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(54) Title: TRANSGENIC ANIMALS CAPABLE OF BEING INDUCED TO DELETE SENESCENT CELLS

(57) Abstract: This document relates to methods and materials involved in the removal of senescent cells within a mammal. For example, transgenic non-human animals that be induced to delete senescent cells are provided.

TRANSGENIC ANIMALS CAPABLE OF
BEING INDUCED TO DELETE SENESCENT CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

5   This application claims the benefit of U.S. Provisional Application Serial No. 61/567,587, filed December 6, 2011 and U.S. Provisional Application Serial No. 61/499,616, filed June 21, 2011. The disclosures of these prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

10   **BACKGROUND**

1. Technical Field

       This document relates to methods and materials involved in the removal of senescent cells within a mammal. For example, this document provides transgenic non-human animals that can be induced to delete senescent cells.

15

2. Background Information

       Cellular senescence, which halts the proliferation of damaged or dysfunctional cells, is widely recognized as an important mechanism to constrain the malignant progression of tumor cells (Campisi, *Curr. Opin. Genet. Dev.*, 21:107-112 (2011); and

20   Kuilman *et al.*, *Genes Develop.*, 24:2463-2479 (2010)). As cells senesce, they can develop a unique phenotype, referred to as the senescence-associated secretory phenotype (SASP, or alternatively called SMS), in which they acquire the ability to secrete a variety of growth factors, cytokines, chemokines, and proteases (Coppe *et al.*, *PLoS Biol.*, 6:2853-2868 (2008)). The observation that senescent cells can accumulate in several

25   tissues and organs during organismal aging and are present at sites of age-related pathologies has led to speculation that they contribute to aging and age-related dysfunction (Campisi, *Cell*, 120:513-522 (2005)).

SUMMARY

30   This document relates to methods and materials involved in the removal of senescent cells within a mammal. For example, this document provides transgenic non-

human animals that can be induced to delete senescent cells (e.g., p16^{Ink4a}-positive
senescent cells). As described herein, transgenic mice can be produced to contain nucleic
acid that allows for the controlled clearance of senescent cells (e.g., p16^{Ink4a}-positive
senescent cells) by controllably inducing apoptosis of senescent cells while inducing

5   little, or no, apoptosis of non-senescent cells. For example, a transgenic non-human
animal provided herein can be allowed to grow and develop for a period of time and then
can be treated with a compound (e.g., AP20187) capable of inducing apoptosis of
senescent cells within the transgenic animal while inducing little, or no, apoptosis of non-
senescent cells within the transgenic animal. As described herein, clearance of senescent

10   cells within a transgenic non-human animal can delay or reduce the likelihood of age-
related disorders and can maximize healthy lifespan. In some cases, a transgenic non-
human animal provided herein can include nucleic acid encoding a marker polypeptide
(e.g., a fluorescent polypeptide such as a green fluorescent protein (GFP)) configured to
be expressed by senescent cells with little, or no, expression by non-senescent cells. In

15   some cases, a transgenic non-human animal provided herein can have a genetic
background (e.g., a BubR1 hypomorphic (BubR1$^{H/H}$) genetic background) that results in a
markedly shortened lifespan with or without exhibiting one or more age-related
phenotypes such as infertility, lordokyphosis, sarcopenia, cataracts, fat loss, cardiac
arrhythmias, arterial wall stiffening, impaired wound healing, and dermal thinning.

20   The transgenic non-human animals provided herein can be used in assays
designed to identify agents having the ability to kill, or to facilitate the killing of,
senescent cells. For example, transgenic non-human animals provided herein can be used
as controls (e.g., positive controls) for the successful clearance of senescent cells. In
some cases, transgenic non-human animals provided herein can be used as controls (e.g.,

25   positive controls) for the successful clearance of senescent cells with minimal or no
killing of non-senescent cells.

          In some cases, transgenic non-human animals provided herein can be used as test
animals in assays designed to identify agents having the ability to kill, or to facilitate the
killing of, senescent cells. In such cases, the ability of a test agent to kill, or to facilitate

30   the killing of, senescent cells can be monitored based, at least in part, on the expression of
a marker polypeptide (e.g., a fluorescent polypeptide such as GFP) configured to be

expressed by senescent cells. In some cases, the ability of the test agent to kill, or to facilitate the killing of, senescent cells can be evaluated by comparing its effects in a particular animal at a first time point to the effects observed in the same animal after treatment with a compound (e.g., AP20187) capable of inducing apoptosis of senescent

5   cells within that transgenic animal at a second time point. Such a comparison can be used to identify test agents that are less effective or at least as effective as the compound capable of inducing apoptosis of senescent cells at the second time point. In some cases, the compound capable of inducing apoptosis of senescent cells can be used at the first time point, and the test agent can be used as the second time point to identify test agents

10   that are more effective than the compound used at the first time point.

In some cases, the transgenic non-human animals provided herein can be used in assays designed to identify agents having the ability to delay or reduce the likelihood of age-related disorders and/or maximize healthy lifespan. For example, transgenic non-human animals provided herein can be used as controls (e.g., positive controls) for the

15   successful delay of age-related disorders and/or for the successful increased duration of a healthy lifespan.

In some cases, transgenic non-human animals provided herein can be used as test animals in assays designed to identify agents having the ability to delay or reduce the likelihood of age-related disorders and/or maximize healthy lifespan. In such cases, the

20   ability of a test agent to delay or reduce the likelihood of age-related disorders and/or maximize healthy lifespan can be monitored based, at least in part, on the expression of a marker polypeptide (e.g., a fluorescent polypeptide such as GFP) configured to be expressed by senescent cells. In some cases, the ability of the test agent to delay or reduce the likelihood of age-related disorders and/or maximize healthy lifespan can be

25   evaluated by comparing its effects in a particular animal at a first time point to the effects observed in the same animal after treatment with a compound (e.g., AP20187) capable of inducing apoptosis of senescent cells within that transgenic animal at a second time point. Such a comparison can be used to identify test agents that are less effective or at least as effective as the compound capable of inducing apoptosis of senescent cells at the second

30   time point. In some cases, the compound capable of inducing apoptosis of senescent cells can be used at the first time point, and the test agent can be used at the second time point

to identify test agents that are more effective at delaying or reducing the likelihood of age-related disorders and/or maximizing healthy lifespan than the compound used at the first time point.

In general, one aspect of this document features a transgenic mouse, the nucleated cells of which contain a transgene. The transgene comprises, or consists essentially of, a promoter sequence operably linked to a nucleic acid sequence encoding a polypeptide having the ability to kill a cell or facilitate the killing of a cell when the transgenic mouse is administered a compound, wherein senescent cells of the transgenic mouse express the polypeptide, and wherein the senescent cells of the transgenic mouse are killed when the compound is administered to the transgenic mouse. Less than 10 percent of non-senescent cells of the transgenic mouse can be killed when the compound is administered to the transgenic mouse. The promoter sequence can be a $p16^{Ink4a}$ promoter sequence. The polypeptide can comprise a caspase 8 polypeptide sequence. The polypeptide can comprise a FKBP polypeptide sequence. The polypeptide can be a FKBP-caspase 8 fusion polypeptide. The compound can be AP20187. The genetic background of the transgenic mouse can be a $BubR1^{H/H}$ genetic background. The transgene can comprise nucleic acid encoding a marker polypeptide. The marker polypeptide can be a GFP polypeptide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1. $p16^{Ink4a}$-specific expression of the INK-ATTAC transgene. A,

Schematic representation of the INK-ATTAC transgenic construct and the mechanism of apoptosis activation. B, GFP intensity of IAT collected from 5-month-old untreated mice with the indicated genotypes. C, qRT-PCR analysis of untreated 9-month-old mouse tissue analyzed for relative expression of $p16^{Ink4a}$ (left), FKBP-Casp8 (middle), and EGFP (right). Error bars, s.d.; n = 3 female mice per genotype. Abbreviation: SkM, skeletal muscle (gastrocnemius). D, Cultured bone marrow cells from 2-month-old WT:INKATTAC-3 mice treated with rosiglitazone for 48 hours and immunostained for Flag antibody (visualizing Flag-FKBP-Casp8). Scale bar, 20 μm.

Figure 2. Validation of $p16^{Ink4a}$-specific expression of the INK-ATTAC-5 transgene. A, GFP intensity of IAT collected from 5-month-old untreated mice with the indicated genotypes. Scale bar, 20 μm. B, qRT-PCR analysis of untreated 9-month-old mouse tissue analyzed for the relative expression of $p16^{Ink4a}$ (left), FKBP-Casp8 (middle), and EGFP (right). Error bars, s.d.; n = 3 female mice per genotype. Abbreviation: SkM, skeletal muscle (gastrocnemius).

Figure 3. Tissues expressing the INK-ATTAC transgene have high levels of cellular senescence markers. A, SA-β-Gal staining of IAT in 9-month-old untreated mice with the indicated genotypes. B, qRT-PCR analysis of the indicated tissues for selected markers of senescence. Tissues were collected from 9-month-old untreated mice with the indicated genotypes. Abbreviation: SkM, skeletal muscle (gastrocnemius). C, Representative FACS profile of single-cell suspensions of IAT reveals two distinct cell populations based on GFP intensity of untreated $BubR1^{H/H}$:INK-ATTAC-3 mice (dashed line indicates GFP$^-$ versus GFP$^+$ cells). D, Sorted GFP$^+$ cells from IAT express higher levels of senescence-associated genes than GFP$^-$ cells. For B and D: error bars, s.d.; n = 3 female mice per genotype.

Figure 4. Tissues expressing the INK-ATTAC-5 transgene display elevated indicators of senescence. A, qRT-PCR analysis of the indicated tissues for selected markers of senescence. Tissues were collected from 9-month-old untreated mice with the indicated genotypes. Abbreviation: SkM, skeletal muscle (gastrocnemius). B, Sorted GFP$^+$ cells from IAT express higher levels of senescence-associated genes than GFP$^-$ cells. For A and B, error bars, s.d.; n = 3 female mice per genotype.

Figure 5. Delayed onset of $p16^{Ink4a}$-mediated age-related phenotypes in AP20187-

treated BubR1$^{H/H}$:INK-ATTAC mice. A, Bone marrow cells collected from 2-month-old WT:INK-ATTAC-3 mice were exposed to rosiglitazone for 5 days to induce senescence and then cultured in the presence or absence of AP20187. After 48 hours, cells were stained for SA-β-Gal. Scale bar, 50 μm. B, Lordokyphosis (left) and cataracts (right) are

5   significantly delayed when BubR1$^{H/H}$:INK-ATTAC transgenic mice were continuously treated with AP20187 from weaning age on. Lordokyphosis: *, $P < 0.0001$ and **, $P = 0.0077$; cataracts: *, $P = 0.0017$ and **, $P = 0.0158$ log-rank tests. C, Representative images of 9-month-old AP20187 treated (top) and untreated (bottom) BubR1$^{H/H}$: INKATTAC-3 mice. D, AP20187 treatment increases average muscle fiber diameter of

10   gastrocnemius (Gastro) and abdominal (ABD) muscles of BubR1$^{H/H}$:INK-ATTAC mice. Error bars, s.e.m.; n = 6 mice per genotype. *, $P = 0.0157$; **, $P = 0.0007$; ***, $P = 0.0239$, unpaired t tests. E, Duration of exercise to exhaustion reveals that AP20187-treated BubR1$^{H/H}$:INK-ATTAC mice have extended running time. Error bars, s.d.; n = 6 mice per genotype; *, $P = 0.0236$; **, $P = 0.0009$, unpaired t tests. F, BubR1$^{H/H}$:INK-

15   ATTAC mice treated with AP20187 travel longer distances on a treadmill. Error bars, s.d.; n = 6 mice per genotype; *, $P = 0.0187$; **, $P = 0.0012$, unpaired t tests. G, AP20187-treated BubR1$^{H/H}$:INK-ATTAC mice exert more energy during exercise ability tests. Error bars, s.d.; n = 6 mice per genotype; *, $P = 0.0065$; **, $P = 0.0002$, unpaired t tests. H, Sizes of various fat depots of BubR1$^{H/H}$:INK-ATTAC transgenic mice are

20   increased in response to AP20187 treatment. Parentheses, s.d.; n = 6 mice per genotype. Asterisks denote significant ($P < 0.05$) changes compared to untreated animals of the same transgenic line, unpaired t tests. Abbreviations: POV, paraovarian; Peri, perirenal; Mes, mesenteric; SSAT, subscapular adipose tissue. Total fat percentage was determined by DEXA scanning. I, Cell diameter of IAT in BubR1$^{H/H}$:INK-ATTAC mice increases in

25   response to AP20187 treatment. Error bars, s.e.m.; n = 6 female mice per genotype; *, $P = 0.0031$; **, $P = 0.0003$, unpaired t tests. J, Dermal and subcutaneous adipose layer thickness of BubR1$^{H/H}$:INK-ATTAC mice indicates consistently increased adiposity with treatment. Error bars, s.e.m.; n = 6 female mice per genotype; *, $P = 0.0016$ and **, $P = 0.0015$, unpaired t tests.

30   Figure 6. Age-associated traits of BubR1 hypomorphic mice that are p16^{Ink4a}-independent are not influenced by clearance of p16^{Ink4a}-positive cells. A, The percentage

of sinus pause rhythm disturbances is similarly increased in both treated and non-treated BubR1$^{H/H}$:INK-ATTAC heart tissue. Abbreviation: BPM, beats per minute. B, Thinning of the aorta is not corrected by drug treatment in BubR1$^{H/H}$:INK-ATTAC animals. Error bars, s.e.m.; n = 6 female mice per genotype. Consistent with this, p16^{Ink4a} and the INK-

5   ATTAC transgene are not expressed in this tissue. Error bars, s.d.; n = 3. Figure 6C is a graph plotting overall survival for the indicated mice.

Figure 7. AP20187-treated BubR1$^{H/H}$:INK-ATTAC animals have reduced numbers of p16^{Ink4a}-positive senescent cells. A, SA-β-Gal staining of IAT reveals that AP20187-treated adipose tissue attenuates the senescent phenotype driven by BubR1

10   hypomorphism. qRT-PCR analysis for indicators of senescence in IAT. B-D, Treatment of BubR1$^{H/H}$:INK-ATTAC animals with AP20187 leads to lower levels of senescence-associated markers in IAT (B), skeletal muscle (C), and eye (D). Error bars, s.d.; n = 3 female mice per genotype. E, BrdU incorporation rates as a measure of replicative senescence are elevated in IAT and skeletal muscle of BubR1$^{H/H}$:INK-ATTAC mice.

15   Error bars, s.e.m.; n = 6 mice per genotype. Statistical analysis was by unpaired t test: *, P = 0.0146 and **, P = 0.0137.

Figure 8 contains graphs plotting the relative expression of the indicated polypeptides in IAT (A), skeletal muscle (B), and eye (C).

Figure 9 is a listing of the nucleic acid sequence of a pBLUESCRIPT II KS vector

20   containing a p16^{Ink4a}-ATTAC-IRES-GFP nucleic acid construct.

Figure 10 is a listing of the nucleic acid sequence of Figure 9 with the various vector components and construct components labeled.

DETAILED DESCRIPTION

25   This document relates to methods and materials involved in the removal of senescent cells within a mammal. For example, this document provides transgenic non-human animals that can be induced to delete senescent cells (e.g., p16^{Ink4a}-positive senescent cells). Such non-human animals can be farm animals such as pigs, goats, sheep, cows, horses, and rabbits, rodents such as rats, guinea pigs, and mice, and non-

30   human primates such as baboons, monkeys, and chimpanzees. The term "transgenic non-human animal" as used herein includes, without limitation, founder transgenic non-human

animals as well as progeny of the founders, progeny of the progeny, and so forth, provided that the progeny retain the transgene. The nucleated cells of the transgenic non-human animals provided herein can contain a transgene that includes a promoter sequence (e.g., a p16^{Ink4a} promoter sequence) operably linked to a nucleic acid sequence encoding a

5   polypeptide capable of killing a cell or capable of facilitating the killing of a cell. A promoter sequence of a transgene described herein can be one that drives polypeptide expression in senescent cells while driving less, little, or no expression in non-senescent cells. Examples of such promoters include, without limitation, a p16^{Ink4a} promoter sequence, a p21^{cip} promoter sequence, and a Pai1 promoter sequence.

10   In some cases, a polypeptide capable of killing a cell or capable of facilitating the killing of a cell can be a polypeptide that includes two polypeptide sequences fused together (e.g., a fusion polypeptide). An example of such a fusion polypeptide can be a FKBP-caspase 8 fusion protein. See, e.g., Pajvani *et al.*, *Nat. Med.*, 11:797-803 (2005). Other examples of polypeptides capable of killing a cell or capable of facilitating the

15   killing of a cell that can be used as described herein include, without limitation, a FKBP-caspase-1 fusion polypeptide or FKBP-caspase-3 fusion polypeptide. In some cases, a polypeptide capable of killing a cell or capable of facilitating the killing of a cell can be engineered to include a tag (e.g., a Flag tag). In some cases, a transgene provided herein can include nucleic acid encoding a marker polypeptide such as a fluorescent polypeptide

20   (e.g., GFP, BFP, or RFP). For example, a transgene provided herein can include nucleic acid encoding a polypeptide capable of killing a cell or capable of facilitating the killing of a cell followed by an internal ribosome entry site followed by a marker polypeptide (e.g., GFP).

           In some cases, a transgene can include a p16^{Ink4a} promoter sequence followed by

25   nucleic acid encoding an FKBP-caspase 8 fusion protein. In such cases, administration of a compound such as AP20187 can result in apoptosis of cells expressing the FKBP-caspase 8 fusion protein. For example, senescent cells of a transgenic non-human animal provided herein can express the FKBP-caspase 8 fusion protein of a transgene by virtue of the p16^{Ink4a} promoter sequence and can be selectively and controllably killed following

30   administration of AP20187. AP20187 can be obtained as described elsewhere (U.S. Patent Application Publication No. 2004/0006233).

The term "operably linked" as used herein refers to positioning a regulatory element (e.g., a promoter sequence, an inducible element, or an enhancer sequence) relative to a nucleic acid sequence encoding a polypeptide in such a way as to permit or facilitate expression of the encoded polypeptide. In the transgenes disclosed herein, for example, a promoter sequence (e.g., a p16^{Ink4a} promoter sequence) can be positioned 5' relative to a nucleic acid encoding a polypeptide (e.g., an FKBP-caspase 8 fusion protein).

Various techniques known in the art can be used to introduce transgenes into non-human animals to produce founder lines, in which the transgene is integrated into the genome. Such techniques include, without limitation, pronuclear microinjection (See, e.g., U.S. Patent No. 4,873,191), retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci. USA, 82:6148-1652 (1985)), gene targeting into embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)), electroporation of embryos (Lo, Mol. Cell. Biol., 3:1803-1814 (1983)), and in vitro transformation of somatic cells, such as cumulus or mammary cells, followed by nuclear transplantation (Wilmut et al., Nature, 385:810-813 (1997); and Wakayama et al., Nature, 394:369-374 (1998)). For example, fetal fibroblasts can be genetically modified to contain an INK-ATTAC construct (Figure 1A), and then fused with enucleated oocytes. After activation of the oocytes, the eggs are cultured to the blastocyst stage. See, for example, Cibelli et al., Science, 280:1256-1258 (1998). Standard breeding techniques can be used to create animals that are homozygous for the transgene from the initial heterozygous founder animals. Homozygosity is not required, however, as the phenotype can be observed in hemizygotic animals.

Once transgenic non-human animals have been generated, expression of an encoded polypeptide (e.g., an FKBP-caspase 8 fusion protein or marker polypeptide) can be assessed using standard techniques. Initial screening can be accomplished by Southern blot analysis to determine whether or not integration of the transgene has taken place. For a description of Southern analysis, see sections 9.37-9.52 of Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, second edition, Cold Spring Harbor Press, Plainview; NY. Polymerase chain reaction (PCR) techniques also can be used in the initial screening. PCR refers to a procedure or technique in which target nucleic acids are

amplified. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic

5   DNA or total cellular RNA. Primers typically are 14 to 40 nucleotides in length, but can range from 10 nucleotides to hundreds of nucleotides in length. PCR is described in, for example PCR Primer: A Laboratory Manual, ed. Dieffenbach and Dveksler, Cold Spring Harbor Laboratory Press, 1995. Nucleic acids also can be amplified by ligase chain reaction, strand displacement amplification, self-sustained sequence replication, or

10   nucleic acid sequence-based amplified. See, for example, Lewis, *Genetic Engineering News*, 12:1 (1992); Guatelli *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:1874-1878 (1990); and Weiss, *Science*, 254:1292-1293 (1991).

Expression of a nucleic acid sequence encoding a polypeptide (e.g., an FKBP-caspase 8 fusion protein or marker polypeptide) in senescent cells of transgenic non-

15   human animals can be assessed using techniques that include, without limitation, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, Western analysis, immunoassays such as enzyme-linked immunosorbent assays, and reverse-transcriptase PCR (RT-PCR). As described herein, expression of an FKBP-caspase 8 fusion protein by senescent cells within the transgenic animal can result in

20   transgenic animals that can be treated with AP20187 such that the senescent cells are killed. Such transgenic animals can exhibit delayed, or a reduced likelihood of, age-related disorders and/or a maximized healthy lifespan. It is understood that a particular phenotype in a transgenic animal typically is assessed by comparing the phenotype in the transgenic animal to the corresponding phenotype exhibited by a control non-human

25   animal that lacks the transgene.

A transgenic non-human animal provided herein can have any appropriate genetic background. In some cases, a transgenic non-human animal provided herein can have a BubR1 hypomorphic (BubR1$^{H/H}$) genetic background, a Tert$^{-/-}$ genetic background, or a bGH^{+} (bovine growth hormone) genetic background.

30   This document also provides tissues (e.g., skin, eye, fat, muscle, lung, heart, bone, liver, intestine, kidney, spleen, brain, cartilage, marrow, adrenal glands, ovaries, and

testes) and cells (e.g., fat cells, preadipocytes, skin or lung fibroblasts, muscle satellite cells, osteoblasts, bone marrow progenitor cells, neuronal progenitor cells, hepatocytes, endothelial cells, chondroblasts, and splenocytes cells) obtained from a transgenic non-human animal provided herein.

5   This document also provides methods for identifying agents having the ability to kill, or to facilitate the killing of, senescent cells and methods for identifying agents having the ability to delay, or reduce the likelihood of age-related disorders, and/or maximize healthy lifespan. Such methods can include, for example, (1) targeting senescent cells based on compounds activated by enzyme activities that are higher in

10   them than other cells (such as senescence-associated β-galactosidase), such compounds killing the senescent cells upon activation, (2) use of compounds that kill cells to which they bind through receptors that are more highly expressed by senescent than other cells (such receptors being identified by proteomic or expression profiling of senescent versus non-senescent cells or other approaches), or (3) compounds that are activated by

15   metabolic processes that are more active in senescent than non-senescent cells (with such metabolic processes being identified through metabolomic, proteomic, expression profiling, or other means), with the compounds so activated killing the senescent cell.

           In some cases, methods for identifying agents having the ability to kill, or to facilitate the killing of, senescent cells and methods for identifying agents having the

20   ability to delay, or reduce the likelihood of age-related disorders, and/or maximize healthy lifespan can include obtaining senescent cells from a mammal (e.g., an animal model or a human). For example, a transgenic mouse provided herein such as a transgenic mouse that expresses a marker polypeptide (e.g., GFP) in senescent cells can be used to obtain senescent cells. Such a transgenic mouse can contain a transgene that

25   includes a marker polypeptide (e.g., GFP) operably linked to a promoter sequence that drives polypeptide expression in senescent cells while driving less, little, or no expression in non-senescent cells. Examples of such promoters include, without limitation, a $p16^{Ink4a}$ promoter sequence, a p21cip promoter sequence, and a Pai1 promoter sequence. The senescent cell can be any appropriate cell type or from any appropriate tissue. For

30   example, senescent cells can be obtained from fat or endothelial tissue. In some cases, senescent cells can be obtained from liver, bone marrow, heart, lung, or skin tissue.

Any appropriate method can be used to obtain senescent cells from a mammal. For example, senescent cells expressing a marker polypeptide (e.g., GFP) under the control of a p16^{Ink4a} promoter sequence can be separated from non-senescent cells using standard techniques such as cell sorting methods based on the expression of the marker

5   polypeptide. In some cases, cell lines of senescent cells can be used in place of freshly obtained senescent cells to identify agents having the ability to kill, or to facilitate the killing of, senescent cells and agents having the ability to delay, or reduce the likelihood of age-related disorders, and/or maximize healthy lifespan as described herein. In some cases, senescent cells can be obtained by cell passage in culture (e.g., greater than about

10   12 to 15 cell passages for mouse embryonic fibroblasts and greater than about 20 cell passages at a 1:2 split ratio for human cells) or by radiation treatment (e.g., treatment with about 5 to about 50 Grays), a ceramide (e.g., C6, C16, or C18) treatment (e.g., treatment with about 7 µM to about 15 µM (e.g., 13 µM) of ceramide such as C16 for at least about 15 days), exposure to oncogenes or increased expression of oncogenes such as H-Ras or

15   K-Ras (e.g., K-RasG12V), exposure to non-oncogenes or increased expression of non-oncogenes such as JAK or STAT, or exposure to glucose (e.g., about 16.5 mM to about 22.5 mM of D-glucose) for at least 10 days (e.g., greater than 30 days). In some cases, senescent cells can be obtained by exposing cells to reactive oxygen species or hydrogen peroxide to induce senescence via a p53 pathway.

20   Once obtained, the senescent cells can be exposed to a library of test agents individually or in pools to identify those agents or pools of agents having the ability to kill, or to facilitate the killing of, the senescent cells. Once identified as having the ability to kill, or to facilitate the killing of, the senescent cells, the identified agent can be applied to comparable non-senescent cells in comparable concentrations to confirm that the agent

25   has a reduced ability to kill, or to facilitate the killing of, non-senescent cells. Those agents having the ability to kill, or to facilitate the killing of, senescent cells with a reduced or no ability to kill, or to facilitate the killing of, non-senescent cells can be classified as being an agent having the ability to delay, or reduce the likelihood of age-related disorders, and/or maximize healthy lifespan. In some cases, senescent cells

30   obtained from a transgenic mammal provided herein and treated in a manner that results in senescent cell death can be used as positive controls.

In some cases, an agent can be identified as having the ability to kill, or to facilitate the killing of, senescent cells or as having the ability to delay, or reduce the likelihood of age-related disorders, and/or maximize healthy lifespan using *in vivo* techniques. For example, an animal model such as wild-type mice or animals, mice with

5   a BubR1 hypomorphic (BubR1$^{H/H}$) genetic background, or other mouse or animal models can be used. In such cases, a library of test agents can be administered individually or in pools to the animals (e.g., mice), and the animals (e.g., mice) can be assessed for indications that the test agent is capable of killing, or facilitating the killing of, senescent cells or is capable of delaying, or reducing the likelihood of age-related disorders, and/or

10   maximizing healthy lifespan. Indications of senescent cell killing or indications of delayed or reduced likelihood of age-related disorders, and/or indications of maximized healthy lifespan can be detected and assessed as described herein. For example, the ability of an agent to increase the length of lifespan can be assessed comparing treated and untreated mice with, for example, a BubR1 hypomorphic (BubR1$^{H/H}$) genetic

15   background.

This document also provides methods and materials for identifying molecules (e.g., polypeptides, carbohydrates, lipids, and nucleic acids) possessed or expressed by senescent cells. For example, senescent cells can be obtained as described herein and assessed to identify molecules (e.g., polypeptides) possessed or expressed by those

20   senescent cells. Any appropriate method can be used to identify molecules possessed or expressed by senescent cells. For example, polypeptide isolation and sequencing techniques can be used to identify polypeptides expressed by senescent cells.

In some cases, a transgenic mouse provided herein can be used to identify molecules (e.g., polypeptides and carbohydrates) possessed or expressed by senescent

25   cells. For example, a transgenic mouse provided herein can be treated with a compound (e.g., AP20187) starting at or before birth (e.g., shortly after fertilization via treatment of the mouse's mother) such that senescent cells are killed or prevented from developing. In such cases, the resulting mouse can be immunologically naïve with respect to the molecules exclusively expressed by senescent cells. The immunologically naïve mouse

30   can then be exposed to senescent cells or components from senescent cells (e.g., plasma membranes) in a manner designed to trigger an immune response. Resulting antibodies

or antibody-producing cells can be isolated and assessed to confirm that the antibodies recognize a molecule presented or expressed by senescent cells. In some cases, the antibodies can be assessed for the ability to not recognize molecules presented or expressed by non-senescent cells. Once such antibodies are obtained, they can be used to

5   identify the molecule present or expressed by the senescent cells.

In some cases, antibodies directed to a molecule present or expressed by senescent cells can be used to kill, or to facilitate the killing of, senescent cells or to delay, or reduce the likelihood of age-related disorders, and/or to maximize healthy lifespan. For example, antibodies directed to a molecule present or expressed by senescent cells can be

10 conjugated with isotopes or toxins to form conjugates having the ability to kill, or to facilitate the killing of, senescent cells or as having the ability to delay, or reduce the likelihood of age-related disorders, and/or maximize healthy lifespan.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

15

EXAMPLES

Example 1 – Clearance of $p16^{Ink4a}$-positive senescent cells delays aging-associated disorders

To examine the role of senescence in aging and age-related pathologies and to test

20 whether elimination of senescent cells has beneficial effects, a transgenic strategy that enabled clearance of senescent cells in mice was designed. A 2617-bp fragment of the $p16^{Ink4a}$ gene promoter, which is transcriptionally active in senescent, but not non-senescent, cells (Wang *et al.*, *J. Biol. Chem.*, 276:48655-48661 (2001)), was engineered into a nucleic acid construct upstream of nucleic acid encoding a FKBP-caspase 8 fusion

25 protein containing a Flag tag (Pajvani *et al.*, *Nat. Med.*, 11:797-803 (2005)) to create an INK-ATTAC construct (Figure 1A). As shown in Figure 1A, the INK-ATTAC construct was designed to express the FKBP-caspase 8 fusion protein within senescent cells. Once myristoylated, the FKBP-caspase 8 fusion protein becomes membrane-bound, and addition of AP20187, a synthetic drug, is capable of inducing dimerization of the

30 membrane-bound myristoylated FKBP-caspase 8 fusion protein, thereby inducing apoptosis.

In addition, an internal ribosome entry site (IRES) followed by an open reading frame coding for EGFP was added downstream of the nucleic acid encoding the FKBP-caspase 8 fusion protein (Figure 1A). The nucleic acid encoding EGFP was added to allow for detection and collection of $p16^{Ink4a}$-positive senescent cells. Injection of the resulting construct into fertilized eggs yielded nine transgenic INK-ATTAC founder lines.

To examine whether removal of $p16^{Ink4a}$-expressing cells is technically feasible and whether this impacts age-associated deficits in mice, each of the founder lines were bred onto a BubR1 hypomorphic ($BubR1^{H/H}$) genetic background. $BubR1^{H/H}$ mice have a markedly shortened lifespan and exhibit a variety of age-related phenotypes including, without limitation, infertility, lordokyphosis, sarcopenia, cataracts, fat loss, cardiac arrhythmias, arterial wall stiffening, impaired wound healing, and dermal thinning (Baker *et al.*, *Nat. Genet.*, 36:744-749 (2004); Hartman *et al.*, *Neurobiol. Aging*, 28:921-927 (2007); and Matsumoto *et al.*, *Stroke*, 38:1050-1056 (2007)). $BubR1^{H/H}$ mice can accumulate $p16^{Ink4a}$-positive cells in several tissues in which age-associated pathologies develop including, without limitation, adipose tissue, skeletal muscle, and eye.

To screen for transgene activity in $p16^{Ink4a}$-positive cells, samples of inguinal adipose tissue (IAT) were collected from each of the nine $BubR1^{H/H}$:INK-ATTAC strains at five months of age and analyzed for GFP expression by fluorescence microscopy. GFP fluorescence was observed in two of these strains, $BubR1^{H/H}$:INK-ATTAC-3 and $BubR1^{H/H}$:INK-ATTAC-5 (Figures 1B and 2A). Next, the extent to which expression of INK-ATTAC and endogenous $p16^{Ink4a}$ overlap was determined using a quantitative (q)RT-PCR approach. Consistent with earlier data from $BubR1^{H/H}$ mice (Baker *et al.*, *Nat. Cell Biol.*, 10:825-836 (2008)), skeletal muscle, IAT, and eye exhibited increased $p16^{Ink4a}$ expression with aging (Figures 1C and 2B). $BubR1^{H/H}$:INK-ATTAC-3 and $BubR1^{H/H}$:INK-ATTAC-5 mice also exhibited elevated INK-ATTAC and GFP transcript levels in these tissues. On the other hand, $BubR1^{H/H}$ tissues in which $p16^{Ink4a}$ is not induced, such as brain, lung, colon, liver, and heart, had no clear induction of INK-ATTAC or GFP in $BubR1^{H/H}$:INK-ATTAC-3 and $BubR1^{H/H}$:INK-ATTAC-5 mice (Figures 1C and 2B).

To confirm that transgenic INK-ATTAC and endogenous p16^{Ink4a} are under the same transcriptional control mechanism outside the context of BubR1 hypomorphism, bone marrow of wildtype (WT):INK-ATTAC transgenic lines 3 and 5 were harvested and cultured in the absence or presence of rosiglitazone, a drug that can induce cellular

5   senescence and p16^{Ink4a} expression through activation of PPARγ (Gan *et al.*, *J. Cell Sci.* 121:2235-2245 (2008)). Immunofluorescence microscopy revealed that a high proportion of cells expressed Flag-tagged FKBP-Casp8 fusion protein in the presence of rosiglitazone, but not in its absence (Figure 1D). Together, these data indicated that INK-ATTAC gene activity in the two transgenic founder lines overlaps with endogenous

10   p16^{Ink4a} expression.

Next, the following was performed to determine whether INK-ATTAC is expressed in senescent cells in BubR1 hypomorphic tissue. Fat tissue of aged BubR1$^{H/H}$:INK-ATTAC mice was strongly positive for senescence-associated-β-galactosidase (SA-β-Gal; Figure 3A). qRT-PCR analysis demonstrated that INK-ATTAC

15   expression correlates with expression of senescence markers in IAT, including p21, Pai1, IL-6, and Igfbp2 (Figures 3B and 4A). Skeletal muscle and lens tissue of aged BubR1$^{H/H}$:INK-ATTAC mice were SA-β-Gal negative, but both these tissues expressed other markers of senescence, including Mmp13, Pai1, p21, and IL6 (Figures 3B and 4A). To obtain additional evidence for selective expression of INK-ATTAC in senescent cells,

20   IAT was collected from aged BubR1$^{H/H}$:INK-ATTAC animals. Single-cell suspensions were prepared by collagenase treatment, and GFP$^+$ and GFP$^-$ cell populations were separated by fluorescence activated cell sorting (FACS; Figure 3C). Each population was analyzed for expression of INK-ATTAC and senescence markers by qRT-PCR. GFP$^+$ cells not only expressed much higher levels of p16^{Ink4a} than GFP$^-$ cells, but also exhibited

25   elevated levels of other key senescence markers for IAT (Figures 3D and 4B). Senescence markers in GFP$^-$ cells from BubR1$^{H/H}$:INK-ATTAC mice were as low as in GFP$^-$ cells from age-matched WT:INK-ATTAC mice. Taken together, these results indicated that INK-ATTAC is selectively expressed in p16^{Ink4a}-positive senescent cells.

To determine whether INK-ATTAC can eliminate senescent cells, bone marrow

30   cells of WT:INK-ATTAC transgenic lines 3 and 5 were cultured in the presence of rosiglitazone to induce senescence, and cell survival was monitored after activating the

FKBP-Casp8 fusion protein by AP20187 treatment. The vast majority of cells from both transgenic lines were found to be either dead or in the process of dying 48 hours after adding AP20187 (Figure 5A). In contrast, parallel cultures that remained untreated consisted almost entirely of viable SA-β-Gal-positive cells. These data demonstrated that

5   FKBP-Casp8 activation efficiently eliminates p16^{Ink4a}-positive senescent cells *in vitro*.

The following was performed to examine whether clearance of p16^{Ink4a}-expressing cells from BubR1$^{H/H}$ mice prevents or delays the onset of age-related phenotypes in this progeroid background. To this end, cohorts of BubR1$^{H/H}$:INK-ATTAC-3 and BubR1$^{H/H}$:INK-ATTAC-5 mice were established, which were either treated with

10   AP20187 every third day beginning at 3 weeks of age or left untreated. Both treated and untreated mice were monitored for development of age-associated deficits known to accompany p16^{Ink4a} induction, including sarcopenia, cataracts, and loss of adipose tissue (Baker *et al.*, *Nat. Cell Biol.*, 10:825-836 (2008)). Treated mice of both BubR1$^{H/H}$:INK-ATTAC lines exhibited substantially delayed onset of lordokyphosis (a measure of

15   sarcopenia in this model) and cataracts compared to untreated mice, which developed these phenotypes at a rate similar to BubR1$^{H/H}$ mice lacking the INK-ATTAC transgene (Figures 5B and 5C). Consistent with decreased lordokyphosis, muscle fiber diameters of AP20187-treated BubR1$^{H/H}$:INK-ATTAC animals were larger than those of untreated counterparts (Figure 5D). In addition to muscle retention, treadmill exercise tests

20   revealed that duration of exercise (Figure 5E), distance traveled (Figure 5F), and overall amount of work performed (Figure 5G) were all significantly increased in the animals treated with AP20187, indicating preservation of muscle function. Dual-energy x-ray absorptiometry (DEXA) scans of BubR1$^{H/H}$:INK-ATTAC mice confirmed that AP20187 treatment prevented loss of adipose tissue (Figure 5H). All major fat deposits were larger

25   in AP20187-treated BubR1$^{H/H}$:INK-ATTAC animals (Figure 5H), and individual adipocytes were markedly increased in size (Figure 5I). Consistent with this generally increased adiposity, dorsal skin contained significantly more adipose tissue (Figure 5J).

Age-related phenotypes of BubR1$^{H/H}$ mice that arise in a p16^{Ink4a}-independent fashion, such as cardiac arrhythmias and arterial wall stiffening (Matsumoto *et al.*, *Stroke*,

30   38:1050-1056 (2007)), were not attenuated in AP20187-treated BubR1$^{H/H}$:INK-ATTAC-3 and BubR1$^{H/H}$:INK-ATTAC-5 mice (Figures 6A and 6B). This correlated with lack of

INK-ATTAC induction in heart and aorta (Figure 1C and 6B). Cardiac failure is presumably the main cause of death in BubR1$^{H/H}$ mice, which could explain why the overall survival of AP20187-treated BubR1$^{H/H}$:INK-ATTAC mice was not substantially extended (Figure 6C). To examine whether clearance of p16^{Ink4a}-positive cells might

5   have any overtly negative side effects, WT:INK-ATTAC mice were continuously treated with AP20187 until eight months of age. No such overtly negative side effects were observed. Taken together, these results indicated that continuous removal of p16^{Ink4a}-expressing cells from BubR1$^{H/H}$:INK-ATTAC mice selectively delays age-related phenotypes that depend on p16^{Ink4a} induction.

10   The following was performed to determine whether the delayed onset of age-related pathologies coincided with a reduction in the number of senescent cells in these tissues. IAT of AP20187-treated BubR1$^{H/H}$:INK-ATTAC mice exhibited a dramatic decrease in SA-β-Gal staining compared with IAT of untreated counterparts (Figure 7A). Corresponding decreases of other senescence-associated markers were also observed, as

15   well as expected reductions in INK-ATTAC and GFP (Figures 7B and 8A). Skeletal muscle (Figures 7C and 8B) and eye (Figures 7D and 8C) exhibited a similar reduction in senescence indicators. BrdU incorporation was lower in IAT and muscle tissue of untreated than in treated animals (Figure 7E), further supporting the contention that senescence-associated replicative arrest is decreased upon administration of AP20187 in

20   BubR1$^{H/H}$:INK-ATTAC transgenic animals. Together, these results indicated that senescent cells were cleared from tissues and that this delays acquisition of age-related dysfunction in BubR1 hypomorphic mice.

The results provided herein demonstrate the generation of a transgenic mouse model that allows for the inducible removal of p16^{Ink4a}-positive senescent cells. By

25   breeding this model into a progeroid mouse genetic background, the clearance of p16^{Ink4a}-expressing senescent cells selectively was shown to delay onset of age-related pathologies in tissues that accumulate these cells, demonstrating that development of age-related pathologies and cellular senescence are clearly linked in this model. These results also demonstrate that therapeutic interventions to clear senescent cells or block their effects

30   represent an avenue for treating or delaying age-related diseases and improving healthy human lifespan.

Methods and materials

   The INK-ATTAC transgenic construct was made as follows. The FKBP-Casp8
fragment was subcloned from the aP2-ATTAC transgenic construct (Pajvani *et al.*, *Nat.
Med.*, 11:797-803 (2005)), and inserted into pBlueScriptII (Stratagene). A 2617-bp
5   segment of the murine p16^{Ink4a} promoter was PCR amplified from BAC DNA to replace
the aP2 promoter. An IRES-EGFP fragment was inserted 3' of the ATTAC. Nine
transgenic founder lines of mice were obtained by injection of this construct into FVB
oocytes using standard methods. A PCR-based method was used for INK-ATTAC
transgene identification. BubR1$^{H/H}$ mice were generated as described elsewhere (Baker *et
10   al.*, *Nat. Genet.*, 36:744-749 (2004)). For AP20187 (ARIAD Pharmaceuticals, Inc.;
Cambridge, MA) treatments, animals were injected intraperitoneally (i.p.) every three
days with 0.2 μg/g body weight of the dimer-inducing drug (Pajvani *et al.*, *Nat. Med.*,
11:797-803 (2005)). All mice were on a mixed 129 x C57BL/6 x FVB genetic
background. Animals were housed in a pathogen-free barrier environment throughout the
15   study. Experimental procedures involving the use of laboratory mice were reviewed and
approved by the appropriate committee. GraphPad Prism software was used for
generating survival curves and for statistical analyses.

Cell culture

20   Bone marrow cells were obtained by flushing of tibia and femur bones of 2-
month-old WT:INK-ATTAC transgenic mouse lines and cultured as described elsewhere
(Soleimani and Nadri, *Nat. Protoc.*, 4:102-106 (2009)). In brief, after washing by
centrifugation at 400 x g for 10 minutes and counting of viable cells with trypan blue,
cells were resuspended in DMEM containing 15% FBS to a final concentration of 5 x 10^6
25   viable cells per mL. Initially, cells were plated in 6-well tissue culture dishes at 3.5
mL/well (1.9 x 10^6 cells/cm^2). Cultures were kept in a humidified 5% CO$_2$ incubator at
37°C for 72 hours, when non-adherent cells were removed by changing the medium.
Assays were performed on cells that had been trypsinized and seeded to confluency in 24-
well plates. To induce senescence and evaluate expression of the INK-ATTAC
30   transgene, cells were treated with 1 μM rosiglitazone (Cayman Chemical Company, Ann
Arbor, MI) or with vehicle. The accumulation of GFP-positive cells was observed by

fluorescence microscopy. After 5 days of rosiglitazone treatment, cells were then washed with PBS and treated with vehicle, 1 μM rosiglitazone, 10 nM AP20187, or both. After 48 hours, cultures were fixed and stained for SA-β-Gal activity as described elsewhere (Dimri *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:9363-9367 (1995)).

5

qRT-PCR and flow cytometry

RNA extraction, cDNA synthesis, and qRT-PCR from whole-mouse tissue were performed as described elsewhere (Baker *et al.*, *Nat. Cell Biol.*, 10:825-836 (2008)). To perform qRT-PCR on GFP[+] and GFP[−] cell populations of IAT, single-cell suspensions of
10   stromal vascular fraction were prepared from about 50 mg IAT as described elsewhere (Kirkland *et al.*, *Int. J. Obes. Relat. Metab. Disord.*, 20(Suppl 3):S102-107 (1996)). GFP[+] and GFP^{−} cells were then separated and collected using a FACS Aria Cell Sorter running FACSDiva software (BD Biosciences). RNA was extracted from these cells using an RNeasy Micro Kit (Qiagen), and cDNA synthesized using a WT-Ovation RNA
15   Amplification kit (NuGEN Technologies, Inc.) according to the manufacturers' protocols.

qRT-PCR primers were as follows: FKBP-Casp8 forward, GAATCACAGACT-TTGGACAAAGTT (SEQ ID NO:25); FKBPCasp8 reverse, GGTCAAAGCCCCT-GCATCCAAG (SEQ ID NO:26); EGFP forward, CAAACTACAACAGCCACAACG (SEQ ID NO:27); and EGFP reverse, GGTCACGAACTCCAGCAG (SEQ ID NO:28).
20   Sequences of other primers used were as described elsewhere (Baker *et al.*, *Nat. Cell Biol.*, 10:825-836 (2008)). Statistical differences were determined using two-tailed unpaired t tests.

Analysis of progeroid phenotypes

25   Bi-weekly checks for lordokyphosis and cataracts were performed as described elsewhere (Baker *et al.*, *Nat. Cell Biol.*, 10:825-836 (2008)). Skeletal muscle fiber diameter measurements were performed on cross sections of gastrocnemius and abdominal muscles of female mice (n = 6 mice per genotype). Fifty total fibers per sample were measured using a calibrated computer program (Olympus MicroSuite Five).
30   Fat cell diameter measurements were performed on IAT according to the same method. Dissection, histology, and measurements of dermal and adipose layers of dorsal skin were

performed as described elsewhere (Baker *et al.*, *Nat. Genet.*, 36:744-749 (2004)).

Measurements of body weight, length, gastrocnemius muscle, and assorted adipose

deposits were performed on 8–10-month-old females (n = 6 per genotype). Bone mineral

content, bone mineral density, and total body adipose tissue were analyzed by DEXA

5   scanning as described elsewhere (Krishnamurthy *et al.*, *J. Clin. Invest.*, 114:1299-1307

(2004)) (n = 6 per genotype). Exercise measurements were performed on 8–10-month-

old mice as described elsewhere (Handschin *et al.*, *J. Biol. Chem.*, 282:30014-30021

(2007); and LeBrasseur *et al.*, *J. Gerontol. A. Biol. Sci. Med. Sci.*, 64:940-948 (2009)).

Animals were acclimated for three days for 5 minutes at a speed of 5 m/minute prior to

10   experimentation. For the experiment, the speed of the treadmill began at 5 m/minute and

was increased to 8 m/minute after 2 minutes. Thereafter, the speed was increased at a rate

of 2 m/minute every 2 minutes, and the time (in seconds) and distance (in meters) to

exhaustion, as defined by an inability to move along the treadmill with stimulation, were

determined. The formula to determine the amount of work (J) performed was: mass (kg)

15   * g (9.8 m/s^2) * distance (m) * sin(θ) (with an incline of θ = 5°).

In vivo BrdU incorporation and SA-β-Gal staining

Analyses for *in vivo* BrdU incorporation were performed in 8–10-month-old

female mice (n = 6 per genotype) as described 13. Adipose tissue depots were stained for

20   SA-β-Gal activity as described elsewhere (Baker *et al.*, *Nat. Cell Biol.*, 10:825-836

(2008)).

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction

25   with the detailed description thereof, the foregoing description is intended to illustrate and

not limit the scope of the invention, which is defined by the scope of the appended claims.

Other aspects, advantages, and modifications are within the scope of the following

claims.

WHAT IS CLAIMED IS:

1.   A transgenic mouse, the nucleated cells of which contain a transgene, said transgene containing a promoter sequence operably linked to a nucleic acid sequence encoding a polypeptide having the ability to kill a cell or facilitate the killing of a cell when said transgenic mouse is administered a compound, wherein senescent cells of said transgenic mouse express said polypeptide, and wherein said senescent cells of said transgenic mouse are killed when said compound is administered to said transgenic mouse.

2.   The transgenic mouse of claim 1, wherein less than 10 percent of non-senescent cells of said transgenic mouse are killed when said compound is administered to said transgenic mouse.

3.   The transgenic mouse of claim 1, wherein said promoter sequence is a $p16^{Ink4a}$ promoter sequence.

4.   The transgenic mouse of claim 1, wherein said polypeptide comprises a caspase 8 polypeptide sequence.

5.   The transgenic mouse of claim 1, wherein said polypeptide comprises a FKBP polypeptide sequence.

6.   The transgenic mouse of claim 1, wherein said polypeptide is a FKBP-caspase 8 fusion polypeptide.

7.   The transgenic mouse of claim 1, wherein said compound is AP20187.

8.   The transgenic mouse of claim 1, wherein the genetic background of said transgenic mouse is a $BubR1^{H/H}$ genetic background.

9.   The transgenic mouse of claim 1, wherein said transgene comprises nucleic acid encoding a marker polypeptide.

10.   The transgenic mouse of claim 9, wherein said marker polypeptide is a GFP
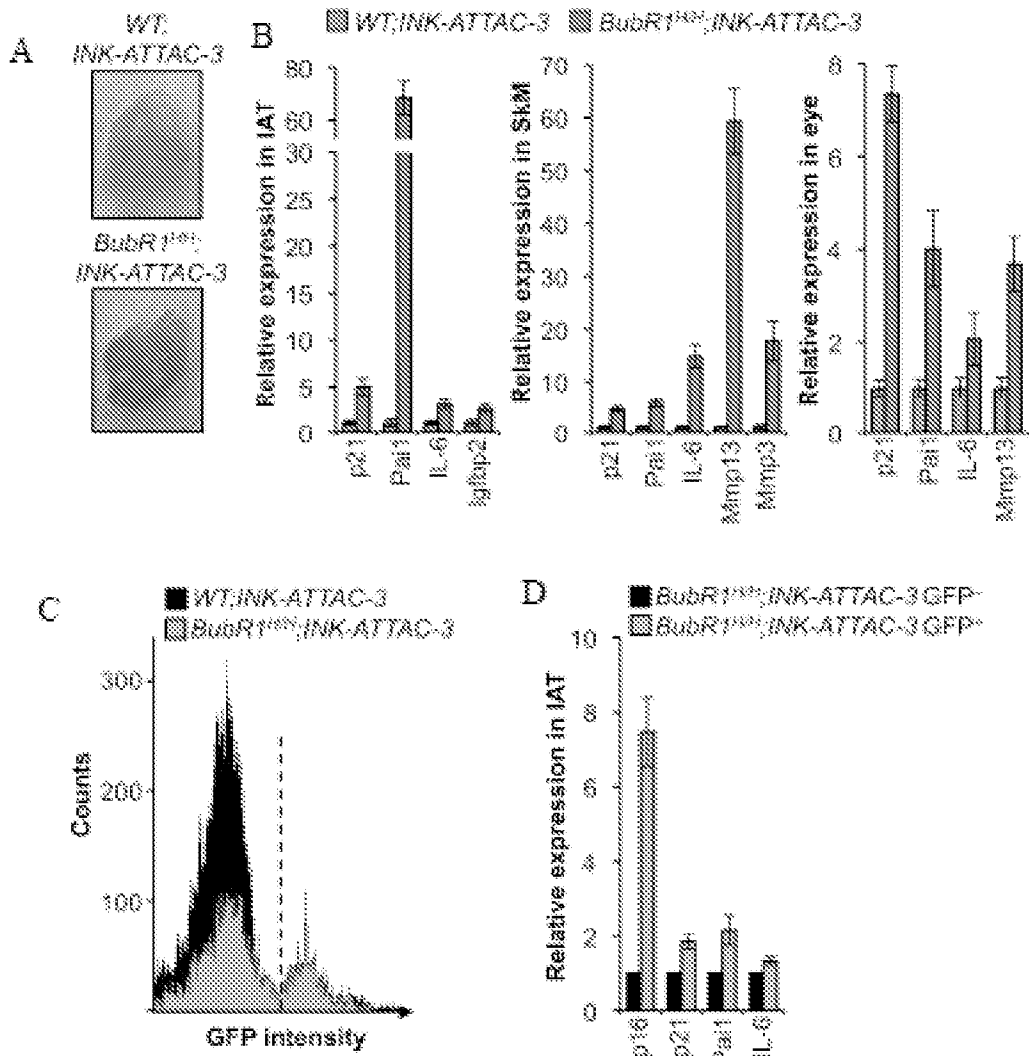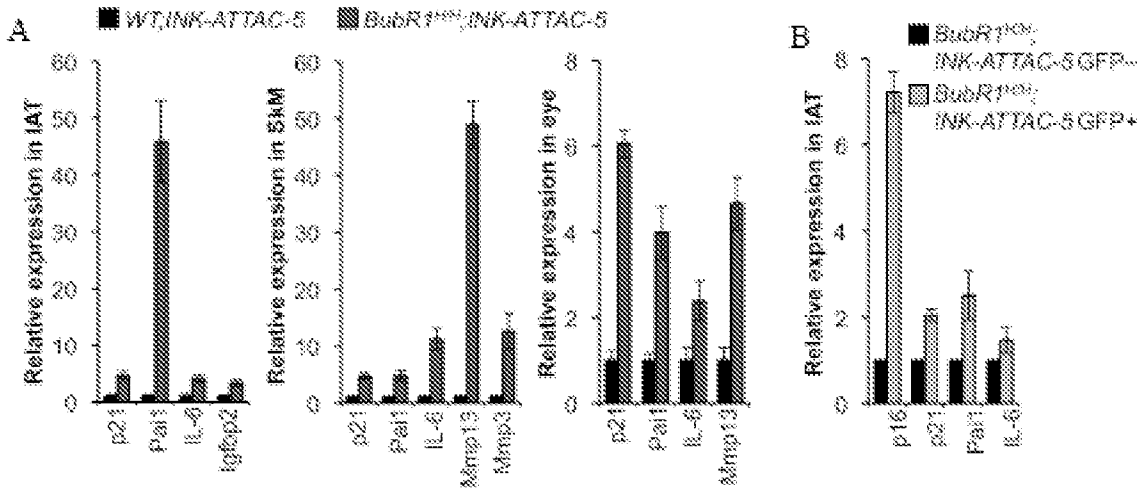5   polypeptide.

Figure 1
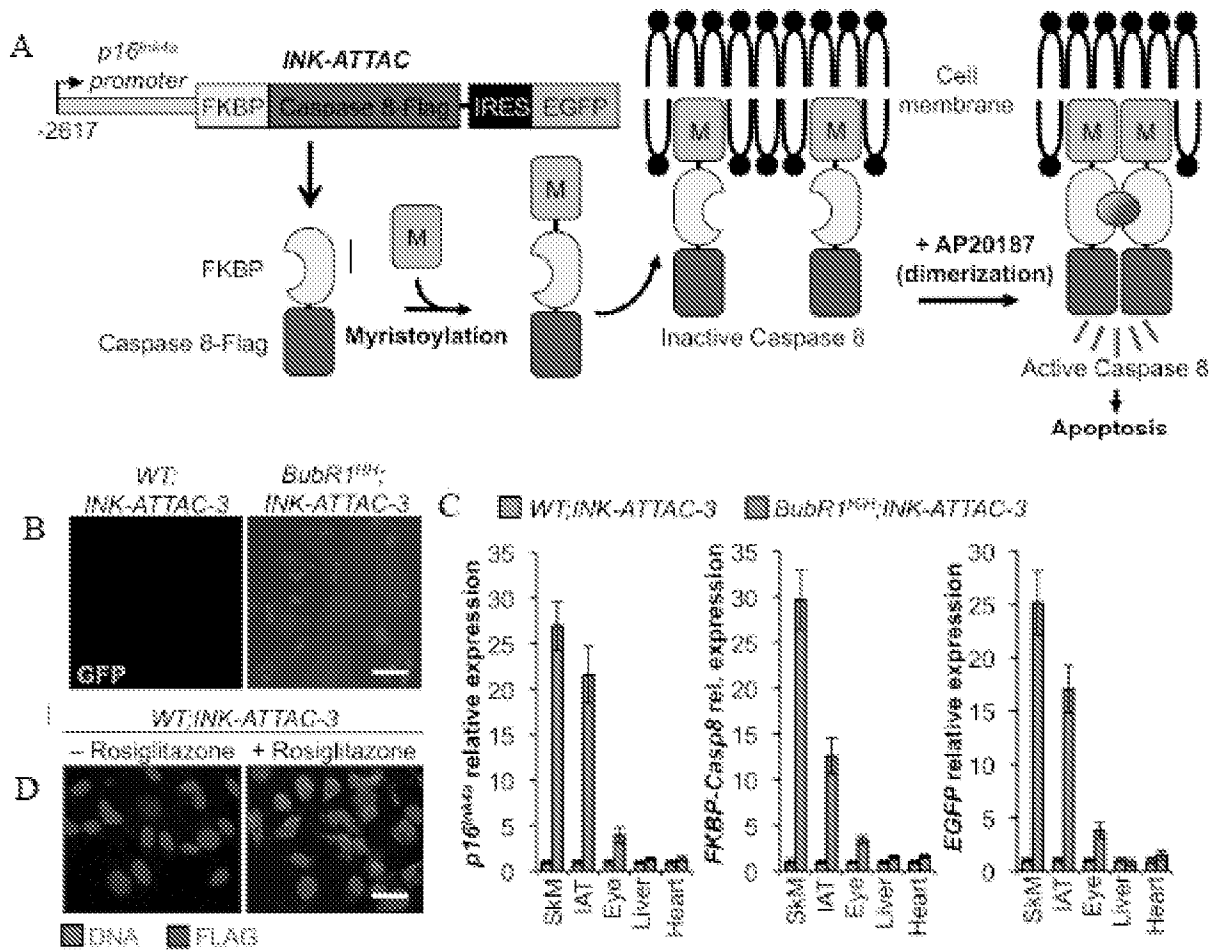
Figure 2
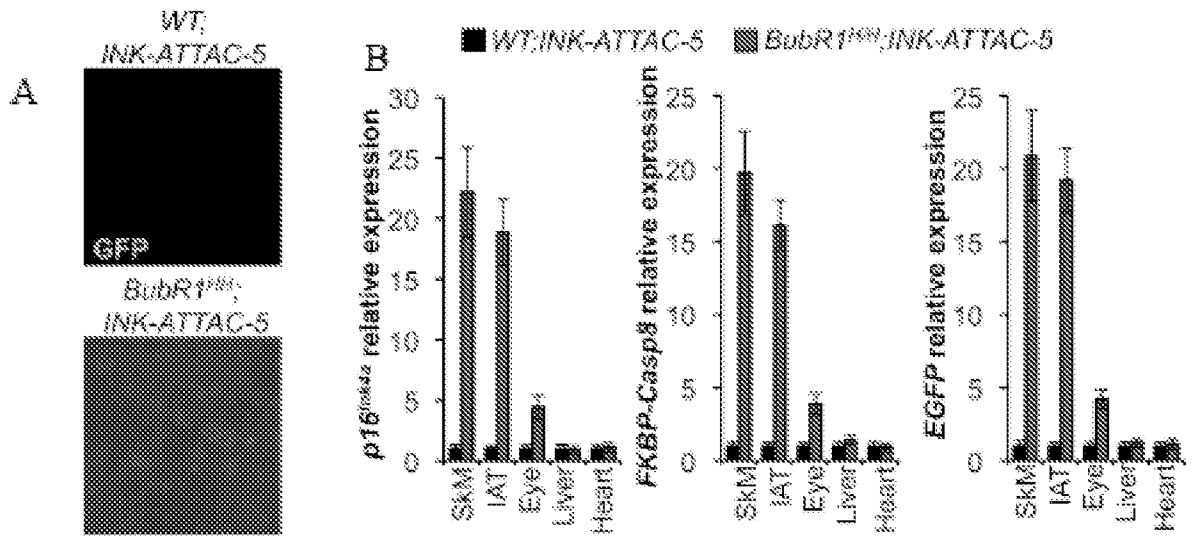
Figure 3

Figure 4

Figure 5

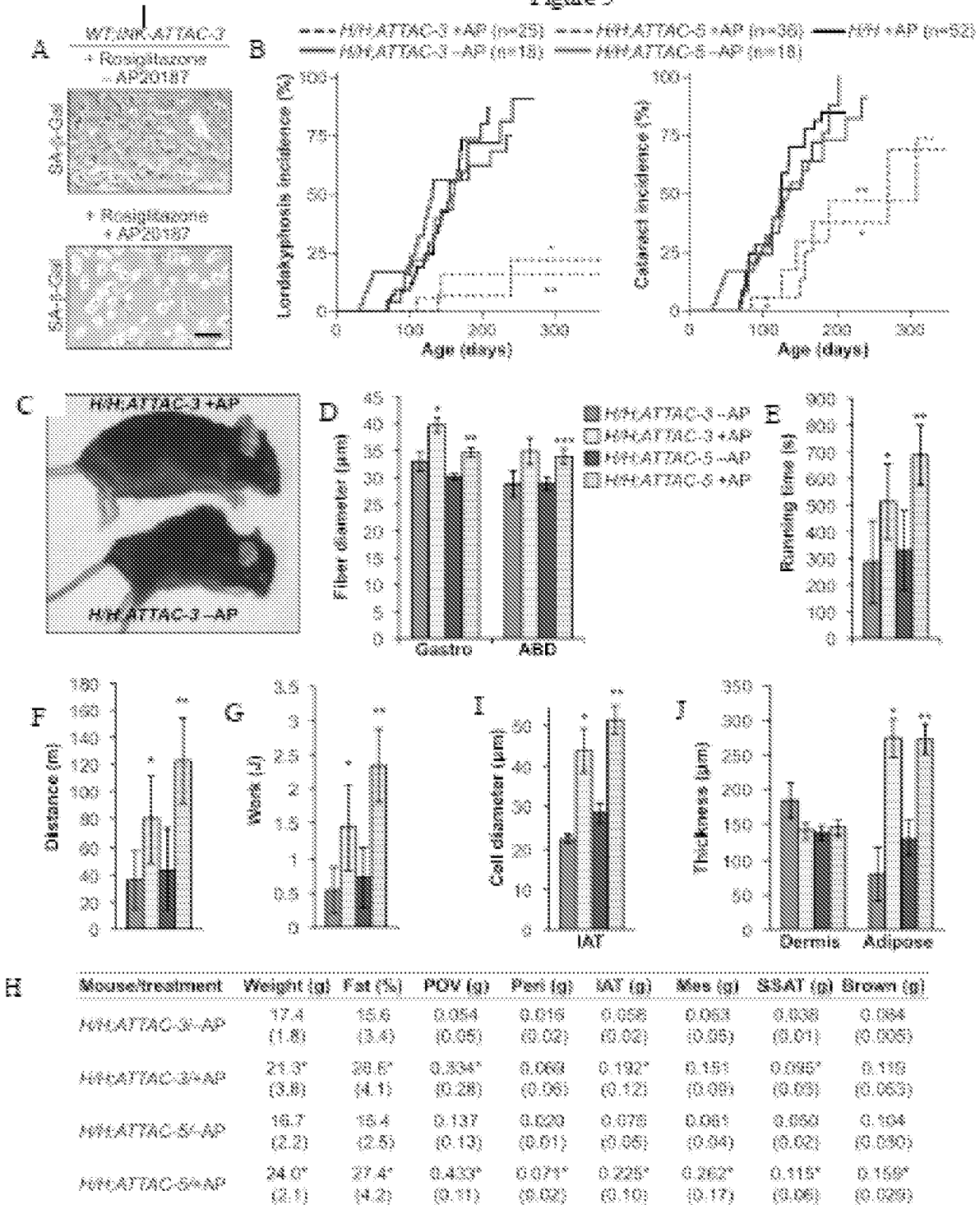## Figure 6

A

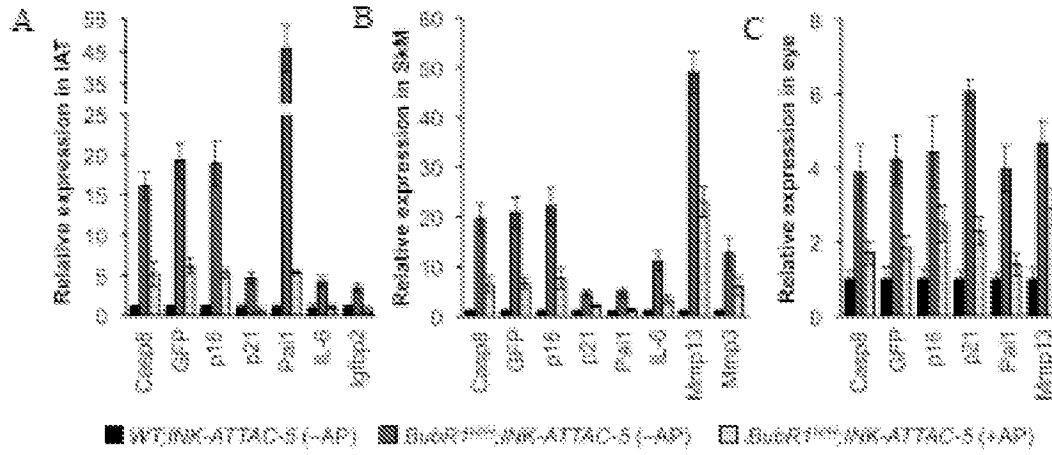| Genotype	BPM (SD)	Sinus pauses % (SD)
WT;INK-ATTAC-3 −AP (n=3)	485.3 (24.7)	0.024 (0.008)
HH;INK-ATTAC-3 −AP (n=6)	482.5 (35.2)	2.237 (0.135)
HH;INK-ATTAC-3 +AP (n=6)	456.7 (36.3)	2.176 (0.181)
WT;INK-ATTAC-5 −AP (n=3)	480.8 (29.3)	0.021 (0.004)
HH;INK-ATTAC-5 −AP (n=6)	456.7 (31.3)	2.124 (0.221)
HH;INK-ATTAC-5 +AP (n=6)	478.2 (33.9)	2.046 (0.189)

B

C
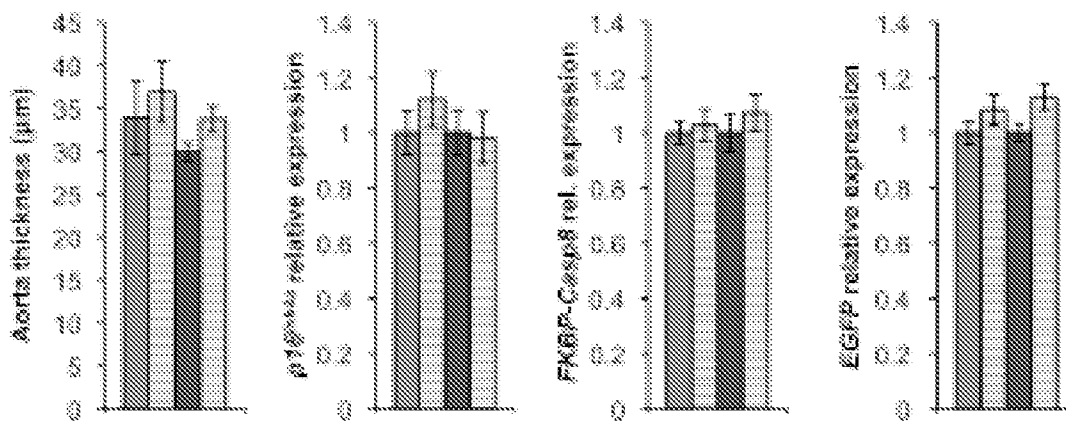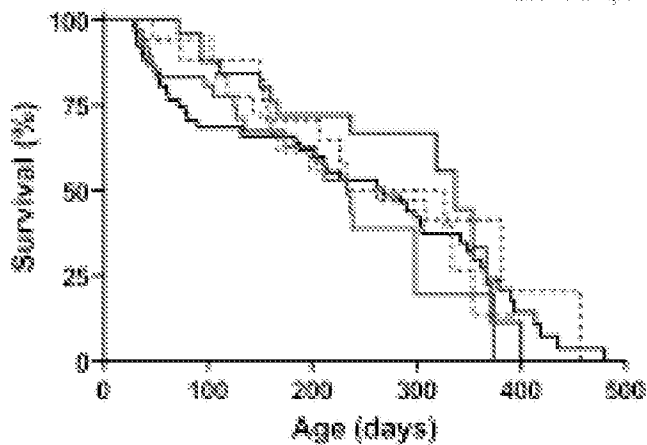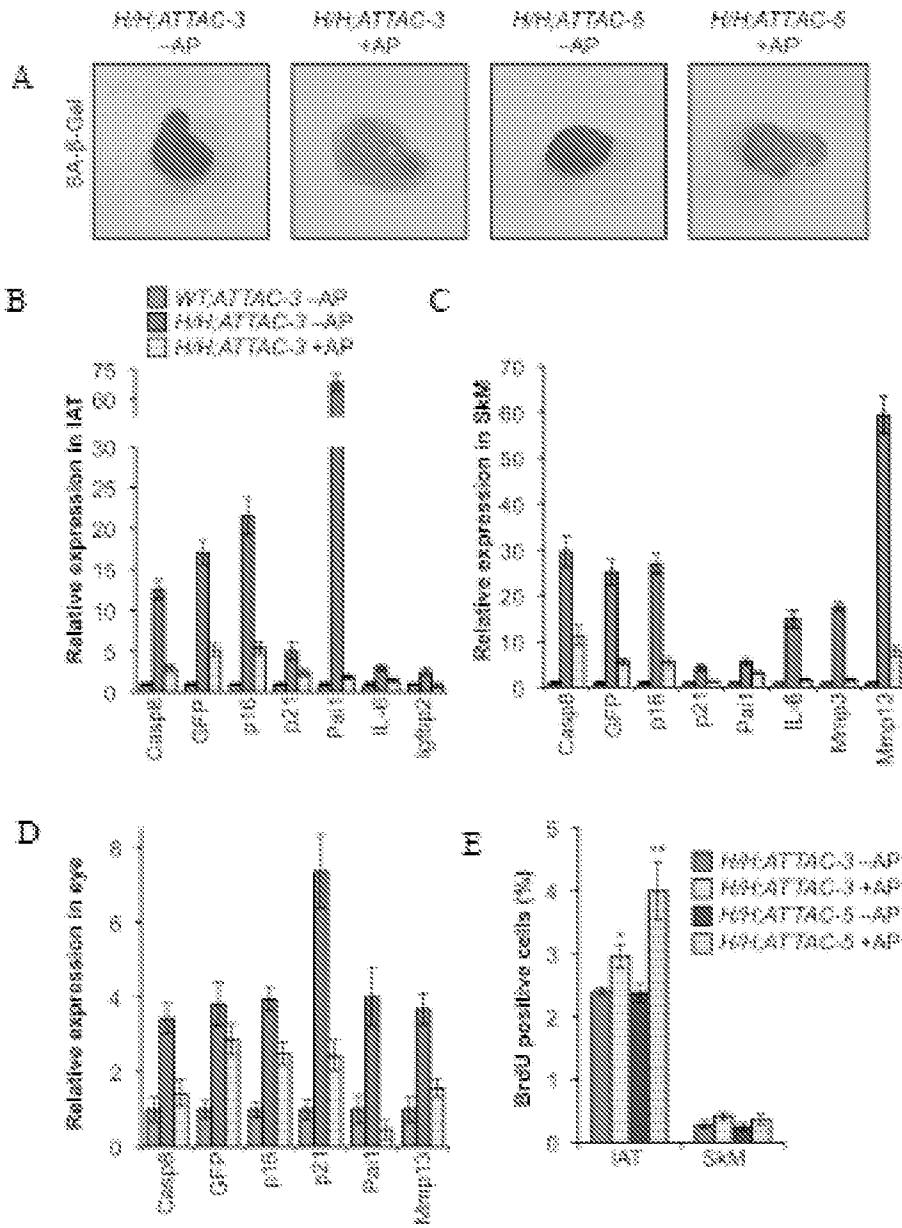
Figure 7

Figure 8

Figure 9

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Entire 9267 nt sequence, uninterrupted
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TGGCCTTTTCTAGGTGAATTTTATTTTCCTTACAGACCTCAAGAAGTTTACATAATTTACTTAA
ACCTGAGGAGAGAGAACAAAGCCTCAGAAAATTTACATAGTTTATTTAAACTAAACTCAGCTTG
CTTGGTAGCAGCTTCTAATCCCAGCAGTTAAAGAGACAGAAGCAGGGCCAACCTGGGGTATAAT
ATAAGGTGAGACTCTCCTTTCTTTCTCTCTGTCTCTGTCTGTCTCTGTCTCTGTGTGTGTGTGT
GTGTGTGTGTGTGTGTGTGTCTCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTG
TCTCTCTCTCCCTCCCCCTCCCTCCCTCTCCCCCTCCTCTCTCCCTCCCTCTCCCTCCCCCCCC
CCCACACATTTGAATTCGTGGAGTTGGTAAATGAGGGGTCAGTTCTCTGTCTGTCTGTAGTTTT
GTGTCCACAGGATATGACTGACATTCTCACCACACACATACAAAGTCAAAATAGCTGTGGCCA
TATAAAGAATATGGGGAGAGAAATTATTCAAAATCTGCAGAAAATAATGCCAGGCCTTTAATC
CTGGCACCCAGGAGGCAGAAGGGAGACAGAGTTCTGAGTTTATGCTGAGTTCCAGGAGTGGAAG
AAAGGGCCATTGCCTTTCTGGTGAGGACTGTCTTTTTAAATCCTCCCTTCTGTCCAGTACTGGT
AACTCTGCCCAAAGCGTGTTCTTCTTCCTGCCTCACAAGATTGCAAAGACGTTTTTAACGAACA
ATTTAAACCGGTGCAACGTTTATGCGCAGCACACCAACTCATTTAAACAAACAACAGCCCCATA
AAATAGAAATACTTTATAAGCAGATTGCCCTCCGATGACTTCACCCCGTCACTTTTTTATAGTT
GTGTACAGAATCCTAGCACTGATACAGCAACATCAGAAATGTTTCTGCAAATCCTTCGCAAAGA
TTCGGATTTCATACTGGGCGTGGTACCCTCCAAAATGAGTTGTTTGAGCTAGGGTTGTTGGGAT
CTCAGCTTGGCGAAGTTGTAGCTCTTTCTTCTGAATAAAAGATGACACAATTTTCTGCTAAGAT
```

Figure 9 (Continued)

GTTAAATACCTTAAGTTTCAGTGTAGTGATGAAAATTACCCTCCTTCGTTTTTCTAATACCTGG
GTGTTGCACTGGGGAGGAAGGAGAGATTTCGAGAAGGACTAGTTCACTTTCTCAGAAGACACGT
GTGCACTTCTTTGCTGTGCGGGTCCAGAAGGAGCCCAGCGTGTCAAAGGGTGACCAGGCATGGG
GGAGGGGTGTTAGCGTGGGTAGCAGGCGGGGGCTGTCCGATCCTTTAGCGCTGTTTCAACGCCC
AGCTCTCCTCCTGAACCCTGCATCTCTTCTGTAGTCCGGGCTCCATCCCTTTCCCCTCCCCCAT
CCGGAGGTGGGGGGAACAGCAGTGTTTTCAGGGGTGTTCAATTCATGCTATATTCAGGGCAAAT
AGCGCCACCTATGGCGGGCTGTGGAGCCAGGTCAGGAGCAGAGTGTGGCTCCCCCCCCCCCCCA
CACCATCCTCAGAGGAAGGAAGGAGGGACCCACTGGTCACACGACTGGGCGATTGGGCGGGCAC
TGAATCTCCGCGAGGAAAGCGAACTCGAGGAGAGCCATCACGCGTAGCatggggagtagcaaga
gcaagcctaaggaccccagccagcgctctagaggcgtccaagtcgaaaccattagtcccggcga
tggcagaacatttcctaaaaggggacaaacatgtgtcgtccattatacaggcatgttggaggac
ggcaaaaaggtggacagtagtagagatcgcaataaacctttcaaattcatgttgggaaaacaag
aagtcattaggggatgggaggagggcgtggctcaaatgtccgtcggccaacgcgctaagctcac
catcagccccgactacgcatacggcgctaccggacatcccggaattattcccctcacgctacc
ttggtgtttgacgtcgaactgttgaagctcgagactagaggagtgcaggtggagactatctccc
caggagacgggcgcaccttccccaagcgcggccagacctgcgtggtgcactacaccgggatgct
tgaagatggaaagaaagttgattcctcccgggacagaaacaagccctttaagtttatgctaggc
aagcaggaggtgatccgaggctgggaagaaggggttgcccagatgagtgtgggtcagagagcca
aactgactatatctccagattatgcctatggtgccactgggcacccaggcatcatcccaccaca
tgccactctcgtcttcgatgtggagcttctaaaactggaaactagtagtgaatcacagactttg
gacaaagtttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaatt
ttgcaaaagcacgggagaaagtgcccaaacttcacagcattagggacaggaatggaacacactt
ggatgcagggctttgaccacgacctttgaagagcttcattttgagatcaagccccacgatgac
tgcacagtagagcaaatctatgagattttgaaaatctaccaactcatggaccacagtaacatgg
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ggaggcccccatctatgagctgacatctcagttcactggtttgaagtgcccttcccttgctgga
aaacccaaagtgttttttattcaggcttgtcaggggaataactaccagaaaggtatacctgttg
agactgattcagaggagcaaccctatttagaaatggatttatcatcacctcaaacgagatatat
cccggatgaggctgactttctgctggggatggccactgtgaataactgtgtttcctaccgaaac
cctgcagagggaacctggtacatccagtcactttgccagagcctgagagagcgatgtcctcgag
gcgatgatattctcaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaa
aaacatggggaaacagatgcctcagcctactttcacactaagaaaaaaacttgtcttcccttct
gatgattacaaggatgacgacgataagtgaggatcaacctcgaggaattcACGCGTTTAATTAA
CTCGAGGTTTTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCGCCCCTCTCCCTCCC
CCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTT
ATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTG
ACGAGCATTCCTAGGGGTCTTTCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGA
AGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCA
GCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCT
GCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGC
TCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCATTGTATGGGATC
TGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGC

Figure 9 (Continued)

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CCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACAACCATG
GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACG
TAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG
ACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGT
CCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAA
GACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATC
GACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG
TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACAT
CGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCC
GTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGA
AGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGA
GCTGTACAAGTAAAGCGGCCGCGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAG
CCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGA
ATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGA
GTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATA
AAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTG
ACTTGAGGTTAGATTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAAT
TTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGT
CCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCT
GTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAG
TGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCG
CTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTC
CCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATG
GCTGACTAATTTTTTTATTTATGCAGAGGCCGAGGCCGCCTAAACGGCCGGCCATcgataccg
tcgacctcgagggggggcccggtacccagcttttgttccctttagtgagggttaattgcgcgct
tggcgtaatcatggtcatagctgtttcctgtgtgaaattgttatccgctcacaattccacacaa
catacgagccggaagcataaagtgtaaagcctggggtgcctaatgagtgagctaactcacatta
attgcgttgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaa
tcggccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctcgctcactga
ctcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacgg
ttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggcca
ggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccctgacgagcatca
caaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgttt
ccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccg
cctttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggt
gtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgcc
ttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcag
ccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtg
gcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagccagttacc
ttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggttttt
ttgtttgcaagcagcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttc
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Figure 9 (Continued)

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tacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatca
aaaaggatcttcacctagatccttttaaattaaaaatgaagttttaaatcaatctaaagtatat
atgagtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctg
tctatttcgttcatccatagttgcctgactccccgtcgtgtagataactacgatacgggagggc
ttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctccagatttat
cagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctc
catccagtctattaattgttgccgggaagctagagtaagtagttcgccagttaatagtttgcgc
aacgttgttgccattgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattca
gctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttag
ctccttcggtcctccgatcgttgtcagaagtaagttggccgcagtgttatcactcatggttatg
gcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagt
actcaaccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccggcgtcaat
acgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaaacgttcttcg
gggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacccactcgtgcac
ccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggca
aaatgccgcaaaaaagggaataagggcgacacggaaatgttgaatactcatactcttcctttt
caatattattgaagcatttatcagggttattgtctcatgagcggatacatttgaatgtattt
agaaaaataaacaaatagggggttccgcgcacatttccccgaaaagtgccac    (SEQ ID
NO:1)
```

Figure 10

ctaaattgtaagcgttaatattttgttaaaattcgcgttaaattttttgttaaatcagctcattt
tttaaccaataggccgaaatcggcaaaatcccttataaatcaaaagaatagaccgagatagggt
tgagtg (SEQ ID NO:2)

F1 ori:
ttgttccagtttggaacaagagtccactattaaagaacgtggactccaacgtcaaagggcgaaa
aaccgtctatcagggcgatggcccactacgtgaaccatcaccctaatcaagttttttggggtcg
aggtgccgtaaagcactaaatcggaaccctaaagggagccccgatttagagcttgacggggaa
agccggcgaacgtggcgagaaaggaagggaagaaagcgaaaggagcgggcgctagggcgctggc
aagtgtagcggtcacgctgcgcgtaaccaccacacccgccgcgcttaatgcgccgctacagggc
gcgtc (SEQ ID NO:3)

LacZ alpha:
ccattcgccattcaggctgcgcaactgttgggaagggcgatcggtgcgggcctcttcgctatta
cgccagctggcgaaaggggggatgtgctgcaaggcgattaagttgggtaacgccagggttttccc
agtcacgacgt (SEQ ID NO:4)

M13 fwd:
tgtaaaacgacggccagtgagcgcgc (SEQ ID NO:5)

T7:
gtaatacgactcactatagggcgaattggagctccaccgcggtggcggccgctctagaactagt
g (SEQ ID NO:6)

BAMH1, p16 promoter:
GATCC (SEQ ID NO:7)

forprimer3, p16 promoter:
GTGTAAAGTCACT (SEQ ID NO:8)

Figure 10 (Continued)

p16 promoter:
CTTTTATAGCTACATCTGCATAGATCCCCTGTATGAAAGCATGTACTACCTGGATAATAATATC
TGTATTTTTCTGTAGTAGGAAATCAGTGTAGTTTTTAAAACCAAAAGTATTGTTATTAATCTA
TCTTTGATCTCAAACAATTTCAATGACCTAGTATAGTGATTTCTACGGAAAGCCCTGCAATTTA
CTCAAAGCAGTTTTTAAATATTGTTTTAAAAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
TGTGTGGTGTTAAAGTCATTTTCAAACCCCTCACAATGTCTTGAATGTGACATTTGAGTCATTT
ATGGTAACTTATAACTCCTTTGAAGAAGTTATTCAGAATTGAGGTTCCAGACACACAAATGCAC
AATACACCATTTTTCCTTCCAGTTAACAATCAGAGGGCAACACTTATTTTTAAAGGAAATCGA
CTCCATAAGGGACTTTATAAAGGGGTAGACATAAACCAGTATCAGGGATAAACTCTCCGTTCCC
CTGTTTAACCTAATTTTCCCAGGGCCATCCTGGAATACGAATTTTCTCTTGAAATACAGTCAAA
GAAAAGTGGTAGGCTACAGAGCAGAGGAAACACTGGACACAGCGACCCACCCCAGAGTCACTT
CCCTTAATCTAATGACTAGGTTTTTTCTGAAAGTTATTTTGTTAGAACACAGGAACTTTTGCGA
CCACAGTGATGCTTTTAGAGGGTTGAATCCTCAAAAAGAAAATTAATCGCAACTAGTAGAAGGG
AGATTACTTATTGATTCTTATAACTTCTGCAGGAATACACAGTTATGAGTTAGGGCAAAGAGAA
AATTGACTTTTAATATTCTCTATCACTAACATGAGAGAACATGTATGTGTTCCAAAATAATTTT
TATTTATTGAAAACCCGCTATATACCTGGATTTTCACAGAATATTCATTACTCTCCAAAATGGC
CTTTTCTAGGTGAATTTTATTTTCCTTACAGACCTCAAGAAGTTTACATAATTTACTTAAACCT
GAGGAGAGAGAACAAAGCCTCAGAAAATTTACATAGTTTATTTAAACTAAACTCAGCTTGCTTG
GTAGCAGCTTCTAATCCCAGCAGTTAAAGAGACAGAAGCAGGGCCAACCTGGGGTATAATATAA
GGTGAGACTCTCCTTTCTTTCTCTCTGTCTCTGTCTGTCTCTGTCTCTGTGTGTGTGTGTGTGT
GTGTGTGTGTGTGTGTGTCTCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTGTCTC
TCTCTCCCTCCCCCTCCCTCCCTCTCCCCCTCCTCTCTCCCTCCCTCTCCCTCCCCCCCCCCCA
CACATTTGAATTCGTGGAGTTGGTAAATGAGGGGTCAGTTCTCTGTCTGTCTGTAGTTTTGTGT
CCACAGGATATGACTGACATTCTCACCACACACATACAAAGTCAAAAATAGCTGTGGCCATATA
AAGAATATGGGGAGAGAAAATTATTCAAAATCTGCAGAAAATAATGCCAGGCCTTTAATCCTGG
CACCCAGGAGGCAGAAGGGAGACAGAGTTCTGAGTTTATGCTGAGTTCCAGGAGTGGAAGAAAG
GGCCATTGCCTTTCTGGTGAGGACTGTCTTTTTAAATCCTCCCTTCTGTCCAGTACTGGTAACT
CTGCCCAAAGCGTGTTCTTCTTCCTGCCTCACAAGATTGCAAAGACGTTTTTAACGAACAATTT
AAACCGGTGCAACGTTTATGCGCAGCACACCAACTCATTTAAACAAACAACAGCCCCATAAAAT
AGAAATACTTTATAAGCAGATTGCCCTCCGATGACTTCACCCCGTCACTTTTTTATAGTTGTGT
ACAGAATCCTAGCACTGATACAGCAACATCAGAAATGTTTCTGCAAATCCTTCGCAAAGATTCG
GATTTCATACTGGGCGTGGTACCCTCCAAAATGAGTTGTTTGAGCTAGGGTTGTTGGGATCTCA
GCTTGGCGAAGTTGTAGCTCTTTCTTCTGAATAAAGATGACACAATTTTCTGCTAAGATGTTA
AATACCTTAAGTTTCAGTGTAGTGATGAAAATTACCCTCCTTCGTTTTTCTAATACCTGGGTGT
TGCACTGGGGAGGAAGGAGAGATTTCGAGAAGGACTAGTTCACTTTCTCAGAAGACACGTGTGC
ACTTCTTTGCTGTGCGGGTCCAGAAGGAGCCCAGCGTGTCAAAGGGTGACCAGGCATGGGGGAG
GGGTGTTAGCGTGGGTAGCAGGCGGGGCTGTCCGATCCTTTAGCGCTGTTTCAACGCCCAGCT
CTCCTCCTGAACCCTGCATCTCTTCTGTAGTCCGGGCTCCATCCCTTTCCCCTCCCCCATCCGG
AGGTGGGGGGAACAGCAGTGTTTTCAGGGGTGTTCAATTCATGCTATATTCAGGGCAAATAGCG
CCACCTATGGCGGGCTGTGGAGCCAGGTCAGGAGCAGAGTGTGGCTCCCCCCCCCCCCCACACC
ATCCTCAGAGGAAGGAAGGAGGGACCCACTGGTCACACGACTGGGCGATTGGGCGGGCACTGAA
TCTCCGCGAGGAAAGCGAACTCGAGGAGAGCCATCACGCGTAGC   (SEQ ID NO:9)

Figure 10 (Continued)

FKBP:
atggggagtagcaagagcaagcctaaggaccccagccagcgctctagaggcgtccaagtcgaaa
ccattagtcccggcgatggcagaacatttcctaaaaggggacaaacatgtgtcgtccattatac
aggcatgttggaggacggcaaaaaggtggacagtagtagagatcgcaataaacctttcaaattc
atgttgggaaaacaagaagtcattaggggatgggaggagggcgtggctcaaatgtccgtcggcc
aacgcgctaagctcaccatcagccccgactacgcatacggcgctaccggacatcccggaattat
tccccctcacgctaccttggtgtttgacgtcgaactgttgaagctcgagactagaggagtgcag
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actacaccgggatgcttgaagatggaaagaaagttgattcctcccgggacagaaacaagccctt
taagtttatgctaggcaagcaggaggtgatccgaggctgggaagaaggggttgcccagatgagt
gtgggtcagagagccaaactgactatatctccagattatgcctatggtgccactgggcacccag
gcatcatcccaccacatgccactctcgtcttcgatgtggagcttctaaaactggaaactagt
(SEQ ID NO:10)

Casp8:
agtgaatcacagactttggacaaagtttaccaaatgaaaagcaaacctcggggatactgtctga
tcatcaacaatcacaattttgcaaaagcacgggagaaagtgcccaaacttcacagcattaggga
caggaatggaacacacttggatgcaggggctttgaccacgacctttgaagagcttcattttgag
atcaagccccacgatgactgcacagtagagcaaatctatgagattttgaaaatctaccaactca
tggaccacagtaacatggactgcttcatctgctgtatcctctcccatggagacaagggcatcat
ctatggcactgatggacaggaggcccccatctatgagctgacatctcagttcactggtttgaag
tgcccttcccttgctggaaaacccaaagtgttttttattcaggcttgtcagggggataactacc
agaaaggtatacctgttgagactgattcagaggagcaaccctatttagaaatggatttatcatc
acctcaaacgagatatatcccggatgaggctgactttctgctggggatggccactgtgaataac
tgtgtttcctaccgaaaccctgcagagggaacctggtacatccagtcactttgccagagcctga
gagagcgatgtcctcgaggcgatgatattctcaccatcctgactgaagtgaactatgaagtaag
caacaaggatgacaagaaaaacatggggaaacagatgcctcagcctactttcacactaagaaaa
aaacttgtcttcccttctgat   (SEQ ID NO:11)

Flag/Tag/Stop:
Gattacaaggatgacgacgataagtga   (SEQ ID NO:12)

3'UTR:
ggatc   (SEQ ID NO:13)

Multiple cloning site (MluI, PacI, XhoI, PmeI)
aacctcgaggaattcACGCGTTTAATTAACTCGAGGTTT (SEQ ID NO:14)

Figure 10 (Continued)

IRES, GFP:
TCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCGCCCCTCTCCCTCCCCCCCCCTAA
CGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACC
ATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTC
CTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGT
TCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCC
CCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGG
CACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAG
CGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGG
CCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACC
ACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACAACCATGGTGAGCAAGG
GCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTC
ATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCG
TGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCC
CGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC
GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGG
AGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCAT
GGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGC
AGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGC
CCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGA   (SEQ ID
NO:15)

Rabbit B-globin PA:
GAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGAC
GAGCTGTACAAGTAAAGCGGCCGCGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA
AGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTG
GAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAAT
GAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTA
TAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAGCCT
TGACTTGAGGTTAGATTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAA
ATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCT
GTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAAT   (SEQ ID
NO:16)

M13-rev:
CATGGTCATAGCTGTTTCCTGTGTGA   (SEQ ID NO:17)

Figure 10 (Continued)

LacO:
AATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGG
AAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTC
CGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTT
TTTTATTTATGCAGAGGCCGAGGCCGCCT   (SEQ ID NO:18)

Fse1, linker:
AAACGGCCGGCCATcgataccgtcgacctcgagggggggcccggtacccagcttttgt   (SEQ
ID NO:19)

T3:
Tccctttagtgagggttaattgcgcgcttggcgtaat   (SEQ ID NO:20)

M13-rev:
Catggtcatagctgtttcctgtgtga   (SEQ ID NO:21)

LacO:
Aattgttatccgctcacaattccacacaacatacgagccggaagcataaagtgtaaagcctggg
gtgcctaatgagtgagctaactcacattaattgcgttgcgctcactgcccgctttccagtcggg
aaacctgtcgtgccagctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtatt
gggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcgg
tatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaa
catgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaa   (SEQ ID NO:22)

ColE1 origin:
Ggccgcgttgctggcgttttttccataggctccgcccccctgacgagcatcacaaaaatcgacgc
tcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttccccctggaagct
ccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttc
gggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgc
tccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaact
atcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacag
gattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggc
tacactagaaggacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagag
ttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagca
gcagattacgcgcagaaaaaaggatctcaagaagatcctttgatcttttctacggggtctgac
gctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaaggatcttca
cctagatccttttaaattaaaaatgaagttttaaatcaatctaaagtatatatgagtaaacttg
gtctgacagtta   (SEQ ID NO:23)

Figure 10 (Continued)

AmpR:
Ccaatgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcc
tgactccccgtcgtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaa
tgataccgcgagacccacgctcaccggctccagatttatcagcaataaaccagccagccggaag
ggccgagcgcagaagtggtcctgcaactttatccgcctccatccagtctattaattgttgccgg
gaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccattgctacaggca
tcgtggtgtcacgctcgtcgtttggtatggcttcattcagctccggttcccaacgatcaaggcg
agttacatgatcccccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtc
agaagtaagttggccgcagtgttatcactcatggttatggcagcactgcataattctcttactg
tcatgccatccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaata
gtgtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacatagc
agaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttac
cgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagcatctttac
tttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaaaaaagggaataagg
gcgacacggaaatgttgaatactcatactcttcctttttcaatattattgaagcatttatcagg
gttattgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaataggggttcc
gcgcacatttccccgaaaagtgccac   (SEQ ID NO:24)

A.	CLASSIFICATION OF SUBJECT MATTER

A01K 67/027(2006.01)i, C12N 15/12(2006.01)i

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)
  A01K 67/027; G01N 33/53; C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
  Korean utility models and applications for utility models
  Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
  eKOMPASS(KIPO internal) & Keywords: transgenic mouse, promoter, killing cell, senescent cell

C.	DOCUMENTS CONSIDERED TO BE RELEVANT	

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	BAKER, D. J. et al., Clearance of p16Ink4a-positive senescent cells delays ageing -associated disorders, Nature, 10 November 2011, Vol.479, pp.232-237. See figure 1, page 232 left column, lines 27-35, left column, line 40 - right column, line 2, right column, lines 16-21, and page 235 right column, lines 53-67.	1-10
A	PAJVANI, U, B. et al., Fat apoptosis through targeted activation of caspase 8: a new model of inducible and reversible lipoatrophy, Nature medicine, July 2005, Vol.11, No.7, pp.797-803. See figure 1, page 797 left column, lines 1-8, and right column, lines 2-13.	1-10
A	DRABEK, D. et al., The expression of bacterial nitroreductase in transgenic mice results in specific cell killing by the prodrug CB1954, Gene therapy, February 1997, Vol.4, No.2, pp.93-100. See figure 1, page 93 right column, lines 25-35, and page 95 left column, line 34 - right column, line 10.	1-10
A	US 2008-0216180 A1 (ABATE-SHEN CORRINE T. et al.) 04 September 2008. See paragraphs 0155, 0167, claims 1, 5.	1-10

☐ Further documents are listed in the continuation of Box C.	☒ See patent family annex.

* Special categories of cited documents:	"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
"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	"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
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)	"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
"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	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 NOVEMBER 2012 (29.11.2012)	**29 NOVEMBER 2012 (29.11.2012)**

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008-0216180 A1	04.09.2008	None	