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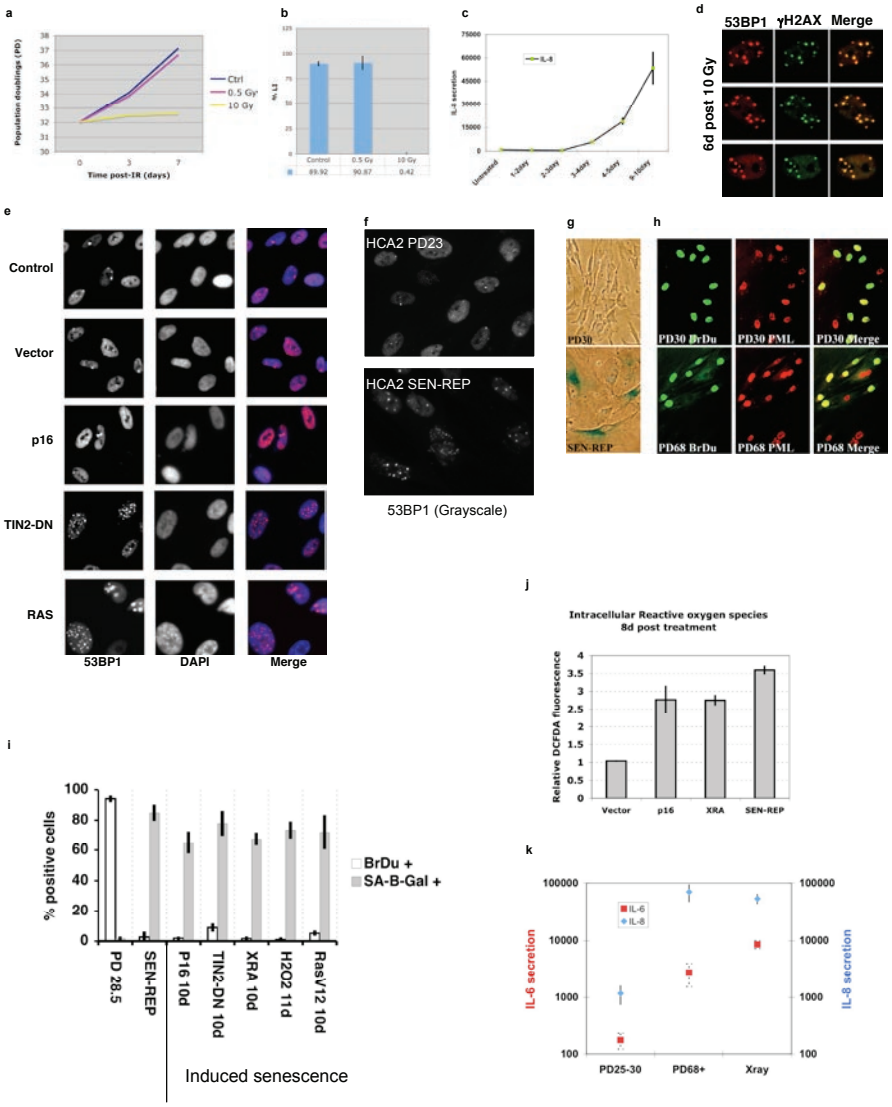


Figure S1 Error bars show the S.D. from 3 or more independent measurements. (**a**) HCA2 cells were irradiated with the indicated doses. Cell number was assessed 3 and 7 d later. The changes in cell number are reported as population doublings. (**b**) Cells were cultured on glass slides, irradiated, allowed to recover for 48 h, and incubated for a further 24 h in the presence of BrdU before being fixed and analyzed by immunofluorescence. The percentage of BrdU positive cells (LI, labeling index) is graphed and the numerical values are given in the boxes below the Y-axis. (**c**) Cells were irradiated with 10 Gy as in Figure 1f, and IL-8 was measured using ELISA and CM collected during the indicated 24 h intervals. The data are reported as 10^{-6} pg IL-8/cell/day. (**d**) Shown are images of three individual cells that were analyzed by immunofluorescence 6 d after 10 Gy irradiation for 53BP1 (red) and γ-H2AX (green) foci. (one cell per panel set). The merged red and green channels display co-localization in yellow. (**e**) HCA2 cells were untreated (control) or infected with lentiviruses expressing no insert (vector) or the indicated proteins. Ten days later, single cells were analyzed by immunofluorescence for 53BP1 foci (red). The nuclei were counterstained using DAPI (blue). (**f-k**) Analysis for additional senescence markers, including DNA damage foci, low BrdU incorporation (growth arrest), high senescence-associated β-galactosidase (SA-Bgal) expression, levels of intracellular reactive oxygen species, and levels of secreted IL-8. (**f**) Early passage (PD23) and replicatively senescent (SEN-REP) HCA2 cells were fixed and stained by immunofluorescence for 53BP1 foci (shown in grayscale). (**g**) Representative SA-ß-Gal staining of early passage (PD30) and replicatively senescent (SEN-REP) HCA2 cells. (**h**) Representative 24 h BrdU labeling of early passage (PD30) and late passage (PD68) HCA2 cells. Cells were fixed and stained for BrdU (green) and a nuclear counterstain (PML, red). (**i**) Percentage of cells expressing SA-ß-Gal or showing positive BrdU staining in early passage (PD28) and replicatively senescent (PD 71+) HCA2 cells, and cells 10-11 d after they were induced to senesce by p16, telomere uncapping due to expression of the dominant negative TIN2-DN protein, 10 Gy X-irradiation (XRA), a one hour exposure to 150 μM H_2O_2, or expression of oncogenic RAS^{V12}. (**j**) Cells were infected or treated to induce senescence as in Figure 1g-i. Levels of intracellular ROS were measured 8 d after treatment using H2-DCFDA and FACS analysis. Fold changes are reported compared to cells infected with an insertless viral vector, which had ROS levels similar to untreated cells (not shown). (**k**) Conditioned media were collected during a 24 h interval from the indicated HCA2 cell populations, and IL-6 and IL-8 were measured using ELISA. The data are reported as 10^{-6} pg/cell/day and plotted on a log scale.

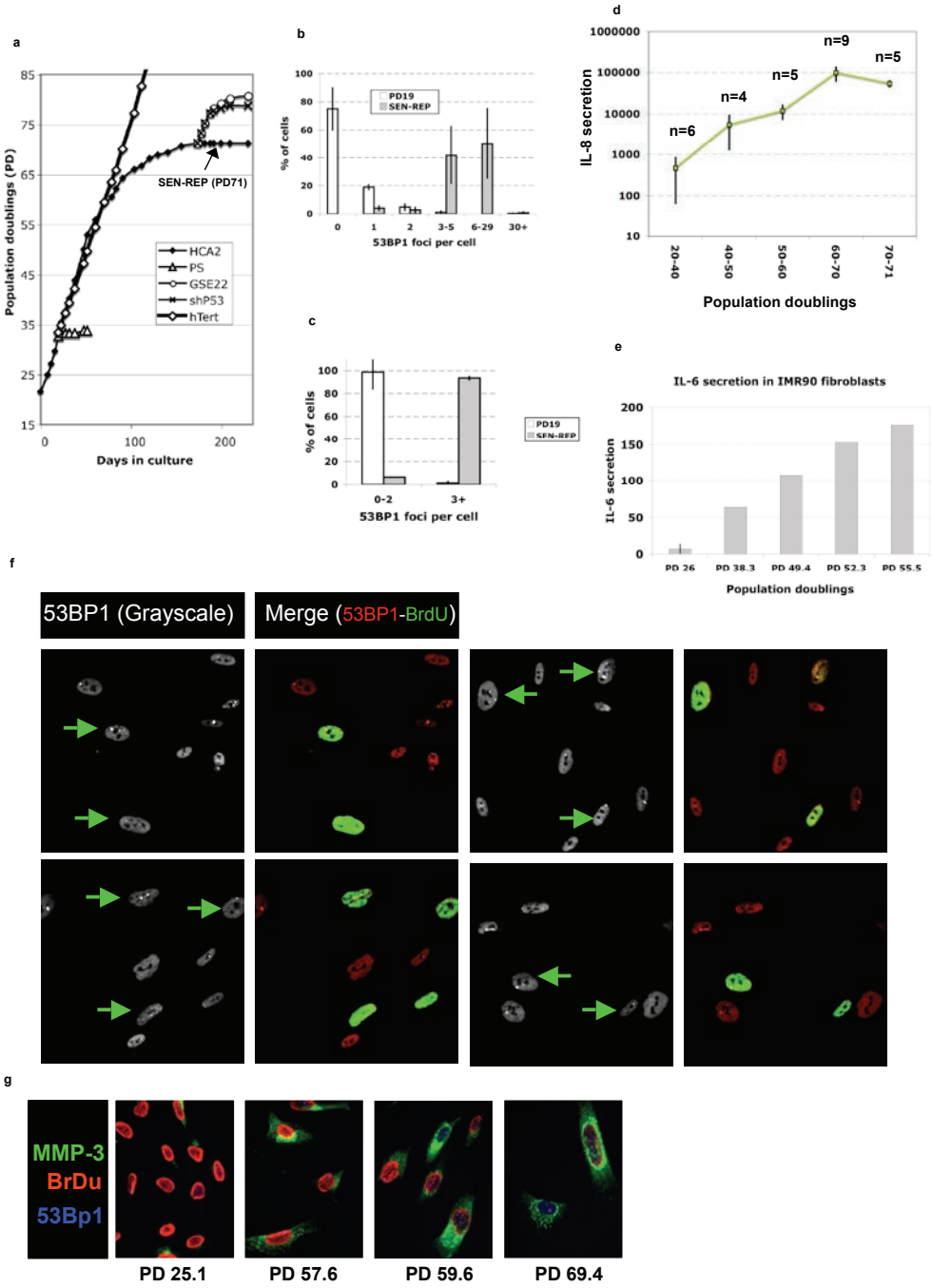
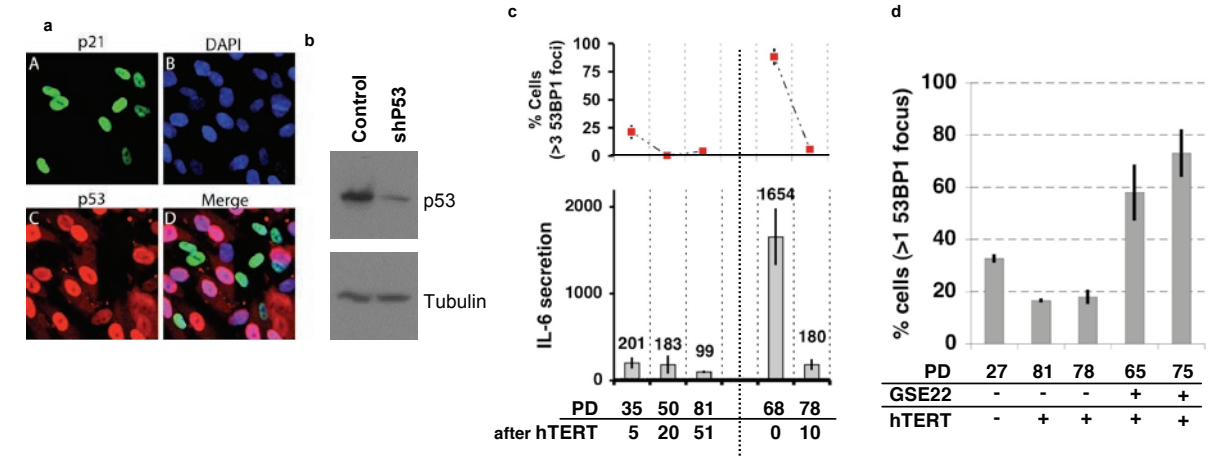
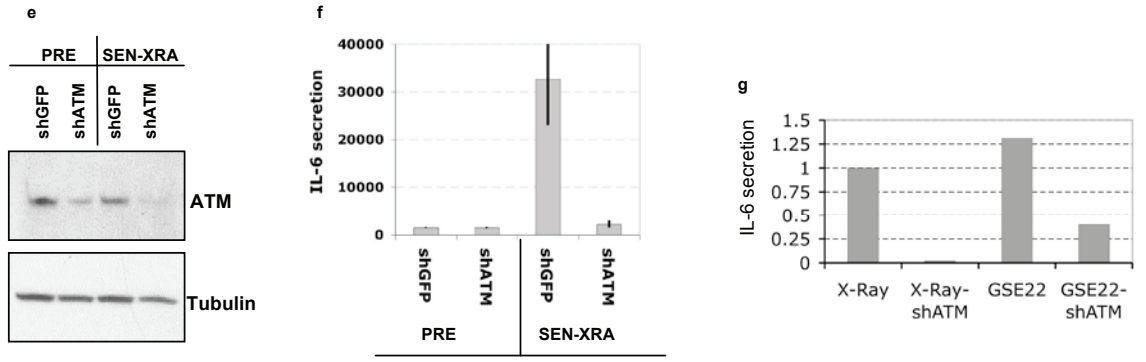


Figure S2 (**a**) Growth curve of HCA2 cells cultured until replicative senescence caused by telomere dysfunction and p53 activation. HCA2 cells at PD27-30 were infected with a retrovirus expressing hTERT (hTERT) or stimulated to rapidly enter senescence (PS) using the stimuli described in sFig1i. HCA2-hTERT cells bypassed telomere- dependent replicative senescence, which occurred in control cells around PD71. Replicatively senescent (SEN-REP) HCA2 (PD71) were infected with a lentivirus expressing GSE22 or a short hairpin RNA that reduces p53 expression by RNA interference (shP53). Loss of p53 function reverses the p53-dependent replicative senescence growth arrest, and allows cells to proliferate until crisis (caused by severely eroded telomeres). (**b**) Distribution of cells harboring varying numbers of 53BP1 foci per nucleus in early passage (PD19) or senescent (SEN-REP) HCA2 populations. (**c**) Distribution of cells harboring either 0-2 or >3 53BP1 foci/nucleus in early passage and replicatively senescent (SEN-REP) populations. (**d**) HCA2 cells at the indicated PD levels were analyzed for IL-8 secretion by assaying CM collected over a 24 h interval by ELISA. The data are reported as 10^{-6} pg IL-8/cell/day. (**e**) IMR90 populations were untreated and cultured until replicative senescence (PD>65). Cell populations were sampled at the indicated PDs and CM were collected over a 24 h interval. IL-6 was measured using ELISA. The data are reported as 10^{-6} pg/cell/day. The error bars on the PD26 control population is the S.D. from 3 independent experiments; all other samples are solitary measurements. (**f**) IMR90 fibroblasts at PD 55 were pulsed with BrdU for 30 min and fixed. At this PD, most cells in the population have at least one permanent 53BP1 DNA damage focus (not shown). Single cells were analyzed simultaneously by immunofluorescence for BrdU incorporation (green) and 53BP1 DNA damage foci (red). Four different representative fields are shown. Cells that are both BrdU positive and harbor at least one 53BP1 focus are indicated by arrows in the left panels, which display the 53BP1 immunofluorescence channel in grayscale for increased resolution of 53BP1 foci. The right panel displays the merged 53BP1 (red) and BrdU (green) channels. (**g**) HCA2 cell populations at the indicated PD levels were pulsed with BrdU for 24 h and fixed. Single cells were analyzed simultaneously by immunofluorescence for intracellular MMP-3 (green) (antibody AF513 R&D), BrdU incorporation (red) and 53BP1 foci (blue).





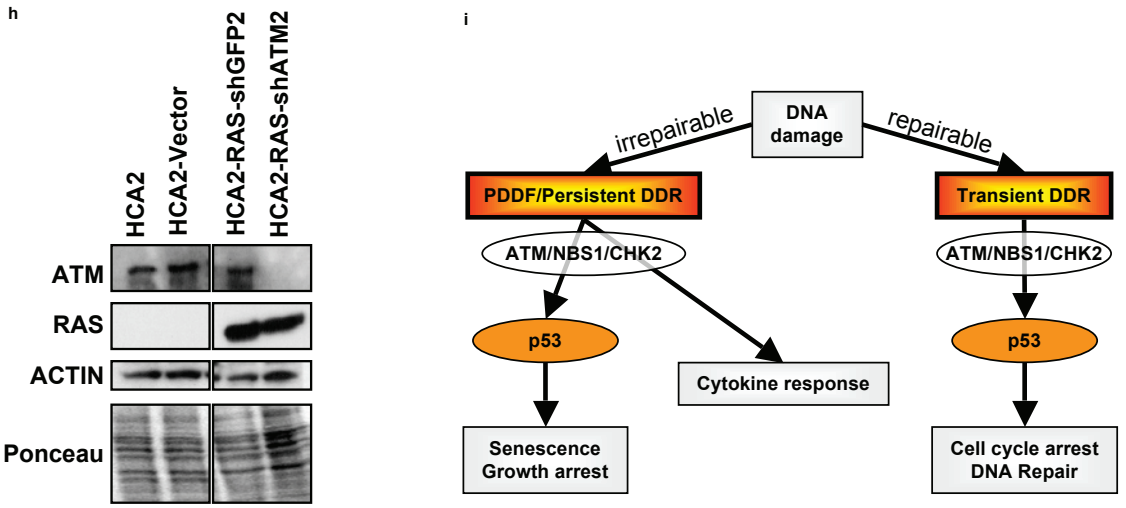


Figure S3 (**a**) HCA2-GSE22 and control HCA2 cells were mixed at a 1:1 ratio and seeded onto a glass slide. Cells were irradiated with 10 Gy and fixed 19 h later. The inactivation and stabilization of p53 in HCA2-GSE22 cells is evident by the strong p53 immunofluorescence staining, visible in the red channel (Panel C). Alternatively, p53-dependent p21 expression in HCA2 control cells was revealed by immunostaining for p21, visible in the green channel (Panel A). Nuclei were counterstained using DAPI, visible in the blue channel (Panel B). Notice the exclusion of red and green staining within single cells, confirming p53 inactivation in HCA2-GSE22 cells (Merge, Panel D). (**b**) Proteins were extracted from untreated HCA2 and HCA2-shP53 (PD 35) and analyzed by western blotting using antibodies against either total p53 (antibody clone DO-1) or tubulin (loading control). (**c**) To further test the idea that both the PDDF that accumulate as cells proliferate, as well as increased IL-6 secretion, are largely the result of dysfunctional telomeres, we asked whether the repair of telomeric PDDF by hTERT suppresses IL-6 secretion in late passage (PD68) HCA2 cells. At this passage, ~99% of cells had >1 53BP1 PDDF and IL-6 secretion was relatively high. Following infection with a lentivirus expressing hTERT, cells recovered a normal growth rate, without obvious colony formation, suggesting that telomerase rescued short dysfunctional telomeres in a large subset of the near-senescent population. The cells were cultured for an additional 10 PDs (right panels) and analyzed for the presence of 3 or more 53BP1 foci (top panels) or IL-6 secretion (lower panels). The left panels are identical to Figure 2e. Both the number of cells with >3 PDDF as well as IL-6 secretion declined, essentially to levels observed in early passage cells (see left panel or Fig. 2e), suggesting that hTERT expression was sufficient to repair telomeric PDDF and decrease IL-6 secretion. hTERT positive cells did not gain a growth advantage in a p53-deficient background. (**d**) Early passage HCA2 and HCA2-hTERT or HCA2-GSE22-hTERT that had undergone approximately 10 doublings in the presence of hTERT were analyzed at the indicated population doubling levels for the presence of 53BP1 foci. Note that GSE22 positive cell populations have more cells with at least one 53BP1 focus suggesting that some PDDF are telomere-independent. (**e**) Early passage WI38 fibroblasts cultured in a 3% oxygen atmosphere were infected with shGFP or shATM encoding lentiviruses and selected for 4 d. Proteins were extracted from untreated or X-irradiated WI38-shGFP and WI38-shATM cells (10 d after 10 Gy X-ray) and analyzed by western bloting using antibodies against either total ATM or tubulin (loading control). (**f**) Cells treated as in (a) were assessed for IL-6 secretion by analyzing CM collected over a 24 h interval using ELISA. The data are reported as fold change over untreated WI38-shGFP controls. Error bars show the S.D. from 3 independent measurements. (**g**) Damaged-induced senescent HCA2 (5 d after 10 Gy X-Ray) or PD50 HCA2-GSE22 cells were left untreated or infected with lenti-shATM12 and let recover for 5d. CM were collected over the next 24 h and analyzed for IL-6 using antibody arrays as in Fig. 5. The data are reported as fold IL-6 secretion compared to control irradiated HCA2 cells. Note that shATM12 reduces IL-6 secretion both in unmodified HCA2 and in HCA2-GSE22 cells, which have dysfunctional p53. (**h**) HCA2 cells were either untreated (HCA2), infected with an insertless lentivirus (HCA2-vector) or infected with lentiviruses expressing oncogenic RAS and shRNAs directed against GFP (shGFP2) or ATM (shATM2). After recovery and appropriate selection (10 d), whole cell lysates were analyzed by western blotting for the presence of the indicated proteins. Ponceau staining shows the total proteins loaded on the gel. (**i**) Model for cytokine response to DDR signaling and chromatin stress. Repairable DNA damage triggers a DDR that transiently prevents cell proliferation while the DNA damage is repaired. Irreparable DNA damage signals through PDDF, which provide persistent DDR signaling. The persistent DDR signals require the ATM, NBS1, and CHK2 proteins, which independently maintain the p53-dependent senescence growth arrest and the p53-independent cytokine response.

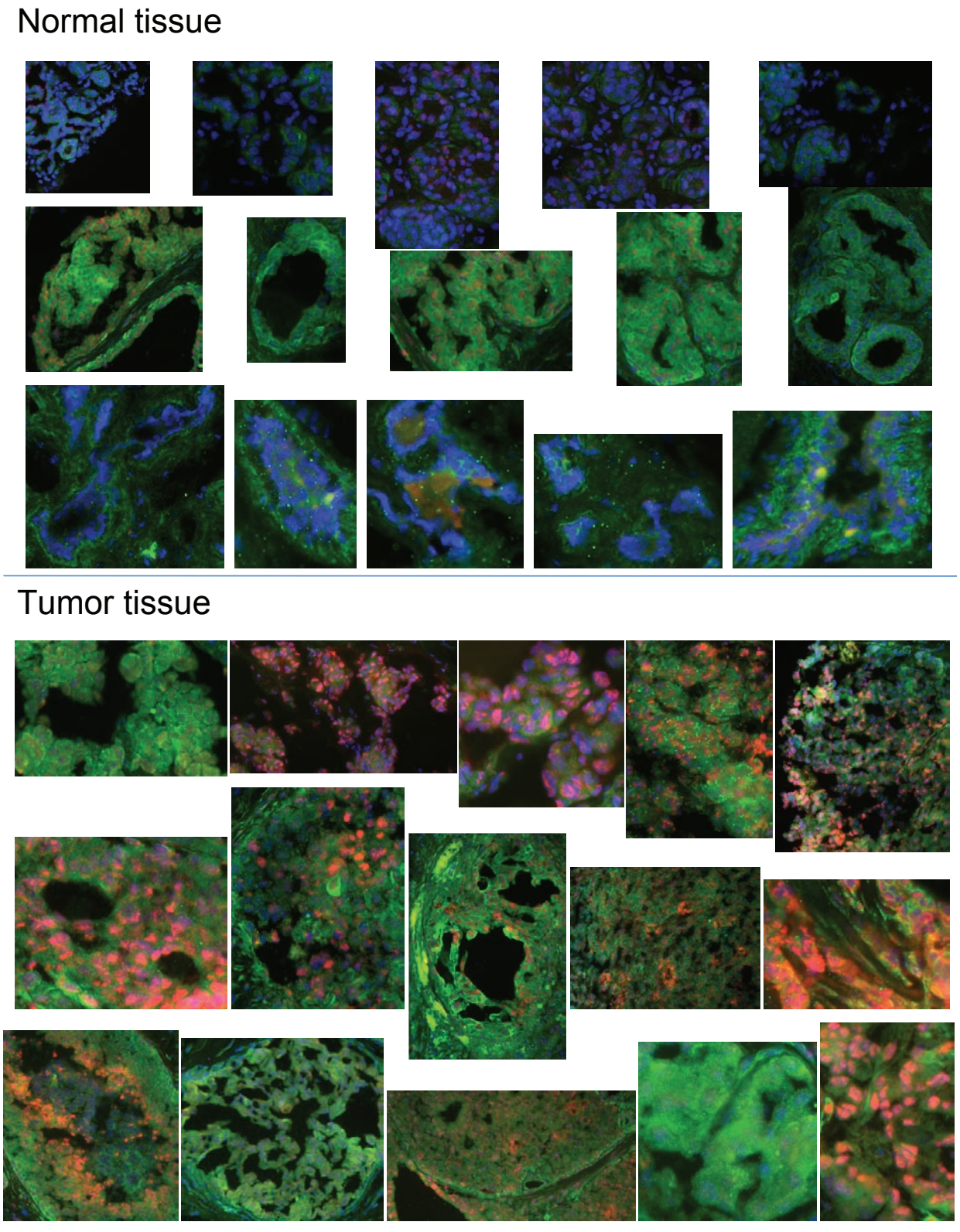


Figure S4 Examples of representative fields imaged from the frozen section tissue array and quantified using the cell profiler software.

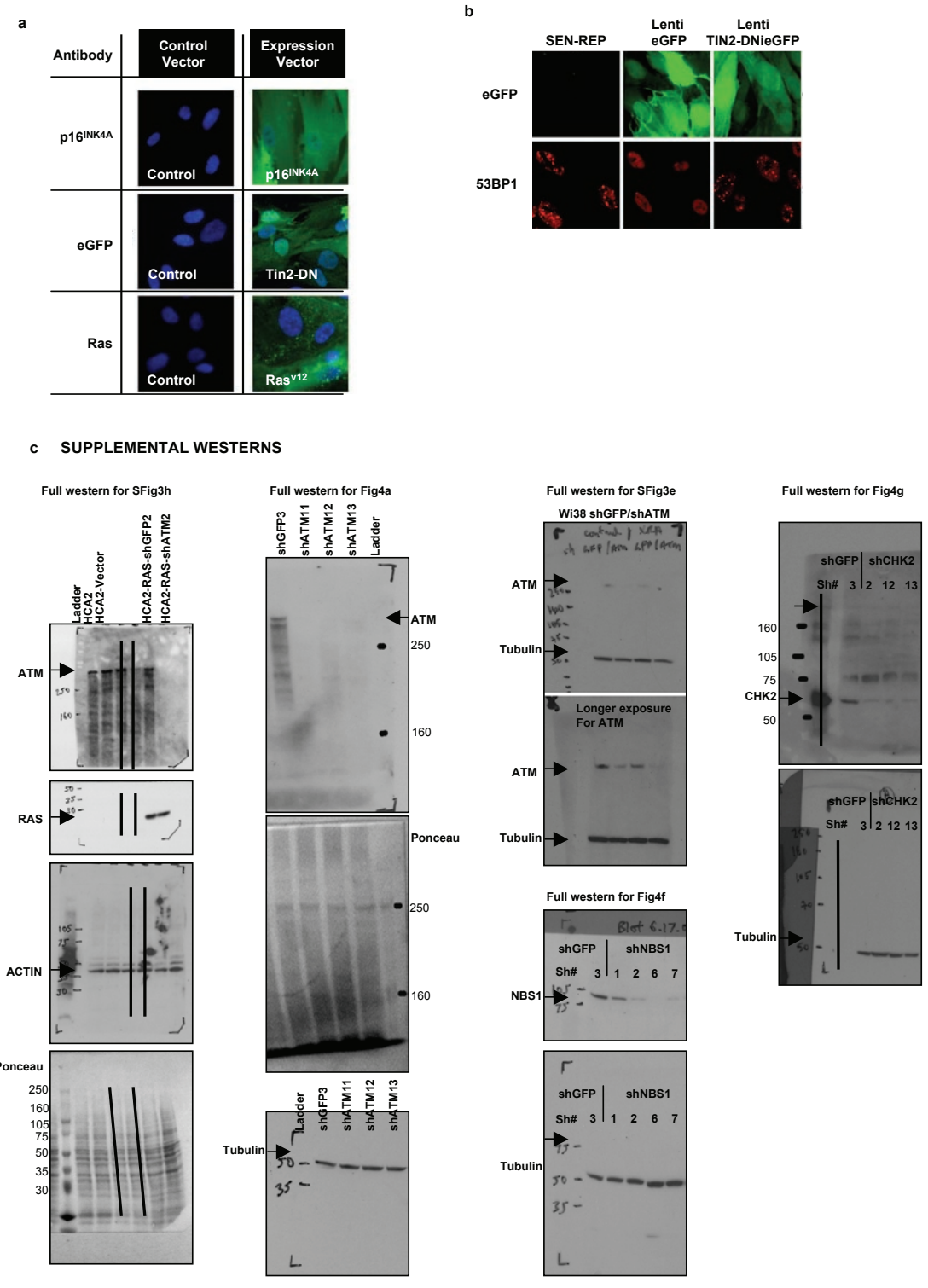


Figure S5 (**a**) HCA2 cells were infected with an insertless lentiviral vector (control), lentiviruses expressing p16, a dominant negative mutant of TIN2 (TIN2DN) coupled to enhanced green fluorescent protein (eGFP) from an internal ribosome entry site (TIN2DNieGFP) or oncogenic RAS^{V12}. Cells were allowed to recover, seeded on glass slides and fixed 7-10 d after infection. Samples were stained by immunofluorescence for p16, Ras or eGFP. Because lenti-TIN2DNieGFP encodes both TIN2DN and eGFP, TIN2DN transgene expression was assessed using eGFP. (**b**) HCA2 were infected with lentiviruses expressing eGFP or TIN2DNieGFP, let recover, seeded on glass slides and fixed 4 d after infection. Samples were stained by immunofluorescence for 53BP1 (red). Note that eGFP expression alone did not induce 53BP1 foci, while TIN2-DN induced multiple 53BP1 foci per nuclei, similar to replicatively senescent HCA2 (SEN-REP). (**c**) Supplementary full scans of western blots verifying gene depletion using RNAi. Full scans were cropped and used in the indicated primary figures.