Supporting Information

Mukherjee et al. 10.1073/pnas.1112414108

SI Materials and Methods

Cells and Cell Culture. Human Mammary Epithelial cells (HMECs) were derived from reduction mammoplasties (Product number CC-2551; Lot numbers 5F0056 and 6F3408; Lonza Walkersville Inc., Walkersville, Maryland, United States). Early passage cells (passage 7), which have already bypassed the first growth plateau (M0) and lost $p16^{INK4a}$ expression were used, because ectopic hTERT expression enables late passage HMECs to become immortalized only after $p16^{INK4a}$ is inactivated (1). Cells were grown in Mammary Epithelial Growth Medium i.e., complete medium, a serum-free basal medium that is supplemented with 10 ng/ml human epidermal growth factor (hEGF), 52 μg/ml bovine pituitary extract, 5 μg/ml insulin, 0.5 μg/ml hydrocortisone, 50 μg/ml gentamicin sulphate and amphotericin B (MEGM bullet kit; CC-3150; Lonza Walkersville Inc.). When studying effects of hTERT in mitogen-limiting conditions (minimal medium), hEGF and bovine pituitary extract were omitted from the medium. Cells were passaged for up to 5 mo as per supplier instructions by counting and plating at 0.2 million cells (or all cells, if less than 0.2 million were present) per 100 mm dish every 7 d and feeding every 2 to 3 d. Live cells counts at each passage were obtained by excluding cells that stained positive with trypan blue. Long term passaging cell counts were reproduced twice using independently transduced cells.

Phoenix packaging cells (Product number SD 3444; American Type Culture Collection (ATCC), Manassas, Virginia, United States) were cultured in DMEM with 10% Bovine Growth Serum (BGS) and plated at 1.4 million cells per 60 mm dish for transfections.

VA13 cells (WI-38 VA-13 subline 2RA; CCL-75.1; ATCC) were first thawed and passaged in DMEM with 10% BGS. To mimic culture conditions used for the HMEC growth assays, cells were passaged in complete primary fibroblast medium with all supplements (Fibroblast Basal medium PCS-201-030 and Fibroblast Growth Kit – serum free, PCS-201-040; ATCC). Cells were cultured in these conditions for an average of 3 wk prior to growth assays to acclimatize them to this medium. When studying effects of hTERT in mitogen-limiting conditions (minimal medium), hEGF and FGF were omitted from the medium.

Retroviral Constructs. Retroviral constructs with hTERT either WT or with mutations in each functional domain and linker region of hTERT were gifts from Dr. C.M. Counter (2, 3) (Duke University Medical Center, Durham, NC, United States). The hTERT-HA and $hTERT^{DN}$ constructs were obtained from Dr. R.A. Weinberg (4, 5) (Whitehead Institute for Biomedical research, Cambridge, MA, United States). The $hTERT^{IA-/DN}$ double mutant was constructed using the Stratagene Quickchange II site-directed mutagenesis kit (Catalog number 200524) instructions, with the only modification being that DMSO (4%) was added to the PCR mix. Mutagenic primers:

5'GAGCTGTACTTTGTCAAGGTGGCTATCACGGGCGCG
TACGACACCATC-3'and
5'GATGGTGTCGTACGCGCCCGTAATAGCCACCTTGAC
AAAGTACAGCTC-3'

were used to change D711 to A and V712 to I. The mutations in the RT and IA (+32) domains were confirmed by sequencing.

The pRetroSuper-puro vector and the sequences described below were used to create the RMRP shRNA constructs. The sequences were selected using the i-Score Designer software (6) and are shown below. As a negative control, the 'RMRP shRNA a' sequence was scrambled using siRNA Wizard software v3.1 (InvivoGen), and BLAST (NCBI) was used to confirm that the scrambled sequence lacks homology to any other gene. The capitalized letters represent the targeting sequences:

RMRP shRNA a:
5'gatccccAGGCCTGTATCCTAGGCTAttcaagagaTAGCCT
AGGATACAGGCCTttttt 3' &
5'agctaaaaaAGGCCTGTATCCTAGGCTAtctcttgaaTAGCC
TAGGATACAGGCCTggg3'

RMRP shRNA b:
5'gatccccCTCCAAAGTCCGCCAAGAAttcaagagaTTCTTG
GCGGACTTTGGAGttttt 3' &
5'agctaaaaaCTCCAAAGTCCGCCAAGAAtctcttgaaTTCTT
GGCGGACTTTGGAGggg3'

RMRP shRNA c:
5'gatccccCGTCAGCTCCCTCTAGTTAttcaagagaTAACTAG
AGGGAGCTGACGttttt 3' &
5'agctaaaaaCGTCAGCTCCCTCTAGTTAtctcttgaaTAACT
AGAGGGAGCTGACGggg3'

RMRP shRNA a scramble control:
5'gatccccGAGCCATGACGCTTCTGTAttcaagagaTACAGA
AGCGTCATGGCTCttttt 3' &
5'agctaaaaaGAGCCATGACGCTTCTGTAtctcttgaaTACAG
AAGCGTCATGGCTCggg3'

The hPOT1 knockdown and control retroviral vectors (Plasmids 11135 and 11136; Addgene Inc., Cambridge, Massachusetts, United States) were obtained from Dr. C.M. Counter (7).

Generation of Cell Lines by Retroviral Transduction. Phoenix packaging cells were transfected with 5-10 μg of

each retroviral vector using Fugene (Catalog number 11988387001; Roche, Indianapolis, Indiana, United States). After 24 h the medium was switched from DMEM with 10% BGS to MEGM (complete medium). After 48 h and again at 66 h, viral supernatants from Phoenix cells were filtered and used to infect HMECs (HMECs were plated at 0.5 million per 60 mm dish and incubated overnight prior to infection), followed by selection for drug resistance after 48 h to obtain stably infected cells. Cells were selected in 2 μg/ml puromycin or 15 μg/ml hygromycin for 3 and 7 days, respectively, and for double infections 1 μg/ml puromycin and 15 μg/ml hygromycin were used.

Cell Growth Assay by Crystal Violet Staining. Stably infected HMECs or VA13 cells were plated in complete medium at 1000 cells/well (maintaining low cell density is critical (8)) in 96 well plates. After 24 h, cells were washed with minimal medium and then cultured in either minimal or retained complete medium. At indicated time points, the cells were washed with PBS, fixed with 10% formalin for 10 min, washed twice with water and stored in PBS. After the time course, the cells were stained with 0.1% crystal violet solution for 15 min. The excess crystal violet was removed with 5 to 6 washes in water, eluted with 200 μl 10% acetic acid, and absorbance measured at 595 nm. Each assay was done in triplicate and the results confirmed with two to three independent sets of transduced HMECs in separate assays. Statistical analyses for the VA13 growth assays were carried out using GraphPad Prism version 4.0 software, with significant differences defined as at least a p value of <0.05 by Analysis of Variance (ANOVA).

BrdU and Annexin V Analyses. Cells were plated at either 0.07 million cells per 60 mm dish or 0.2 million per 100 mm dish in complete medium. After 24 h, cells were washed, the medium was changed to minimal medium (or retained in complete medium for hPOT1 knockdown experiments), and cells refed after 2 d and cultured for an additional 2 d followed by either BrdU labeling for 3 h as described previously (9) or by harvesting for annexin V staining. To obtain a positive control for annexin V, HMECs were treated with 100 μM cisplatin (Product number P4394; Sigma-Aldrich Co., St. Louis, Missouri, United States) for 18 h prior to annexin V staining. Annexin V staining was carried out as per manufacturer instructions (Annexin-V FITC apoptosis detection kit; Catalog number: APOAF; Sigma-Aldrich Co.). Stained cells were analyzed on a FACScan flow cytometer using CellQuest Pro software (Becton Dickinson, Mountain View, California, United States). Each cell division and cell death assay was done in duplicate and reproduced at least twice with independently transduced cells.

Western Blotting. HMECs were plated at 0.2 million per 100 mm dish in complete medium. After 24 h, cells were washed and the medium was changed to minimal medium. Cells were refed after 2 d and cultured for an additional 2 d followed by harvesting in PBS to obtain cell pellets. Cells were freeze-thawed and lysed using 1% Nonidet P-40 lysis buffer containing a complete protease inhibitor cocktail (Catalog number 11836170001; Roche) and mixing at 4°C for 1 h. Protein in the cell lysates was quantitated using Bradford protein assay (Bio-Rad). Total cell lysates (20-40 μg) were resolved by SDS-polyacrylamide gels (7.5% for retinoblastoma protein hyperphosphorylation and 12% for all other proteins), transferred to PVDF membranes (Catalog number NEF1002; PerkinElmer, Waltham, Massachusetts, United States), and probed with one of following primary antibodies (listed below as company; catalog number; clone number): cyclin D1 (Santa Cruz Biotechnology Inc., Santa Cruz, California, United States; sc-8396; A-12), cyclin A2 (Santa Cruz Inc.; sc-751; H432), pRB (BD Pharmingen, San Diego, California, United States; 554136; G3-245), E2F1 (Santa Cruz Inc.; sc-251; KH195), BRG1 (Santa Cruz Inc.; sc-17796; G-7), Actin (Santa Cruz Inc.; sc-1615; C-11) or GRB2 (Santa Cruz Inc.; sc-255; C-23). After washing, membranes were incubated with the following horseradish peroxidase-conjugated secondary antibodies from Santa Cruz Inc.: goat anti-mouse (sc-2064), goat anti-rabbit (sc-2030) or donkey anti-goat (sc-2056) secondary antibodies. Bands were visualized using the Amersham ECLPlus Western Blotting Detection System (Product code RPN2132; GE Healthcare, Piscataway, New Jersey, United States). Results were confirmed in two additional independent experiments.

Telomerase Repeat Amplification Protocol (TRAP) Assay and Telomere Length Determination by Terminal Restriction Fragment (TRF) Analysis. HMECs plated in complete medium were harvested at the indicated passages. 0.2 million cells were used for the TRAP assay, and 1 μg genomic DNA per sample was used to determine telomere length by TRF analysis. Telomerase activity and telomere length were measured using the Telomerase TeloTAGGG PCR ELISA and Telomere Length Assay kits, respectively, according to manufacturer instructions (Catalog numbers 11854666910 and 12209136001; Roche). Briefly, the TRAP assay involved PCR followed by ELISA. hTERT activity using the photometric immunoassay was measured by reading absorbance at 450 nm. Absorbance readings were normalized to WT hTERT (or $hTERT^{IA-}$ for Fig. 3*A*), which was set at 100%. The TRF analysis involved Southern blotting for telomeric terminal restriction fragments. Telomere lengths were indicated by size and intensity of the fragments. TRAP assay PCRs were each done in duplicate, and both TRAP and TRF assays were reproduced two to four times with independently transduced cell populations.

Quantitative Real Time RT-PCR. HMECs were plated at 0.2 million per 100 mm dish in complete medium. For hPOT1 knockdown experiments, HMECs were refed every 2 to 3 d and harvested after 7 d. For RMRP knockdown experiments and Wnt/ß-catenin pathway analyses, HMECs were washed 24 h after plating, and the medium

changed to minimal medium, cells were refed after 2 d and cultured for an additional 2 d before harvest. For LiCl treatment, LiCl was dissolved in water and 5mM, 20mM and 40mM of LiCl (Catalog number 2370-01; J.T. Baker Inc., Phillipsburg, New Jersey, United States) was added to the HMECs 18 h prior to harvest.

Cells were harvested using Qiazol lysis reagent (Catalog number 79306; Qiagen, Valencia, California, United States) and total cellular RNA was isolated using the miRNeasy mini kit (Catalog number 1038703; Qiagen). cDNA was synthesized from 1μg total RNA using Taqman Reverse transcription reagents (Part number N808-0234; Applied Biosystems, Foster City, California, United States) as per manufacturer instructions. Real time PCR was carried out using the Taqman Universal PCR Master Mix (Part number 430447; Applied Biosystems), and the following primer probe sets from Applied Biosystems (listed below as gene names, ID numbers): HPRT1, HS99999909_m1; LEF1, HS01547250_m1; SMAD7, HS00998193_m1; WNT11, HS00182986_m1; Axin2, HS00610344_m1; WNT4, HS00229142_m1; and Pot1, HS00209984_m1. Real time PCR for RMRP knockdown experiments was carried out using Platinum SYBR Green qPCR SuperMix-UDG w/ROX (Catalog number 11744-100; Invitrogen, Carlsbad, CA, United States) and the primer sets for total RMRP (F5 and R257) and β-actin (F673, R947) detection described previously (10). Real time PCR was performed using Applied Biosystems 7900HT Real-time PCR system. Samples were normalized using expression levels of human HPRT1 or ß-actin. Results were confirmed in two to five independent experiments with cells from two separate transductions.

DNA Damage Analysis. Cells were plated at 1000 cells per well (Catalog number 154534; Lab-Tek II chamber slides, Nalgene Nunc International, Rochester, NY, United States). After 5 to 7 d, they were either left untreated or irradiated (10 Gy) and allowed to recover at 37°C for 30 min. Cells were washed with PBS, fixed in 2% formalin for 20 min, permeabilized with 0.05% TritonX-100 for 20 min, and incubated with blocking solution (PBS with 0.5% Sodium azide, 1% BSA, 2% Triton X-100, 0.4% Tween-20, and 5% goat serum) for 10 min. Cells were then incubated with anti-phospho-histone H2AX Serine 139 antibody (Catalog number 05-636; Upstate Biotechnology, Lake Placid, NY, United States) at 1:200 dilution in blocking solution for 45 min, washed, incubated with Alexa Fluor 488-conjugated goat anti-mouse antibody (A11029; Invitrogen/Molecular Probes) at 1:400 dilution in blocking solution for 20 min and washed. Finally, nuclei were stained with TO-PRO 3 (T-3605; Invitrogen/Molecular Probes) and slides mounted using ProLong Gold anti-fade reagent (P36930; Invitrogen/Molecular Probes). Nuclei were imaged and the numbers of phospho-H2AX positive foci were determined for at least 100-300 cells per cell type. Duplicate wells were imaged for each experiment, and the results confirmed with three independent experiments.

Chromosome Analysis. HMECs were plated at 0.3 million cells per T-75 flask in complete medium and analyzed when they reached 80 to 85% confluence. Metaphase spreads were prepared from cells treated with colcemid (0.2 μg/ml) for 2 h, followed by a 0.075 M KCl hypotonic treatment for 30 min. Standard G-banding karyotypic analysis was performed on 20 metaphase spreads for each cell type.

Senescence-Associated β-Gal Staining. HMECs were fixed in 2% formaldehyde, 0.2% glyceraldehyde and stained with fresh SA-β-gal stain at 37°C (no CO_2) for 14 h to 16 h as described previously (11). Images of cells were captured using a Nikon SMZ1500 microscope. Experiments were reproduced with two to three sets of independently transduced cells.

1. Romanov SR, *et al.* (2001) Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. *Nature* 409:633-637.
2. Armbruster BN, Banik SS, Guo C, Smith AC, Counter CM (2001) N-terminal domains of the human telomerase catalytic subunit required for enzyme activity in vivo. *Mol Cell Biol* 21:7775-7786.
3. Banik SS, *et al.* (2002) C-terminal regions of the human telomerase catalytic subunit essential for in vivo enzyme activity. *Mol Cell Biol* 22:6234-6246.
4. Hahn WC, *et al.* (1999) Inhibition of telomerase limits the growth of human cancer cells. *Nat Med* 5:1164-1170.
5. Stewart SA, *et al.* (2002) Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. *Proc Natl Acad Sci U S A* 99:12606-12611.
6. Ichihara M, *et al.* (2007) Thermodynamic instability of siRNA duplex is a prerequisite for dependable prediction of siRNA activities. *Nucleic Acids Res* 35:e123.
7. Veldman T, Etheridge KT, Counter CM (2004) Loss of hPot1 function leads to telomere instability and a cut-like phenotype. *Curr Biol* 14:2264-2270.
8. Smith LL, Coller HA, Roberts JM (2003) Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nat Cell Biol* 5:474-479.
9. Coller HA, Sang L, Roberts JM (2006) A new description of cellular quiescence. *PLoS Biol* 4:e83.
10. Maida Y, *et al.* (2009) An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature* 461:230-235.
11. Dimri GP, *et al.* (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 92:9363-9367.

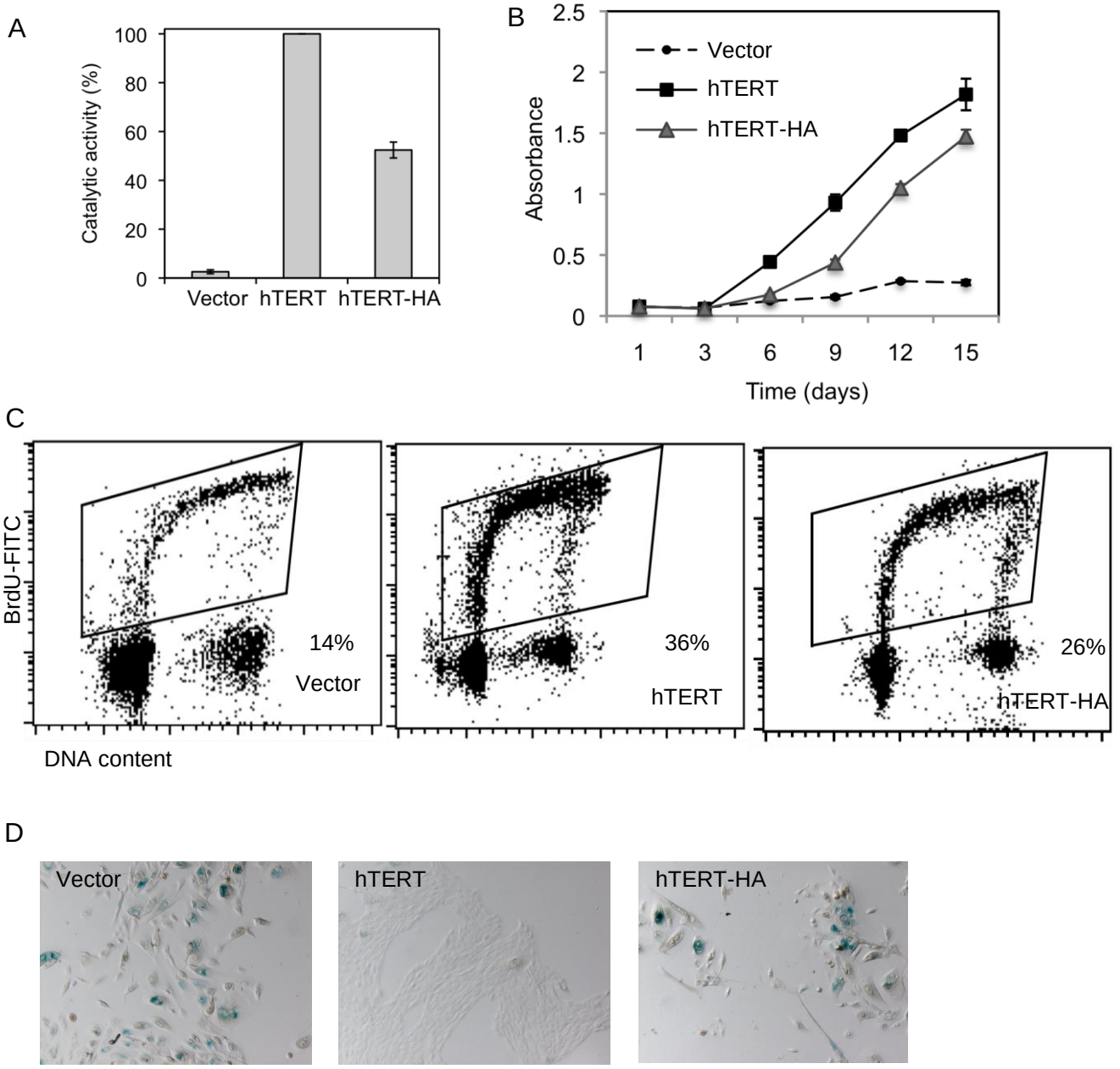


Fig. S1. hTERT-HA enhances proliferation in mitogen-limited conditions but does not extend cellular lifespan. HMECs were stably transduced with the indicated retroviral vectors. *(A)* hTERT catalytic activity. The graph shows the averages ± SEM of three TRAP assays. *(B)* Proliferation rates of cells in minimal medium by crystal violet staining. Results are the averages ± SD from one experiment, and are representative of three of five experiments. *(C)* Cells were labeled with BrdU. Representative flow cytometry profiles and the percentage of BrdU-positive cells are shown. *(D)* Staining of late passage (passage 17) cells for SA-β-gal (blue).

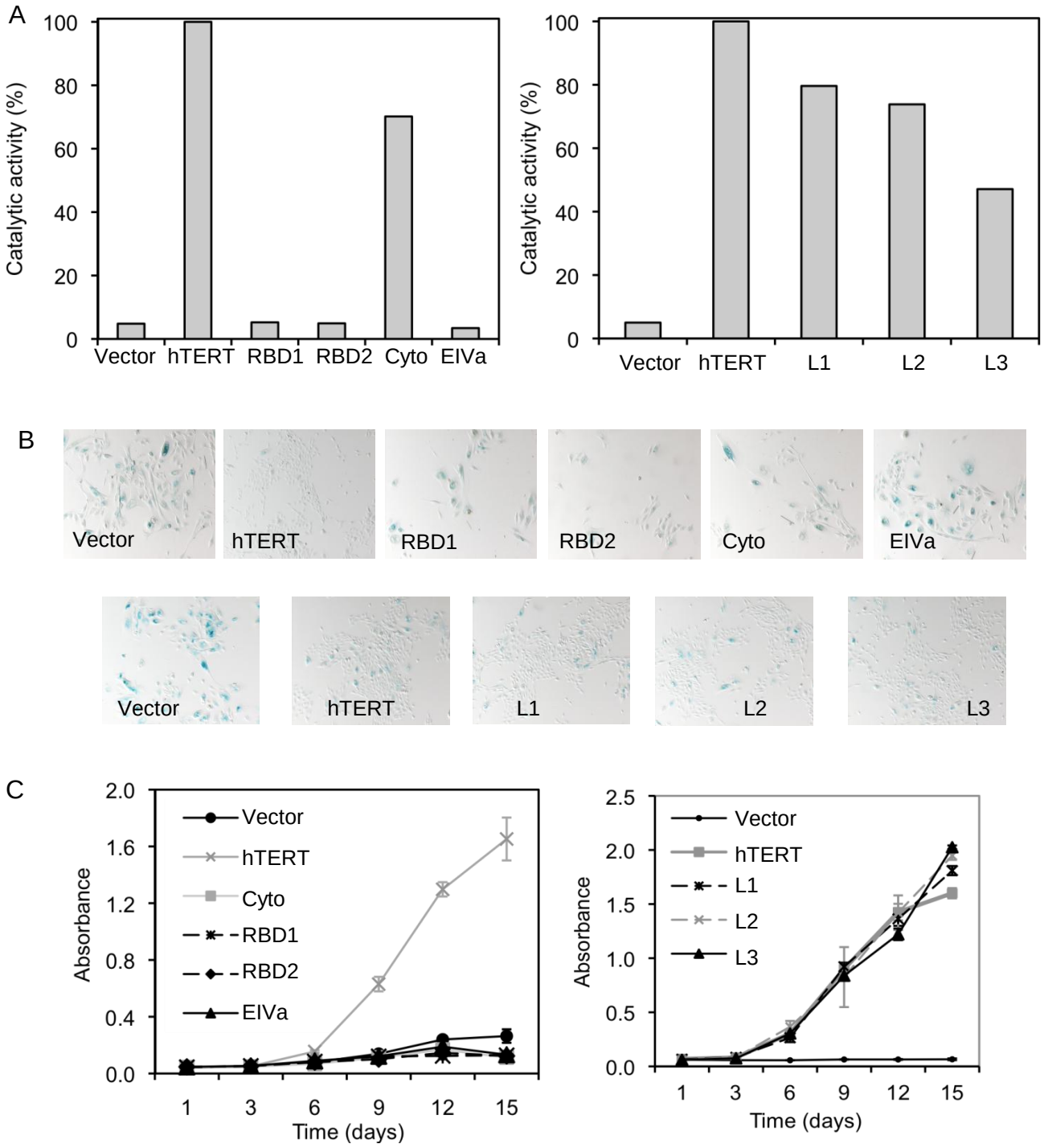


Fig. S2. hTERT catalytic activity, RNA binding, and nuclear localization are required for proliferation enhancement. HMECs were retrovirally transduced with either empty vector (i.e., Vector), WT hTERT (i.e., hTERT) or with a panel of hTERT mutants. These included four functional domain mutants (RBD1-386 and RBD2-512 mutated in the RNA Binding Domain, Cyto-1034 cytoplasmic hTERT, mutated in the domain for 14-3-3-mediated nuclear localization, and EIVa-1088 mutated in the C-terminal domain) and three linker region mutants (L1-296, L2-422, L3-1022). HMECs were assayed for *(A)* hTERT catalytic activity by TRAP assay (functional domain mutants-*Left*, linker region mutants-*Right*), *(B)* Senescence of late passage cells by staining for SA-β-gal (blue) (functional domain mutants-*Upper*, linker region mutants-*Lower*), and *(C)* Proliferation rate of early passage cells by crystal violet staining (functional domain mutants-*Left*, linker region mutants-*Right*). Results shown are ± SD from one growth assay and are representative of two independent experiments.

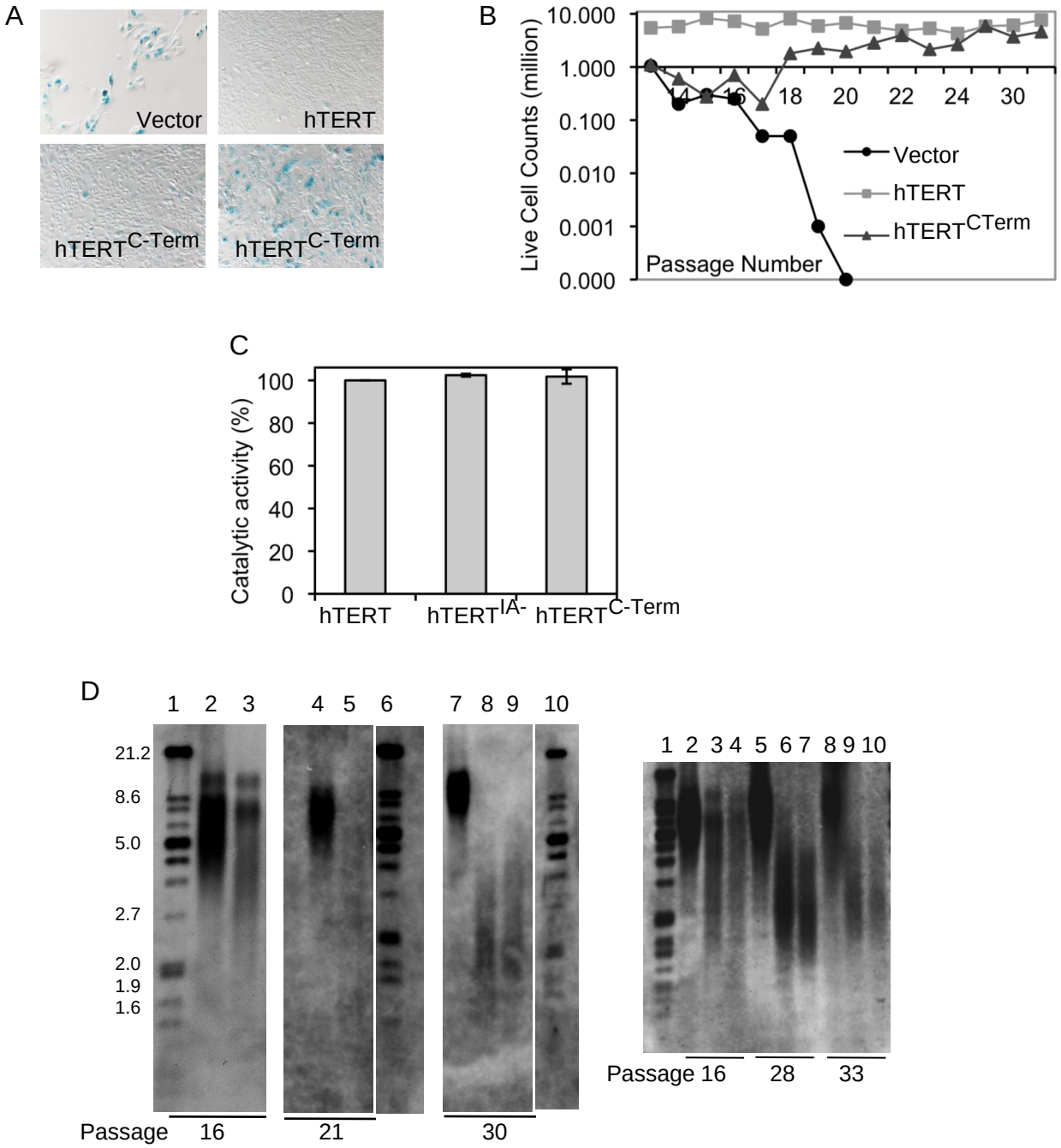
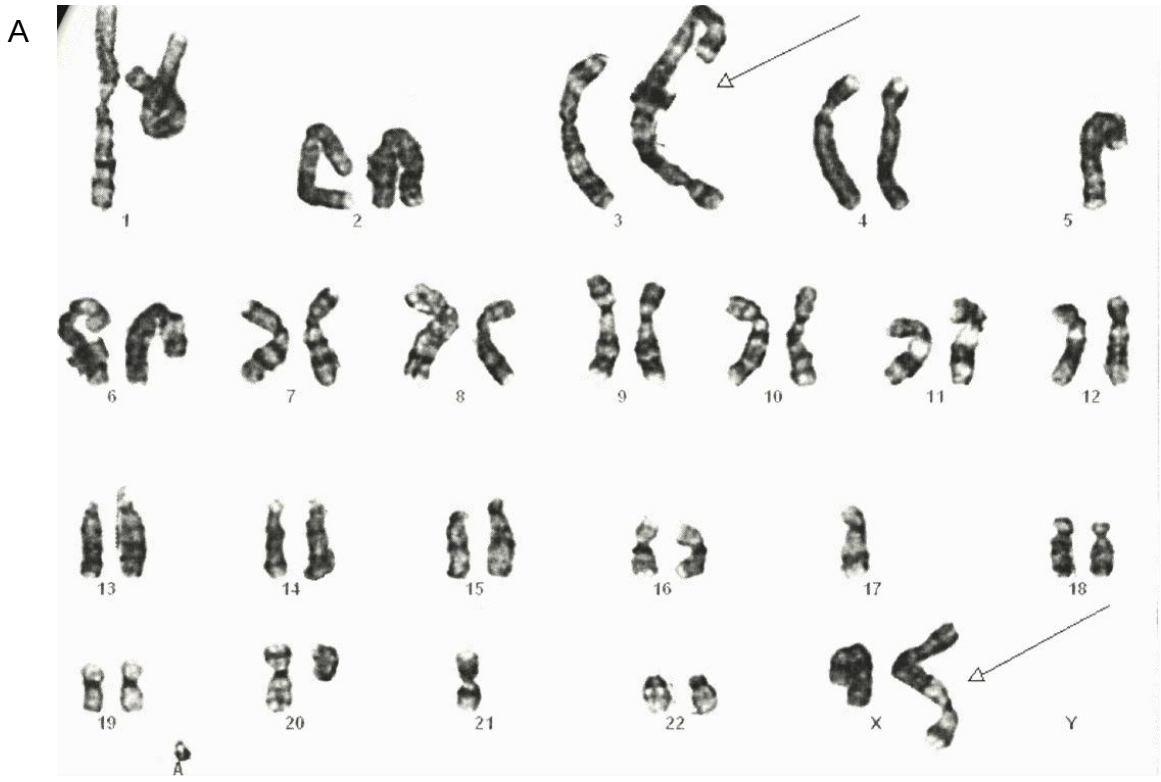
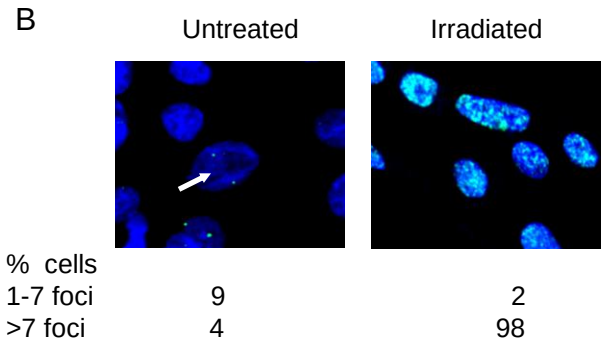


Fig. S3. The hTERT^{CTerm} mutant extends lifespan but does not maintain telomeres. HMECs were stably transduced with the indicated retroviral vectors. *(A)* HMECs at passage 16 were stained for SA-β-gal (blue). *(B-D)* HMECs were passaged as described in Figure 4*B*. *(B)* Live cell counts were determined at the specified passages. *(C)* hTERT catalytic activity in HMECs after 5 mo in culture was measured by TRAP assay. *(D) Left:* HMECs with WT hTERT (lanes 2, 4, 7) or hTERT^{CTerm} (lanes 3, 5, 9) or hTERT^{IA-} (lane 8) were harvested at the indicated passages and their telomere lengths compared. Lanes 1, 6 and 10 are the molecular weight markers (kb). *Right*: Longer exposure of Southern blot shown in Fig 4*D*.





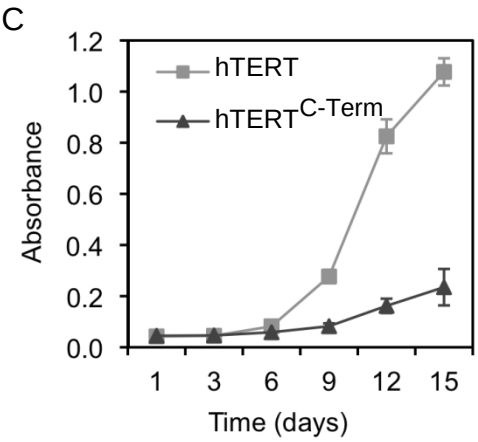


Fig. S4. Lifespan extension occurs without the chromosome fusions seen in senescing HMECs, activation of DNA damage pathways or enhanced proliferation. *(A)* A photomicrograph of one representative metaphase spread showing standard G-banding karyotype analysis of positive control, late passage, senescing HMECs. Tas = telomere-associated events and the arrows indicate the fusions between chromosomes 3 and 5 as well as chromosomes X and 17. *(B-C)* HMECs transduced with the indicated vectors were passaged for either 4 mo *(B)* or 5 mo *(C)*. *(B)* Cells were left untreated or irradiated and stained for phospho-H2AX foci. Phospho-H2AX foci in at least 300 cells for each of the cell populations were scored and the average percentage of cells containing foci are shown. *(C)* Cells were analyzed for proliferation rates in minimal medium by crystal violet staining. The graph shows the averages ± SD from one representative of two growth assays, each with independently transduced cells.

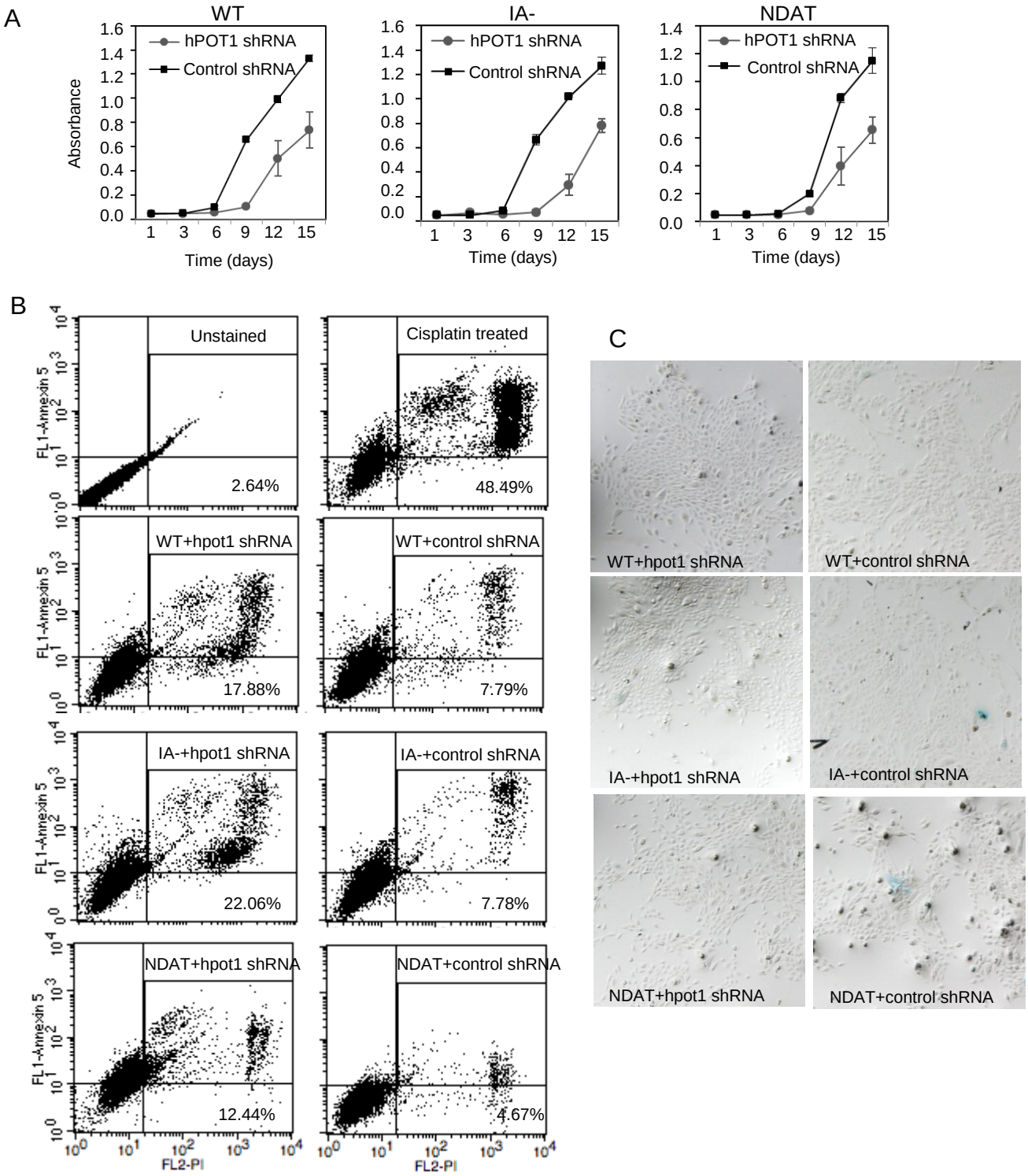


Fig. S5. HMECs with short telomeres show decreased proliferation and increased apoptosis upon hPOT1 knockdown. After 5 mo in culture HMECs expressing indicated hTERT constructs (WT, WT hTERT; IA-, hTERT^{IA-}; NDAT, hTERT$^{\Delta DAT\ 116}$) were stably transduced with retroviral vectors expressing hPOT1 or control shRNAs. *(A)* Cells were analyzed for proliferation rates in complete medium by crystal violet staining. The graph shows the averages ± SEM from one representative of two experiments with independently transduced populations. *(B)* Cells were stained for annexin V. Control HMECs were either left unstained (negative control) or treated with cisplatin 18 h before staining (positive control). Representative flow cytometry profiles and percentages of annexin V-positive cells are shown. *(C)* HMECs were stained at a late time point (12 d after drug selection) to detect SA-β-gal activity (blue). Images are representative of two independent transductions.

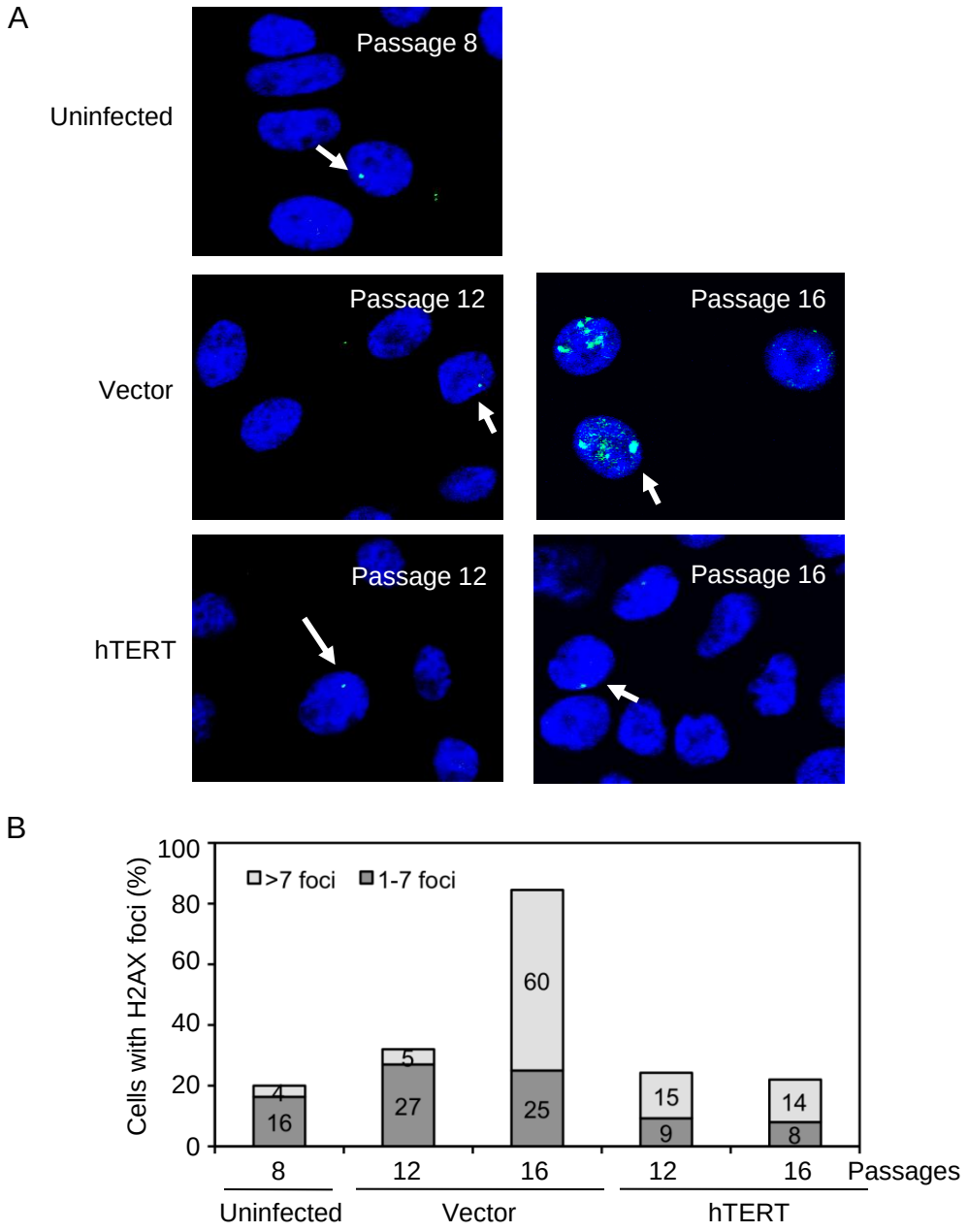


Fig. S6. HMECs with or without hTERT have similar numbers of DNA damage foci except at late passages. *(A)* HMECs either uninfected (early presenescent at passage 8) or retrovirally infected with empty vector or wild type hTERT (presenescent at passage 12 and late passage at passage 16) were assessed for DNA damage foci. Foci were visualized by staining for phospho-H2AX. *(B)* The graph shows the average phospho-H2AX DNA damage foci in at least 100 cells each.

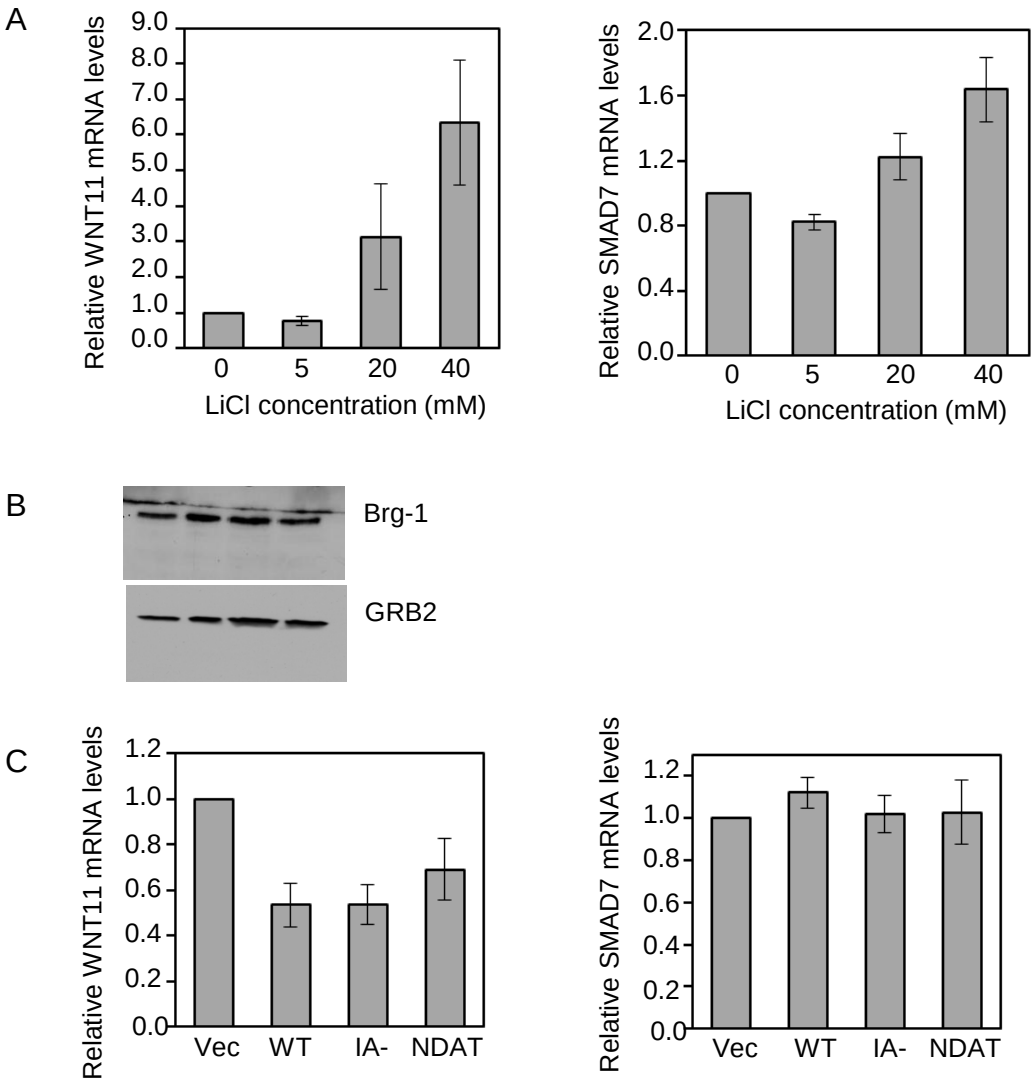


Fig. S7. Wnt pathway gene expression can be induced in HMECs by LiCl but not by hTERT. *(A-B)* The mRNA levels of the specified genes were measured by qPCR and were normalized to HPRT1 in each sample. *(A)* HMECs were treated with the indicated concentrations of LiCl for 18 h prior to harvesting and RNA isolation. The results are the averages ± SEM of four experiments and show the fold change in mRNA levels relative to untreated HMECs. *(B-C)* HMECs were stably transduced with the indicated retroviral vectors (Vec , vector; WT, WT hTERT; IA-, $hTERT^{IA-}$; NDAT= $hTERT^{N\text{-}DAT\ 116}$). *(B)* Cell lysates were analyzed by Western blotting for Brg-1 protein levels. GRB2 is the loading control. *(C)* HMECs were harvested and total RNA isolated. The results are the averages ± SEM of five experiments and show the fold change in mRNA levels relative to HMECs with the vector control.

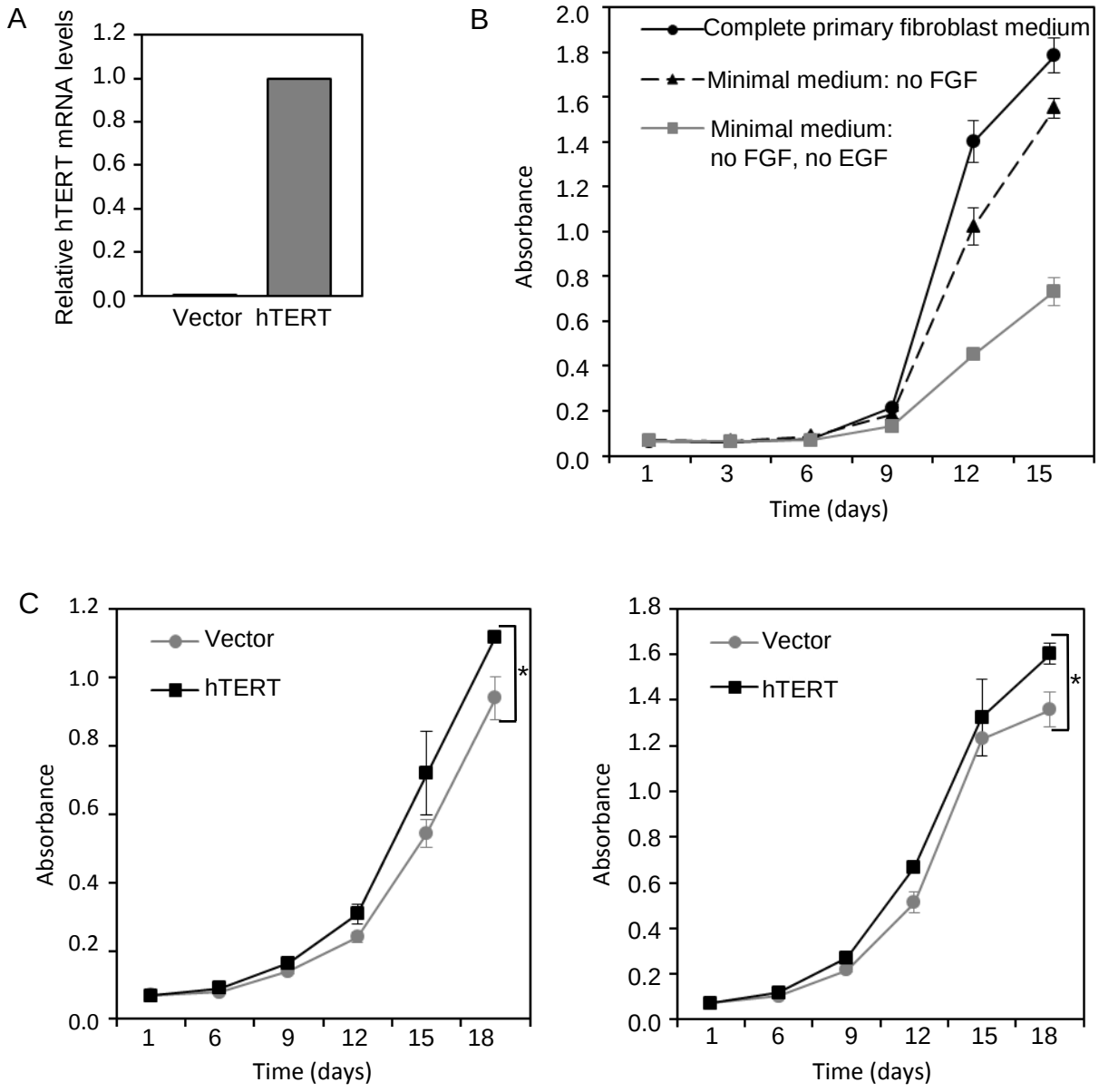


Fig. S8. hTERT can modestly enhance proliferation in mitogen-limited conditions in the absence of hTR. VA13 cells were stably transduced with retroviral vectors (empty vector or with WT hTERT). *(A)* hTERT gene expression was measured by qPCR and normalized to HPRT1 in each sample. *(B)* Cells were cultured in either complete medium or mitogen-limited (minimal) media over the indicated time course. Crystal violet staining was used to assess their proliferation rate. Results are shown as averages ± SEM. *(C)* Cells were cultured in mitogen-limited medium and proliferation measured. Results from two representatives of three growth assays are shown as averages ± SEM, * = statistically significant difference (p value <0.05, ANOVA).

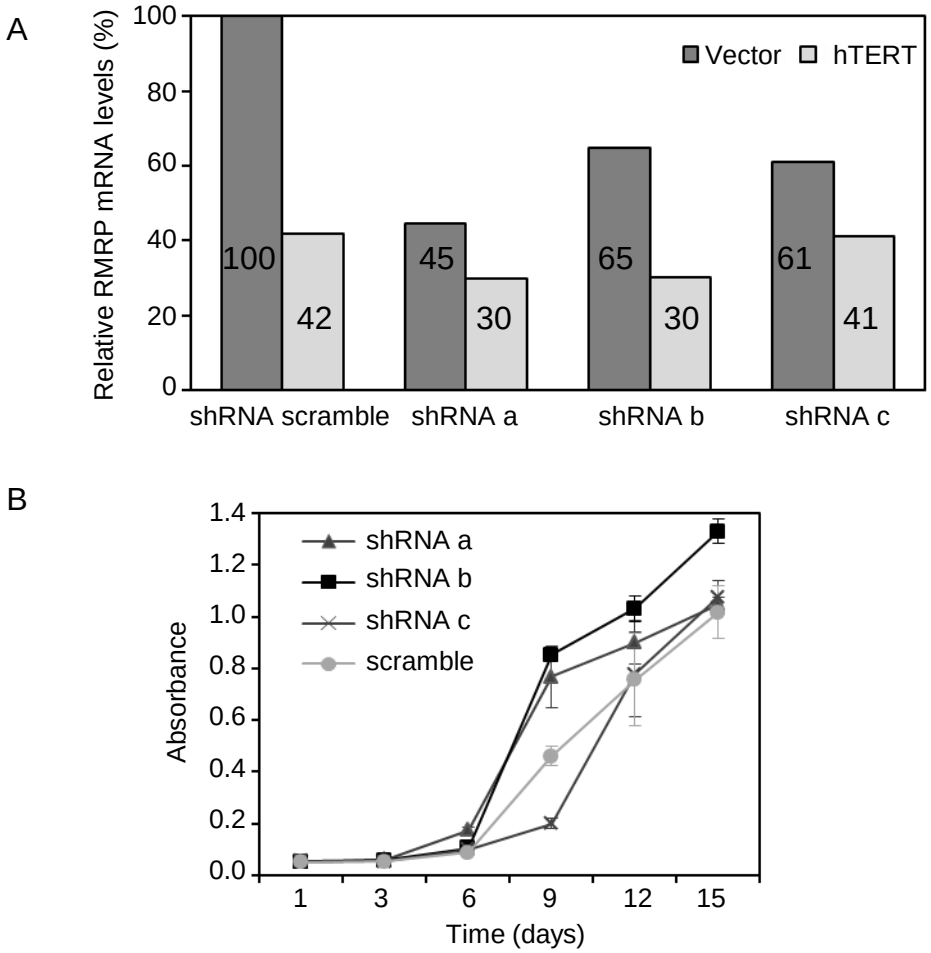


Fig S9. RMRP knockdown marginally enhances cell proliferation in HMECs with hTERT. HMECs were stably transduced with a combination of empty vector/hTERT and the indicated RMRP shRNAs. *(A)* The knockdown in RMRP was measured by qPCR and normalized to β-actin in each sample. The graph shows the averages of the duplicates and represents the percent RMRP knockdown relative to HMECs with empty vector plus RMRP scramble shRNA control. *(B)* HMECs transduced with hTERT and RMRP shRNAs were cultured in minimal medium and cell proliferation was measured by crystal violet staining. Results are averages ± SEM and were confirmed in an independent growth assay.

Table S1. hTERT functional domains and linker regions, showing amino acid locations of the mutants analyzed *(Upper)*. hTERT mutants and their phenotypes *(Lower)*.

IA	DAT	IB	L1	RBD	L2	RBD		RT	EI	L3	EII	EIII	EIV	DAT
32	92	116	296	386	422	512		711-712		1022	1034	1088	1106	1127

1-NH2 C00H-1132

	Name	Domain	Mutation start	Catalytic activity (TRAP)	Telomere synthesis (TRF)	Lifespan extension (Passage & SA-β-gal)	Proliferation advantage (Growth assay & BrdU FACS)	DNA damage
1.	hTERT		Wild type	+	+	+	+	+
2.	$hTERT^{IA-}$	IA	+32	+	-	+	+	
3.	$hTERT^{N\text{-}DAT\ 92}$	N-DAT	+92	+	-	-	-	+
4.	$hTERT^{N\text{-}DAT\ 116}$	N-DAT	+116	+	-	+	-	
5.	$hTERT^{L1}$	L1	+296	+	+	+	+	
6.	$hTERT^{RBD1}$	RBD	+386	-	-	-	-	
7.	$hTERT^{L2}$	L2	+422	+	+	+	+	
8.	$hTERT^{RBD2}$	RBD	+512	-	-	-	-	
9.	$hTERT^{DN}$	RT	711	-	-	-	-	-
10.	$hTERT^{IA-/DN}$	IA/DN	+32/711	-	-	-	-	
11.	$hTERT^{L3}$	L3	+1022	+	+	+	+	
12.	$hTERT^{Cyto}$	(EII) 14-3-3 binding	+1034	+	-	-	-	
13.	$hTERT^{EIV}$	EIV	+1088	-	-	-	-	
14.	$hTERT^{CTerm}$	Cterm	+1106	+	-	+	-	
15.	$hTERT^{CDAT1127}$	C-DAT	+1127	+	-	-	-	+
16.	hTERT-HA	HA tag	+1132	+	-	-	+	

DAT= Dissociation of activities of Telomerase; L= Linker region; RBD= RNA-binding domain
RT= Reverse transcriptase active site; DN= dominant negative;
TRAP= Telomerase repeat amplification protocol; TRF= Terminal restriction fragment analysis
SA-β-gal = Senescence-associated beta-galactosidase
BrdU FACS = Bromodeoxyuridine, Fluorescence activated cell sorting