Supplementary Methods

Measure of telomerase activity by Telomerase Repeat Amplification Protocol (TRAP)

Cells were collected and kept as frozen pellets. Pellets were suspended in ice-cold 1X CHAPS lysis buffer (10mM Tris-HCl, pH7.5, 1mM MgCl2, 1mM EGTA, 0.1mM benzamidine, 5mM β-mercaptoethanol, 0.5% CHAPS, and 10% glycerol) containing RNase inhibitor at a final concentration of 150U/mL. After 30mn incubation on ice, the lysate was centrifuged at 14,000 g for 25mn at 4° C, and the supernatant was transferred into a fresh tube. Protein concentrations of the extract were measured by the Bradford method (Bio-Rad, Hercules, CA), and an aliquot of extract containing 1µg of protein was used for each TRAP assay. TRAP assays were performed using TRAPeze ELISA kit (S7750.Chemicon Int.,Temecula,CA), according to manufacturer's instructions with minor modifications. Twenty µL of reaction mixture containing 1µg of protein extract, 4µL of 5X TRAP reaction mix (Tris buffer, primers, biotinylated TS primer and RP primer, dNTPs and DNPdCTP, and oligomer mix for amplification of 36-bp internal control band), and 1 unit of amplitaqGOLD Taq DNA polymerase (Applied Biosystems Foster City, CA,), was incubated for 30mn at 30° C and subsequently subjected to tree-step PCR (after activation of the hot start Taq) at 94° C for 30 seconds, 56° C for 30 seconds and 72°C for 66 seconds for 40 cycles. Each assay design included (i) a test extract of 1µg of protein in duplicate, (ii) a heat-inactivated lysate at 85° C for 15mn before the assay, (iii) a telomerase-positive control cell extract MDA-MB 231 corresponding to a range of 125 to 31 cells, (iv) a primerdimer/PCR contamination control where 2µL of 1X CHAPS lysis buffer was substituted to the extract, and (v) the TSR8 PCR/ELISA-positive control supplied in the kit. Nonradioactive detection of the telomerase products was performed by ELISA protocol. TRAP products (fifteen percent) were immobilized onto streptavidin-coated microtiter plates and then detected by anti-DNP

antibody conjugate to horseradish peroxidase (HRP). The amount of TRAP products was determined by means of the HRP activity using 3,3',5,5'-tetramethylbenzidine substrate and subsequent color development. Absorbance was measured at 450nm and 690nm and given as A_{450}-A_{690}.

FACS sorting of senescent cells and staining for cell filiation tracing

Senescent cells were electrostatically sorted in air, out of a presenescent population and according to their forward and scatter factors, on a Coulter EPICS XL-MCL or a Coulter EPICS ALTRA. The 25% cells with the highest factors were collected in complete culture medium and again placed in culture. After plating, cells were incubated with 10 µM Vybrant CFDA SE (Molecular Probes V-12883) for 15 min at 37°C or with Vybrant DiI cell-labelling solution (1/200 dilution, Molecular Probes) for 30 min at 37°C, washed, and monitored for emergence.

Transcriptomics analysis

cDNA preparation - Total RNAs were extracted using RNeasy mini-columns (QIAGEN). Double stranded cDNA was synthesised from total RNA using a SMART protocol (1). Two first strand reactions were set up starting with 500ng of total RNA. Four microlitres of each first strand reaction was used in the amplification step which was performed using 17 rounds of cycling. The ds cDNA was checked on a 1.2% agarose gel before the samples were purified using Qiagen Qiaquick clean up columns. The amount of cDNA amplified was then checked using the Nanodrop ND1000.

cDNA labelling and microarray hybridisation - Using the Bioprime labelling kits (Invitrogen), 10ul of purified cDNA was labelled, where 2µl of Cye dye (GE) was incorporated. After incubating for 3 hr at 37^{o}C, the labels were purified using ProbeQuant

G50 micro columns (GE). Incorporation rates were determined using a Nanodrop ND1000 before specific labels were pooled and dried down to completion. The labels were re-suspended in 40µl of hybridisation buffer (40% deionised formamide; 5x Denhart's; 5x SSC; 1mM Na pyrophosphate; 50mM Tris pH 7.4; 0.1% SDS) and hybridised onto a RNG-MRC mouse set 25K microarray printed on GE Codelink slide (http://www.mgu.har.mrc.ac.uk/facilities/microarray/rng.html), overnight at 48^{o}C in a water bath using the Corning hybridisation chambers. After hybridisation, the arrays were then washed initially in 2xSSC until the coverslip had come off, then 5 min with vigorous shaking in 0.1x SSC; 0.1% SDS and then finally in 0.1xSSC for 2 min with vigorous shaking. The arrays were then spun dry and scanned using a ProScanArray HT (Perkin Elmer, Beaconsfield, UK) at 7 different PMT gain settings from 40 to 70. The images were then processed using ImaGene 6.0.1 (Bio Discovery, El Segundo, CA, USA), where all 14 images were overlaid and gridded and the feature data extracted.

Analysis of microarray - The RNA data were processed using Mavi 2.6.0 (MWG Biotech AG, Ebersberg, Germany), which increases the dynamic range whilst avoiding saturation problems. The data was then loaded into R Project for Statistical Computing (http://www.r-project.org) for further analysis. A two dimensional loess normalisation, from the YASMA5 (Yet Another Statistical Microarray Analysis) library, was performed on each array to correct for any spatial variation within the slide. The LIMMA library (Linear Models for Microarray Analysis (2) from the BioConductor software project (http://www.bioconductor.org) was used to further normalise the data and to select differentially expressed genes. In brief, a linear model is fitted to the data for each gene to fully model the systematic part of the data and provide estimates for each coefficient (samples in this case). These coefficients can then be compared and differentially expressed genes selected using an empirical bayes moderated t-statistic. Differential genes were selected for the comparisons of interest based on their

moderated t-statistic (3) after using a false discovery rate control of 5 % (4). The fitted values for each sample were then converted back to red and green intensities and loaded into GeneSpring GX (Agilent technologies, Stockport, UK) to allow for easy comparison of lists of differential genes.

Telomere length determination

We used the TeloTAAAG telomere length assay kit (Roche), according to manufacturer's instructions. Briefly, genomic DNA was isolated using a standard SDS-proteinaseK protocol. Two µg of purified DNA was digested with a HinfI/RsaI enzyme mixture 2hrs at 37°C and separated by an overnight electrophoresis on a 0.8% agarose gel in TBE buffer at 5Volts/cm. The Southern capillary transfer was done using 20xSSC buffer on positively charged nylon membranes. The blot was hybridized with a digoxygenin-labeled probe specific for telomeric repeats and incubated with a digoxygenin-specific antibody coupled to alkaline phosphate. For TeloFISH, slides were prepared as for M-FISH. The hybridization mixture, containing 70% formamide, the nucleic acid probes labelled with Cy3 at 0.3µg/µL (Perceptive Biosystems, Ramsey, MN), 1% (W/V) blocking reagent (Boehringer-Mannheim, Gmbh) in 10mM Tris pH7.2, was laid down, a coverslip was added and DNA was denatured for 3min at 80^{o}C. After 2hrs hybridization at RT, slides were washed with 70% formamide/10mM Tris pH 7.2 (2*15 min) and with 0.05M Tris 0.15M NaCl pH 7.5 containing 0.05% Tween-20 (3*5 min). Slides were then counterstained with 1µg/mL DAPI and mounted in antifade solution (VectaShield, Vector Laboratories Inc., Burlingame, CA).

Reverse Transcription and Quantitative-Polymerase Chain Reaction

Total RNAs were extracted using RNeasy mini-columns (QIAGEN). One µg of RNA was reverse-transcribed using random hexamers, Superscript III and dNTPs (Invitrogen) in a final

volume of 20 µl according to the manufacturer's instructions. The quantitative PCRs were performed on an Opticon 2 Thermocycler (Bio-Rad) with the following primers : p21 (fd 5′-ATGAAATTCACCCCCTTTCC; rs 5′- CCCTAGGCTGTGCTCACTTC), p16 (fd 5′-TGCCTTTTCACTGTGTTGGA; rs 5′-GCCATTTGCTAGCAGTGTGA); PCNA (fd 5′-TCTCAGCCATATTGGAGATG ; rs 5′-CAGGTACCTCAGTGCAAAAG), ARNpol2 (fd 5′- GTGCGGCTGCTTCCATAA; rs 5′-GCACCACGTCCAATGACAT), RPL13a (fd 5′-AGCTCATGAGGCTACGGAAA; rs 5′- CTTGCTCCCAGCTTCCTATG), Actine β (fd 5′-TCCCTGGAGAAGAGCTACGA ; rs 5′- AGCACTGTGTTGGCGTACAG). PCRs were performed in duplicate on 10ng of cDNA using Invitrogen (for p16 and PCNA) and Epicentre reaction mixture with L buffer (for p21), with H buffer (for ARNpol2, RPL13a and Actine β) following the producer instructions. Cycle conditions were set as follows: 94ºC for 15s and 60°C to 66°C (depending on the primers) for 1min, 40 cycles. Gene expression levels were normalized by geometric averaging of the 3 internal control genes, ARNpol2, RPL13a and Actine β, as recommended by Vandesompele et al.(5). Gene expression stability of ARNpol2, RPL13a and Actine β was validated with two softwares: geNorm(5) and NormFinder(6).

BrdU-incorporation assays

BrdU (Roche) was added to cell cultures at 10µM for 6hrs. Cells were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.2% Triton X-100, and incubated with 40U/ml DNase I (Promega) and 20U/ml Exonuclease III (Roche) for 30min at 37°C. BrdU was revealed with anti-BrdU mouse IgG (Dako) and Rhodamine Red-conjugated antimouse IgG (Jackson Immunoresearch laboratories).

Metaphase assays

Presenescent cells were plated in Lab-tek II Chamber slide (Nalge Nunc International) and let grown until senescence. Slides were incubated with Karyomax Colcemid (Invitrogen

Corporation) 1hr at 37°c, incubated in 60mM KCl solution at 37°C, fixed with 4% paraformaldehyde in PBS for 20min, and stained with 1μg/ml Hoechst 33258 for 3min.

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