Supplementary information, Data S1.

Materials and Methods

Antibodies

Antibodies for western blotting and immunostaining were purchased from the following companies. Abcam: anti-NRF2 (ab62352), anti-Lamin B1 (ab16048), anti-WRN (ab200), anti-H3K9me3 (ab8898), anti-NANOG (ab21624), anti-SMA (ab32575), anti-P65 (ab16502); Santa Cruz Biotechnology: anti-OCT4 (sc-5279), anti-SOX2 (sc-17320), anti-Lamin A/C (sc-6215), anti-lamin B1 (sc-6217), anti-β-tubulin (sc-5274), anti-Actin (sc-69879), anti-GAPDH (sc-25778); anti-NQO1 (sc-32793); Cell Signaling Technology: anti-FOXA2 (8186S), anti-HP1α (2616S), anti-P21 (2947s); BD Bioscience: anti-LAP2 (611000), anti-CD73 (550741), anti-CD90 (555595), anti-CD31 (555445), anti-P16 (550834), anti-CD34 (555821), anti-CD43 (555475), anti-CD45 (555482), anti-CD144 (560410); Sigma: anti-TuJ1 (T2200), anti-Laminin (L9393); Vector Laboratories: anti-Ki67 (VP-RM04); ZSGB-Bio: anti-hSMA (ZM-0003); Bethyl Laboratories: anti-53BP1 (A300-273A); DSHB: anti-PAX7 (AB528428); eBioscience: anti-CD105 (17-1057); Enzo: anti-HO-1 (ADI-SPA-895-D); Biolegend: anti-Nuclear Pore Complex Proteins (902904); Dako: anti-VWF (A082).

Cell culture

Human H9 ESCs (WiCell Research) and their genetic modified derivatives were maintained on mitomycin C-inactivated mouse embryonic fibroblast (MEF) feeder in human ESC medium (DMEM/F12 (Invitrogen), 20% Knockout Serum Replacement (Invitrogen), 0.1 mM non-essential amino acids (NEAA, Invitrogen), 2 mM GlutaMAX (Invitrogen), 55 μM β-mercaptoethanol (Invitrogen), and 10 ng/ml bFGF (Joint Protein Central)) or on Matrigel (BD Biosciences) in mTeSR medium (STEMCELL techonology). All hMSCs were cultured in hMSC culture medium (αMEM (Gibco), 10% fetal bovine serum (FBS, Hyclone), 1% penicillin/streptomycin (Gibco) and 1 ng/mL bFGF (Joint Protein Central)). TMSCs were cultured in hMSC medium without bFGF.

Gene editing at *NRF2* locus

Helper-dependent adenoviral vector (HDAdV) for *NRF2* A245G knock-in was generated same as previous report with some modifications [1-6]. In brief, intron 1-2 of *NRF2* was PCR-amplified from RP11-483K11 BAC DNA (BACPAC Resources) and subcloned into the pCR2.1-TOPO vector (Invitrogen). The A245G mutation at exon 2 was introduced with 2 primers (AACTAGATGAAGAGACAGGTGGATTTCTCCCAA and ACCTGTCTCTTCATCTAGTTGTAACTGAGC) using the GeneTailor Site-Directed Mutagenesis System (Invitrogen). An *FRT*-PGK-EM7-neo-bpA-*FRT* fragment was subcloned into a site 397 bp downstream of exon 2 in the A245G mutated plasmid, and the generated DNA fragment was recombined into RP11-483K11 BAC DNA using BAC recombineering [7]. A total of 22.7 kb of *NRF2* homology, including the A245G mutation and marker cassette, was subcloned into the HDAdV plasmid pCIHDAdGT8-4 (kindly provided by Dr. Kohnosuke Mitani) [8]. The generated plasmids were linearized by PI-SceI (NEB) and transfected into 116 cells (kindly provided by Dr. Philip Ng) in the presence of helper virus AdHPBGF35 (kindly provided by Dr. André M. Lieber) [9, 10]. Crude virus extracts were serially amplified in 116 cells and then purified according to a previously described method [9]. β -gal -transducing units (btu) were determined in 293 cells to define infectious vector titers.

To further generate heterozygous *NRF2* A245G mutation knock-in hESCs, 2×10^6 feeder-free cultured H9 hESCs were dissociated by TrypLE, and resuspended in 1 mL MEF-conditioned medium containing 10 μM Y-27632. Cells were infected with *NRF2* A245G knock-in-HDAdV at MOI of 0.6-6 btu/cell at 37 ℃ for 1 h, and after brief centrifugation resuspended in 10 mL MEF-conditioned medium containing 10 μM Y-27632. Cells were plated onto 100 mm dishes precoated with 1×10^6 irradiated neomycin-resistant MEFs. 2 days after infection, G418 (100 μg/mL) was added to the medium to start positive selection. After 9 days, GANC (2 μM) in addition to G418 was added to the medium to start negative selection. After an additional 7 days, G418/GANC double-resistant clones were transferred to 96-well plates and expanded for further characterization. Gene-targeting efficiency was determined by PCR of genomic DNA from drug-resistant clones with the primers (5' gene targeting, NRF2A245G5'KOF: CTTGCCCCTACAAAATGTGCTGCCAGTTCCA and neoF2: CTACCTGCCCATTCGACCACCAAGCGAAACATC; 3' gene targeting, PGK-R:

CCCCAAAGGCCTACCCGCTTCCATTGCTCA and NRF2A245G3'KOR: ACCTGATGACACCATTTGTTTCCCTGCAAGTCT) using PrimeSTAR GXL DNA Polymerase (Takara). To determine mutation knock-in events, genomic DNA was extracted from the gene-targeted clones. Exon 2 of *NRF2* was PCR-amplified with NRF2A245GseqF (ACCATTTGTGACTTTGCCCTTTAGTGACCTCTACCATC) and NRF2A245GseqR (AACCTGCCATAACTTTCCCAAGAACTGA) using PrimeSTAR GXL DNA Polymerase. Amplicons were sequenced with an ABI 3730 sequencer (Applied Biosystems). To remove the neomycin-resistance cassette from intron 2, we followed the same procedure as previously described [1]. To generate homozygous *NRF2* A245G knock-in hESCs, we repeated 2nd round of mutation knock-in using the generated heterozygous *NRF2* A245G knock-in clones.

Knockout of *WRN* gene in *NRF2* A245G knock-in hESCs

CRISPR/Cas9-mediated gene editing was performed as previously described with several modifications [11]. In brief, WRN gRNA (TACATAAACAGGTGGATAC) targeting Exon 14 of *WRN* was cloned into gRNA-mCherry vector (WRN-gRNA-mCherry). *NRF2* A245G knock-in hESCs were treated with ROCK inhibitor Y-27632 (TOCRIS) for 24 h. The next day, individualized hESCs (5×10^6) were resuspended in 100 μL opti-MEM (Gibco) containing 12 μg Cas9-GFP and 8 μg WRN-gRNA-mCherry and were then electroporated by 4D-Nucleofector (Lonza). After electroporation, cells were seeded on Matrigel-coated plates in mTeSR. After 48 h expansion, dual-positive cells were collected by FACS machine (BD FACS Aria II) and plated on MEF feeder in human ESC medium. Emerging clones were manually picked into 96-well plates and expanded on Matrigel-coated plates for western blotting.

Generation and characterization of hMSCs.

hMSCs were differentiated from hESCs as previously described [4, 12, 13]. Briefly, embryoid bodies were plated on Matrigel-coated plates in hMSC differentiation medium (αMEM (Gibco), 10% fetal bovine serum (FBS, Hyclone), 1% penicillin/streptomycin (Gibco), 1 ng/mL bFGF (Joint Protein Central) and 5 ng/mL TGFβ (Humanzyme)) for around 10 days till fibroblast-like cells were confluent. These fibroblast-like cells were maintained in hMSC culture medium for two passages and then were sorted by FACS machine (BD FACS Aria II) to purify CD73/CD90/CD105 tri-positive hMSCs. hMSCs were further differentiated towards osteoblasts, chondrocytes and adipocytes to verify their multiple-lineage differentiation capacity [4, 12]. Histochemical staining with Von Kossa (Osteogenesis), Toluidine blue (Chondrogenesis), and Oil red O (Adiopogenesis) was performed respectively.

Nuclear protein extraction

Fresh cells were lysed in buffer A (10 mM HEPES-KOH pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.4% NP-40, 1 mM DTT) with protease inhibitor cocktail (Roche, 04693159001). Supernatants after centrifuge were collected as cytoplasmic fraction. Precipitation was further lysed in buffer B (20 mM HEPES-KOH pH 7.9, 420 mM NaCl, 0.2 mM EDTA, 0.1 mM EGTA, 25% glycerin, 1 mM DTT) with protease inhibitor cocktail (Roche, 04693159001) to extract the nuclear fraction. Both the cytoplasmic and nuclear fractions were quantified by a BCA kit (Thermo Fisher Scientific) for western blotting and Sanger sequencing.

Gero-protective chemicals screening

4×10^3 WS hMSCs (P4) were seeded on gelatin-coated 96-well plate. The next day, hMSCs were treated with various gero-protective chemicals, fresh medium containing chemicals were changed every two days. After 2 weeks, CellTiter 96 AQueous One Solution Cell Proliferation Kit (Promega) was used to measure cell viability according to the manufacturer's protocol. Chemicals employed in this assay were as follows: OLZ (5/10/20 μM, Oltipraz, Sigma), MET (50/100/200 μM, Metformin, TOCRIS), RAPA (0.1/0.5/1μM, Rapamycin, TOCRIS), RES (1/2/5 μM, Resveratrol, Sigma) and SPD (0.5/1/2 μM, Spermidine, Sigma).

Flow cytometric analysis.

For cell cycle analysis, Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay Kit (C-10419, Molecular Probes) was used according to the manufacturer's instruction. For ROS measurement [12], living cells were incubated with ROS indicators (1 μM CM-H2DCFDA, C6827, Molecular Probes).

For detection of oxidative DNA damage, OxyDNA Test Kit (BIO81DNA, Argutus Medical) was used according to the instructions. For apoptosis analysis, supernatant and attached cells were collected freshly and stained with Annexin V-EGFP and PI (A002, Vigorous). All experiments were measured by LSRFortessa™ cell analyzer (BD) and data were analyzed by FlowJo software.

MDA measurement

For malondialdehyde measurement, lipid peroxidation (MDA) colorimetric assay kit (Biovision, K739-100) was used according to the manufacturer's instruction. Briefly, cell lysates mixed with thiobarbituric acid (TBA) were incubated at 95 ℃ for 1 h. Then the absorbance of the generated MDA-TBA adduct was measured at 532 nm using a microplate reader (BioTek).

Protein, DNA and RNA analysis

For western blotting, cells were lysed in RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) with protease inhibitor cocktail (Roche). Protein quantification was performed using a BCA Kit (Thermo Fisher Scientific). Protein lysate was subjected to SDS-PAGE and subsequently electrotransferred to a PVDF membrane (Millipore). Then primary antibodies and HRP conjugated secondary antibodies were incubated with the 5% milk blocked membrane. The imaging and quantification of target proteins was obtained by Image Lab software for ChemiDoc XRS system (Bio-Rad). For RT-PCR, total RNA was extracted in TRIzol (Invitrogen) and contaminating DNA was removed by TURBO DNA-free Kit (Ambion). Then GoScript Reverse Transcription System (Promega) was used to generate cDNA. RT-qPCR was performed with THUNDERBIRD qPCR Mix (TOYOBO) in CFX384 Real-Time system (Bio-Rad). Semi-quantitative RT-PCR was carried out with PrimeSTAR (TAKARA) in 96 well Thermal Cycler (Applied Biosystems). For telemetric qPCR [4, 14], genomic DNA was extracted by a DNA extraction kit (TIANGEN) and qPCR was performed with THUNDERBIRD qPCR Mix. All primer sequences for PCR are given in Table S1.

Immunofluorescence microscopy

Cells and tissue sections were fixed in 4% paraformaldehyde at room temperature (RT) for 15 min, permeabilized in 0.4% Triton X-100/PBS at RT for 10 min. After blocking with 10% donkey serum (Jackson ImmunoResearch Labs)/PBS for 1 h, cells and sections were incubated with primary antibodies at 4 ℃ overnight and the corresponding secondary antibody (Invitrogen) at RT for 45 min. Nuclei were stained with Hoechst 33342 (62249, Thermo Fisher) and F-actin was labeled by phalloidin (A12381, Molecular Probes). Dil-Ac-LDL (L23380, Thermo) was used in lipoprotein uptake assay.

Luciferase reporter assay

To determine the transcriptional activity of NRF2, NQO1-ARE-Luc and HO-1-ARE-Luc [15] were transiently transfected with Renilla plasmid into hMSCs with Lipofectamine 2000 (Invitrogen). 48 h after transfection, relative luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega).

SA-β-gal staining assay

The SA-β-gal staining of hMSCs was conducted as previously described [4, 16].

ChIP-qPCR

ChIP-qPCR was performed according to a previous protocol with slight modifications [17]. Briefly, 1×10^6 hMSCs or TMSCs were cross-linked in 1% vol/vol formaldehyde/PBS for 15 min at room temperature and then quenched by 125 mM Glycine. Samples were lysed on ice for 5 min. Subsequently, lysates were sonicated using a Bioruptor® Plus sonication device (Diagenode). The collected supernatants were incubated overnight with Protein A dynabeads (Life technology, 10001D) associated with 2.4 μg NRF2 antibody (ab62352, Abcam) or rabbit IgG (SC-2027, Santa Cruz). Next the input sample and chromatin-beads complexes sample were digested, eluted and cross-link-reversed at 68 ℃ for 2 h on a thermomixer. DNA was finally purified by phenol-chloroform-isoamylalcohol and chloroform-isoamylalcohol extractions. The enriched DNA was subjected to qPCR to evaluate NRF2 occupation at endogenous ARE motifs. The primer sequences used for ChIP-qPCR are listed in Table S1.

Lentivirus and retrovirus preparation.
For generating lentiviral vectors encoding shRNA targeting P53, shRNA oligos [13, 18] were cloned into the pLVTHM/GFP vector (12247, Addgene) pre-cleaved by ClaI and MluI [4]. Lentiviral vectors expressing luciferase were described in our previous study [4, 12, 13]. For packaging of lentiviruses, HEK293T cells were co-transfected with lentiviral expression vectors or shRNA vectors, as well as psPAX2 (Addgene, 12260) and pMD2.G (Addgene, 12259). The pBABE-neo-hTERT, pBABE-zeo-largeTgenomic and pBABE-puro-RasV12 retroviral vectors were purchased from Addgene (1774, 1778, and 1768, respectively). For packaging of retrovirus, HEK293T cells were co-transfected with retroviral vectors, as well as gag/pol (Addgene, 14887) and VSV.G (Addgene, 14888). Viral particles were collected by ultracentrifugation at 19,400 g for 2.5 h.

Oncogenic transformation
The *in vitro* oncogenic transformation was performed as previously described with minor modifications [19]. hMSCs at passage 3 were transduced sequentially with pBABE-neo-hTERT, pBABE-zeo-largeTgenomic and pBABE-puro-RasV12 retroviruses. Cells were respectively selected in G418 (100 μg/mL, 7 days, Invitrogen), zeocin (100 μg/mL, 7 days, Invitrogen) and puromycin (1 μg/mL, 3 days, Invitrogen). The drug-resistant cells were further treated with three drugs simultaneously for one passage and transduced with pLVTHM-shP53 vector expressing both P53-specific shRNA and GFP. The generated TMSCs were regarded as passage 1 (P1), and maintained in TMSC medium.

Anchorage-independent growth assay
For soft agarose assay, 1×10^5 TMSCs suspending in warmed 2×TMSC medium mixed with melting equal volume 0.7% agarose (BIOWEST) were seeded onto the 0.6% agarose layers in 6 well plate. TMSC medium was changed every 4 days. 4 weeks later, colonies were stained with 0.001% crystal violet for photographing. The relative colony diameters were calculated by ImageJ. For sphere-growth assay, 150 μL cell suspension containing 2×10^4 TMSCs were seeded in each well of 96 well low-attach plate without centrifuge. Fresh TMSC medium was changed every 3 days and images were captured every 5 days. The sphere diameters were calculated by ImageJ.

RNA-seq
Total RNA was isolated with the RNeasy Mini Kit (Qiagen) from 1×10^6 cells per duplicate following the manuals. Then the quality of RNA was checked with Fragment Analyzer (Advanced Analytical), following by RNA library construction by NEBNext® Ultra™ RNA Library Prep Kit for Illumina (NEB) according to the manufacture's protocol. Paired ends reads were generated from Illumina sequencing platform and low quality reads (reads with high percentage of N bases and with low phred33) were removed. The remaining reads were aligned to the human reference (hg19 from UCSC genome browser) by TopHat [20-22] and transcripts were assembled with Cufflinks [23]. Differentially expressed gene lists were obtained with Cuffdiff [24] and assessed for Gene Ontology (GO) enrichment by DAVID Bioinformatics Resources [25, 26].

CNV
DNeasy Blood & Tissue Kit (Qiagen) was applied to extract genomic DNA from 1×10^6 cells per duplicate. The fragments of DNA ranging in 150-200 bp was obtained with Covaris S2 and constructed into sequencing libraries using NEBNext® DNA Library Prep Reagent Set for Illumina® (NEB). The analyses of the libraries assessed on Illumina sequencing platform were performed as previously described [4] with minor modifications. Briefly, a HUES9 Parental line without CNVs was served as a standard control [27]. The CNVs in 500 kb windows was calculated, with mappability and GC content controlled by the R package HMMcopy [28].

Identification of putative tumor-associated genes
Tumor-promoting genes on the duplicated region of chromosome 4 (4q26-4q35) were selected from the Tumor Associated Gene database (Listed by chromosome) (http://www.binfo.ncku.edu.tw/TAG/GeneFinder_chr.php) [29].
For NRF2-responsive TSGs, the uniquely activated genes in TMSCs were compared with the "tumor suppressor gene (TSG) signature sets" which combined two reference gene lists including

"All the 1217 human tumor suppressor genes with basic annotations" and "All the precomputed P-values for gene expression difference between tumor and normal samples for all human tumor suppressor genes in pan-cancer" in Tumor Suppressor Gene Database (https://bioinfo.uth.edu/TSGene/index.html) [30, 31]. Afterwards, transcription regulation domain sequences of potential TSG genes were obtained from NCBI and scanned in JASPR database (http://jaspar.genereg.net/, ID: MA0150.1, NAME: NFE2L2, Species: Homo Sapiens) [32] to find putative NRF2 binding motifs for further validation.

Animal experiments

For teratoma formation assay, 3×10^6 hESCs suspending in Matrigel/mTeSR (1:4) were subcutaneously injected into male NOD/SCID mice (6-8 weeks). Animals were sacrificed at around 8 weeks to collect teratomas. Teratomas were fixed in 4% paraformaldehyde, dehydrated in 30% sucrose and embedded in O.C.T. (4583, TISSUE-TEK) for further analysis.

For hMSC transplantation assay, 1×10^6 hMSCs transduced with lentivirus expressing luciferase were injected into the midportion of the tibialis anterior (TA) muscle (6-8 weeks, n=5). 0/3/5/7 days after transplantation, mice were treated with D-luciferin (GoldBio) and imaged by IVIS spectrum imaging system (XENOGEN, Caliper). Bioluminescence images were acquired at AUTO mode.

For hindlimb ischemia model, limb ischemia was induced in male nude mice (8-10 weeks) according to the visualized protocol [33]. In brief, proximal femoral artery and distal femoral artery were ligated by double knots using 7-0 silk. Arterial ligated mice were assigned to three groups: the negative control group (200 μL PBS), the *NRF2*$^{+/+}$ hMSC group (P8, 3×10^6 cells in 200 μL PBS) and the *NRF2*$^{AG/AG}$ hMSC group (P8, 3×10^6 cells in 200 μL PBS). PBS and cell suspension were injected immediately into four sites of the thigh muscles. Blood perfusion was monitored every 4 days after the surgery by a laser Doppler blood perfusion (Moor instruments). 4 weeks after surgery, the whole thigh muscles were dissected and frozen in O.C.T. for section staining.

For hMSC tumor susceptibility test, 3×10^6 hMSCs (P5) suspending in 100μL Matrigel/PBS (1:4) were injected into tibia proximal to knee-joint of male BALB/c nude mice (4-6 weeks, n=12). Animals were checked every month to continuously monitor the long-term *in vivo* consequences.

For TMSC tumor-forming assay, 3×10^6 TMSCs suspending in 100μL Matrigel/PBS (1:4) were injected into tibia proximal to knee-joint of male BALB/c nude mice (4-6 weeks, n=8). 10 weeks after transplantation, animals were sacrificed to evaluate the tumor-forming ability, legs with tumors were fixed in 4% paraformaldehyde and embedded in O.C.T for section staining.

All animal experiments were conducted with approval of the Institute of Biophysics, Chinese Academy of Science.

Statistical analysis.

The statistical analyses were performed using PRISM software (Graphpad Software). Comparisons were performed with two-tail student's t-test unless otherwise stated. $P<0.05$ was defined as statistically significant.

Accession numbers

All sequencing data have been deposited in the National Center for Biotechnology Information under the accession number GSE84694.

Reference

1 Liu GH, Qu J, Suzuki K *et al.* Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. *Nature* 2012; **491**:603-607.

2 Liu GH, Suzuki K, Qu J *et al.* Targeted gene correction of laminopathy-associated LMNA mutations in patient-specific iPSCs. *Cell stem cell* 2011; **8**:688-694.

3 Liu GH, Suzuki K, Li M *et al.* Modelling Fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. *Nature communications* 2014; **5**:4330.

4 Zhang W, Li J, Suzuki K *et al.* Aging stem cells. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. *Science* 2015; **348**:1160-1163.

5 Kubben N, Zhang W, Wang L *et al.* Repression of the Antioxidant NRF2 Pathway in Premature Aging. *Cell* 2016; **165**:1361-1374.

6 Suzuki K, Yu C, Qu J *et al.* Targeted gene correction minimally impacts whole-genome mutational load in human-disease-specific induced pluripotent stem cell clones. *Cell stem cell* 2014; **15**:31-36.

7 Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proceedings of the National Academy of Sciences of the United States of America* 2000; **97**:6640-6645.

8 Suzuki K, Mitsui K, Aizawa E *et al.* Highly efficient transient gene expression and gene targeting in primate embryonic stem cells with helper-dependent adenoviral vectors. *Proceedings of the National Academy of Sciences of the United States of America* 2008; **105**:13781-13786.

9 Palmer DJ, Ng P. Physical and infectious titers of helper-dependent adenoviral vectors: a method of direct comparison to the adenovirus reference material. *Molecular therapy : the journal of the American Society of Gene Therapy* 2004; **10**:792-798.

10 Shayakhmetov DM, Li ZY, Gaggar A *et al.* Genome size and structure determine efficiency of postinternalization steps and gene transfer of capsid-modified adenovirus vectors in a cell-type-specific manner. *Journal of virology* 2004; **78**:10009-10022.

11 Ding Q, Regan SN, Xia Y, Oostrom LA, Cowan CA, Musunuru K. Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. *Cell stem cell* 2013; **12**:393-394.

12 Pan H, Guan D, Liu X *et al.* SIRT6 safeguards human mesenchymal stem cells from oxidative stress by coactivating NRF2. *Cell research* 2016; **26**:190-205.

13 Duan S, Yuan G, Liu X *et al.* PTEN deficiency reprogrammes human neural stem cells towards a glioblastoma stem cell-like phenotype. *Nature communications* 2015; **6**:10068.

14 Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic acids research* 2002; **30**:e47.

15 Liu GH, Qu J, Shen X. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. *Biochimica et biophysica acta* 2008; **1783**:713-727.

16 Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. *Nature protocols* 2009; **4**:1798-1806.

17 Dahl JA, Collas P. A rapid micro chromatin immunoprecipitation assay (microChIP). *Nature protocols* 2008; **3**:1032-1045.

18 Raya A, Rodriguez-Piza I, Guenechea G *et al.* Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* 2009; **460**:53-59.

19 Scaffidi P, Misteli T. In vitro generation of human cells with cancer stem cell properties. *Nature cell biology* 2011; **13**:1051-1061.

20 Meyer LR, Zweig AS, Hinrichs AS *et al.* The UCSC Genome Browser database: extensions and updates 2013. *Nucleic acids research* 2013; **41**:D64-69.

21 Karolchik D, Hinrichs AS, Furey TS *et al.* The UCSC Table Browser data retrieval tool. *Nucleic acids research* 2004; **32**:D493-496.

22 Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 2009; **25**:1105-1111.

23 Trapnell C, Williams BA, Pertea G *et al.* Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature biotechnology* 2010; **28**:511-515.

24 Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nature biotechnology* 2013; **31**:46-53.

25 Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID

bioinformatics resources. *Nature protocols* 2009; **4**:44-57.

26 Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic acids research* 2009; **37**:1-13.

27 Veres A, Gosis BS, Ding Q *et al.* Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing. *Cell stem cell* 2014; **15**:27-30.

28 Ha G, Roth A, Lai D *et al.* Integrative analysis of genome-wide loss of heterozygosity and monoallelic expression at nucleotide resolution reveals disrupted pathways in triple-negative breast cancer. *Genome research* 2012; **22**:1995-2007.

29 Chen JS, Hung WS, Chan HH, Tsai SJ, Sun HS. In silico identification of oncogenic potential of fyn-related kinase in hepatocellular carcinoma. *Bioinformatics* 2013; **29**:420-427.

30 Zhao M, Sun J, Zhao Z. TSGene: a web resource for tumor suppressor genes. *Nucleic acids research* 2013; **41**:D970-976.

31 Zhao M, Kim P, Mitra R, Zhao J, Zhao Z. TSGene 2.0: an updated literature-based knowledgebase for tumor suppressor genes. *Nucleic acids research* 2016; **44**:D1023-1031.

32 Mathelier A, Fornes O, Arenillas DJ *et al.* JASPAR 2016: a major expansion and update of the open-access database of transcription factor binding profiles. *Nucleic acids research* 2016; **44**:D110-115.

33 Niiyama H, Huang NF, Rollins MD, Cooke JP. Murine model of hindlimb ischemia. *Journal of visualized experiments : JoVE* 2009.