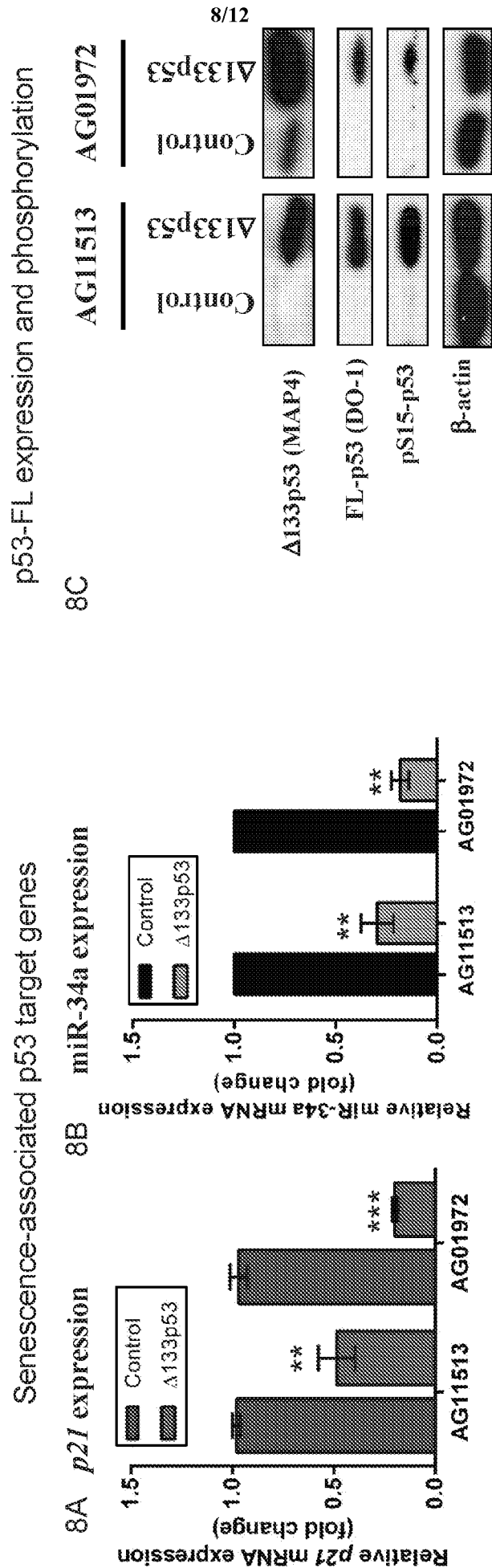


Figure 9A-9B

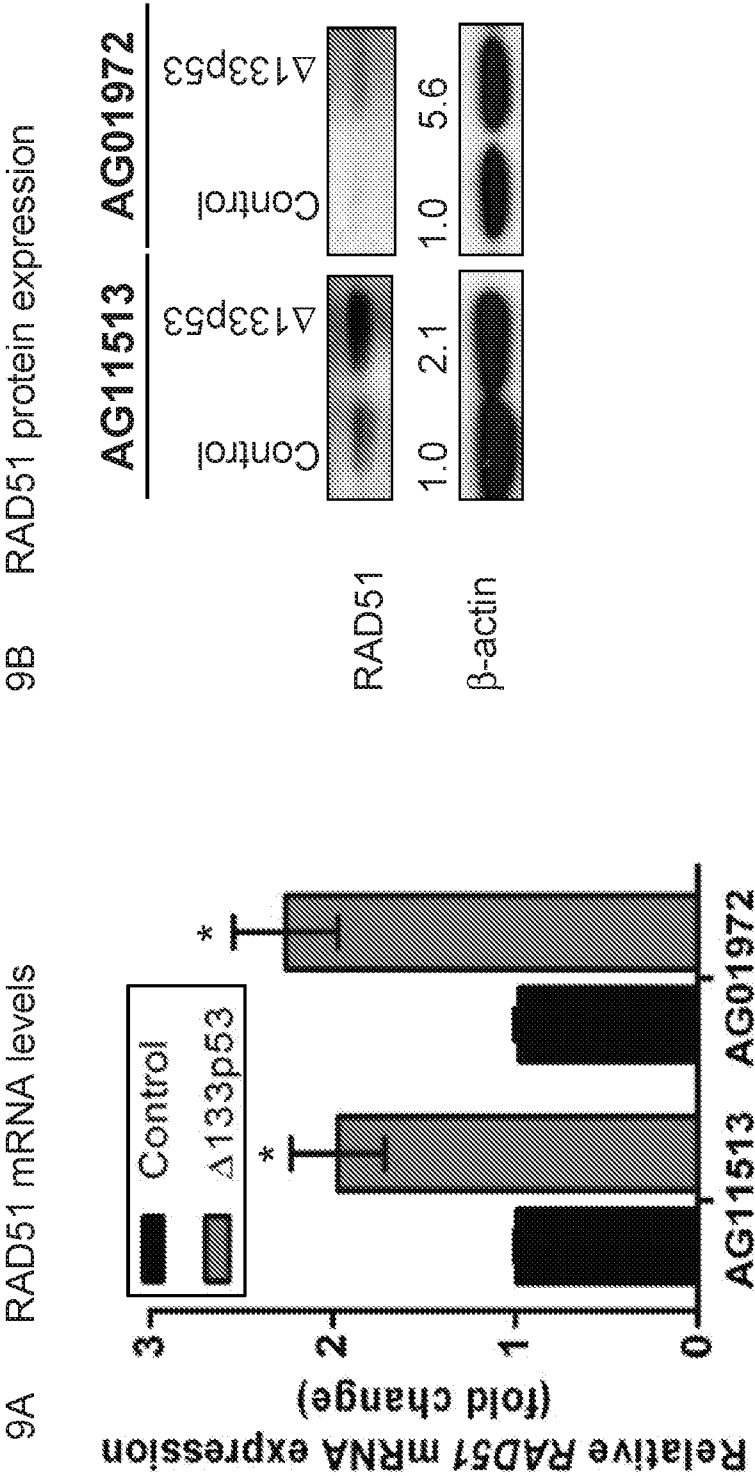


Figure 10A-10C

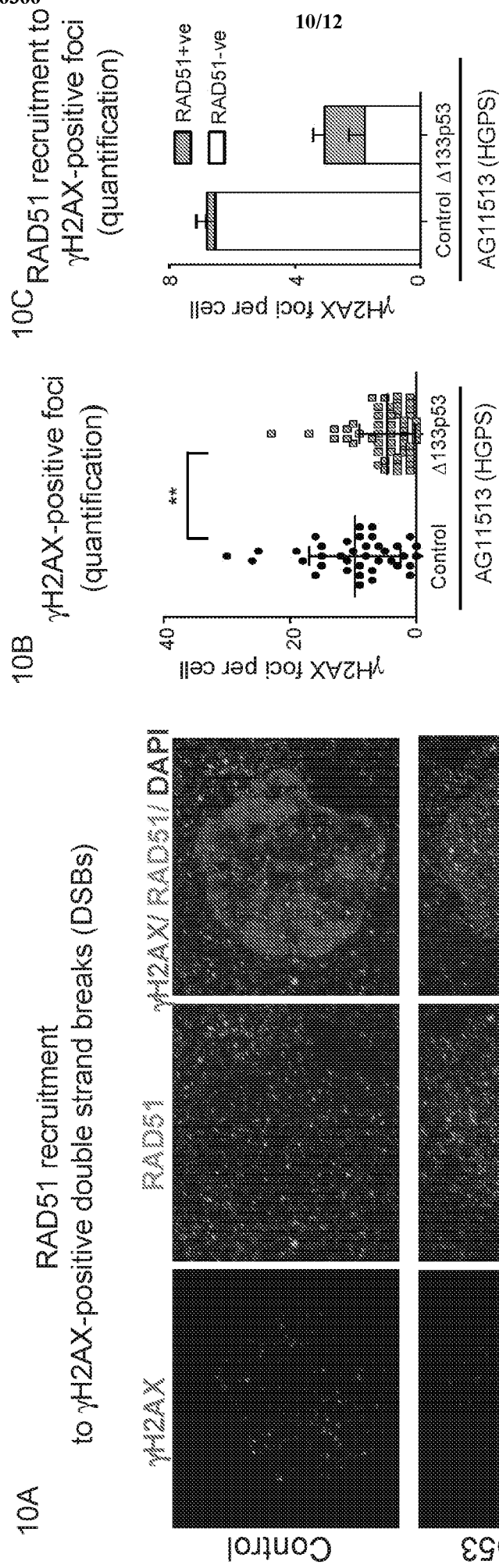


Figure 11A-11D

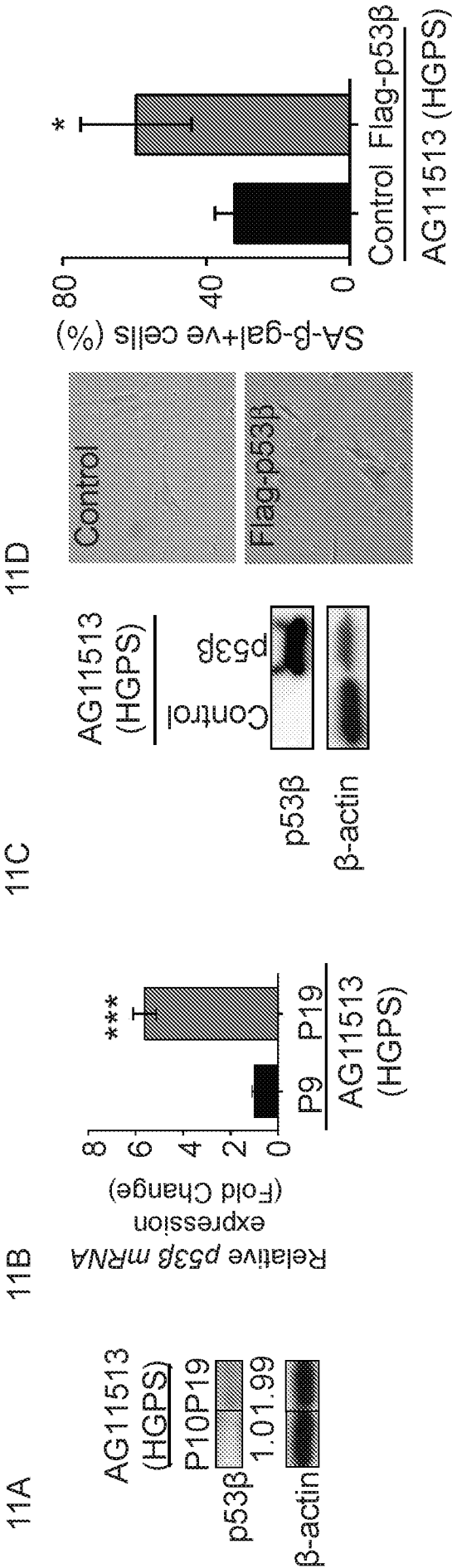
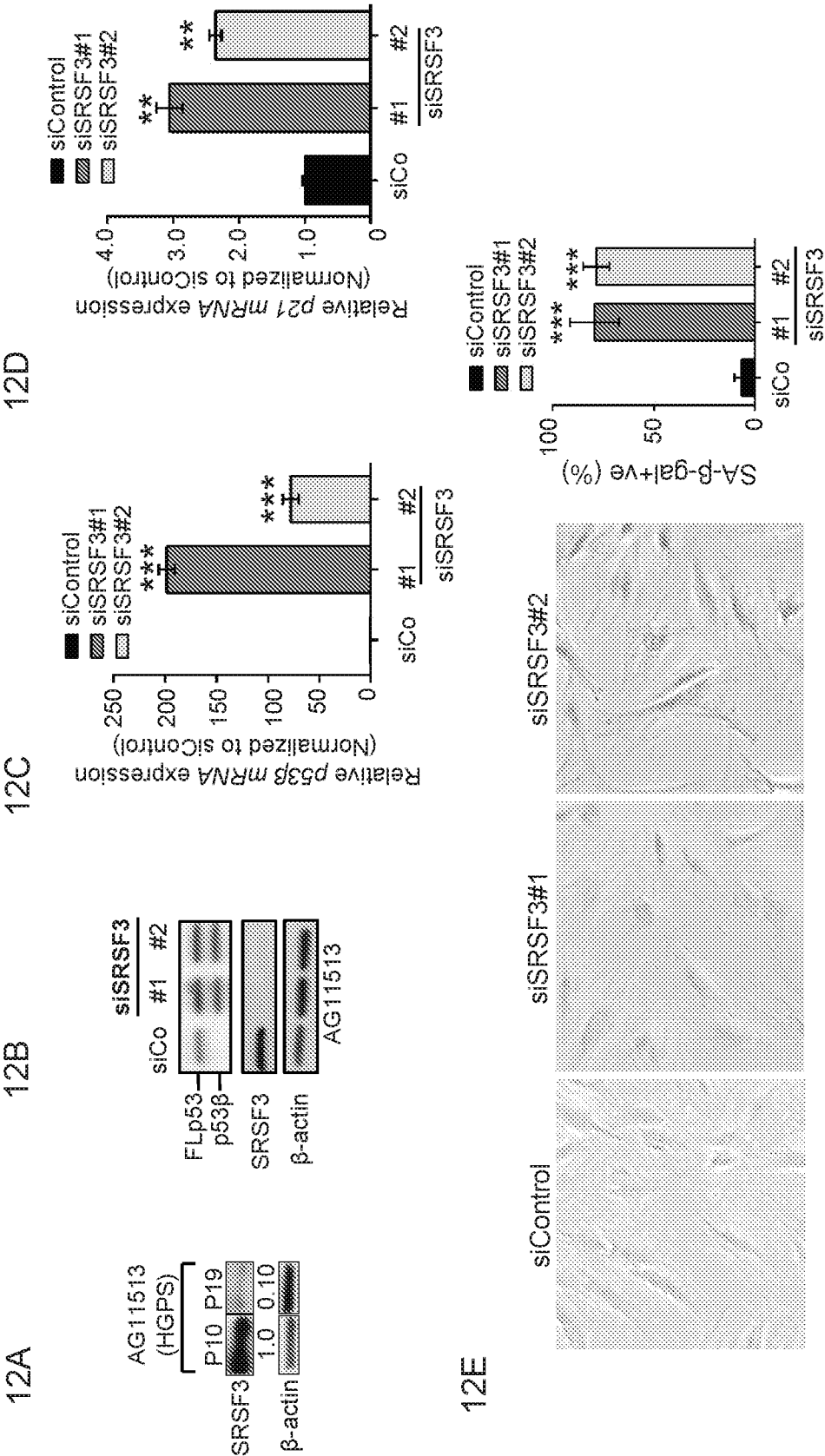


Figure 12A-12E



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/034821

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

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

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

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

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

see additional sheet

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/034821

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/47 A61K48/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/064590 A2 (US GOV HEALTH & HUMAN SERV [US]; VOJTESEK BORIVOJ [CZ]; HARRIS CURTIS) 22 May 2009 (2009-05-22) cited in the application	1,4,5,9, 10,13, 14,18, 19,22, 25,26, 30,31
Y	paragraphs [0008], [0012], [0013], [0016], [0019], [0020] examples 3, 6 figures 2, 9, 14, 16, 27 claims 14, 15, 16, 24, 25-27, 28, 32, 33 ----- -/--	2,3,6-8, 11,12, 15-17, 20,21, 23,24, 27-29, 32,33



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

7 September 2016

Date of mailing of the international search report

20/09/2016

Name and mailing address of the ISA/

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Authorized officer

Rojo Romeo, Elena

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/034821

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AKIRA SHIMAMOTO ET AL: "Immunogenicity of induced pluripotent stem cells.", FRONTIERS IN GENETICS, vol. 474, no. 3678, 29 January 2015 (2015-01-29), page 212, XP055300087, DOI: 10.1038/nature10135 abstract -----	2,6,11, 15,20, 23,27,32
Y	CARMOSINO MONICA ET AL: "Role of nuclear Lamin A/C in cardiomyocyte functions", BIOLOGY OF THE CELL (PARIS), vol. 106, no. 10, October 2014 (2014-10), pages 346-358, XP002761471, ISSN: 0248-4900 abstract -----	3,7,12, 16,21, 24,28,33
Y	MICHAEL J MOSER ET AL: "Genetic Instability and Hematologic Disease Risk in Werner Syndrome Patients and Heterozygotes 1", CANCER RESEARCH, vol. 60, 1 May 2000 (2000-05-01), pages 2492-2496, XP055300141, abstract -----	2,8,11, 17,20, 23,29,32

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/034821

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009064590 A2	22-05-2009	AU 2008321253 A1	22-05-2009
		CA 2705488 A1	22-05-2009
		EP 2217706 A2	18-08-2010
		US 2011243968 A1	06-10-2011
		US 2013302892 A1	14-11-2013
		US 2015299706 A1	22-10-2015
		WO 2009064590 A2	22-05-2009

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-33

All claims searched

1.1. claims: 1-9, 19-21(all partially)

Inventions 1-6: A method of extending the replicative lifespan of a cell of a subject that has a disease of premature aging, the method comprising contacting the cell with an agent that activates the function or expression of p53, thereby inhibiting cell senescence and extending the replicative lifespan of the cell; in which the disease is Werner syndrome or Hutchinson-Gilford progeria syndrome and the cell is a stem cell, or a cardiac progenitor cell or an endothelial progenitor cell or a bone-marrow-derived progenitor cell.

1.2. claims: 10-21(partially)

Inventions 7-12: A method of extending the replicative lifespan of a cell of a subject that has a disease of premature aging, the method comprising contacting the cell with an agent that inhibits the function or expression of miR-34a, thereby inhibiting cell senescence and extending the replicative lifespan of the cell; in which the disease is Werner syndrome or Hutchinson-Gilford progeria syndrome and the cell is a stem cell, or a cardiac progenitor cell or an endothelial progenitor cell or a bone-marrow-derived progenitor cell.

1.3. claims: 22-33(partially)

Inventions 13-18: A method of extending the replicative lifespan of a cell of a subject that has a disease of premature aging, the method comprising contacting the cell with an agent that inhibits the function or expression of p53, thereby inhibiting cell senescence and extending the replicative lifespan of the cell; in which the disease is Werner syndrome or Hutchinson-Gilford progeria syndrome and the cell is a stem cell, or a cardiac progenitor cell or an endothelial progenitor cell or a bone-marrow-derived progenitor cell.
