SUPPLEMENTAL FIGURE LEGENDS

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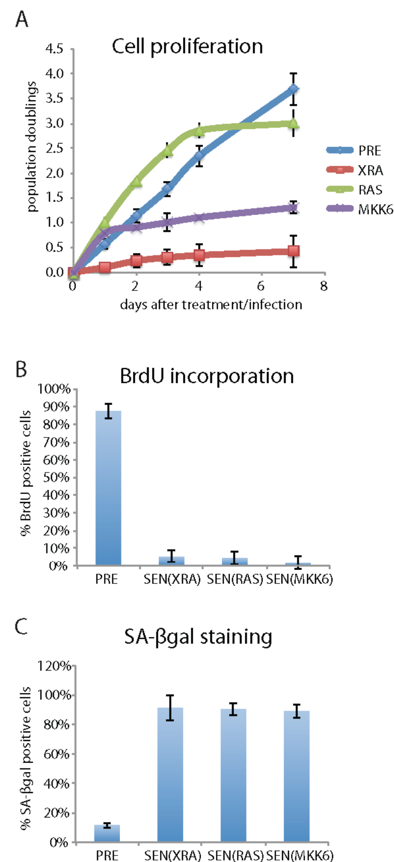
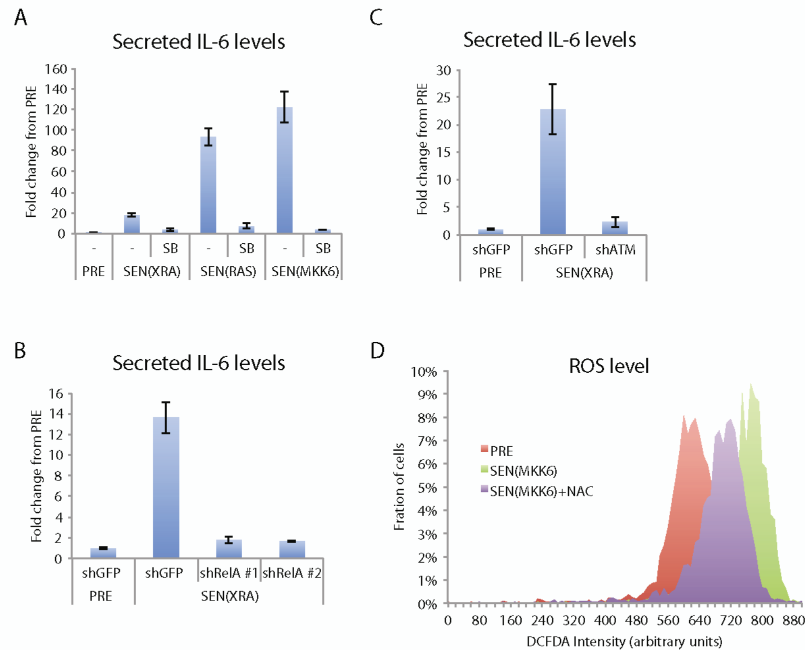


Supplemental Figure 1

(A) Cell proliferation. HCA2 cells (PRE) were irradiated (XRA), infected with a lentivirus expressing oncogenic RASV12 (RAS) or MKK6EE (MKK6) and counted at the indicated intervals thereafter.

(B) BrdU incorporation. HCA2 cells were treated as in (A), allowed to senescence for 7-10 d, cultured with BrdU for 24 h, fixed, and immunostained for incorporated BrdU. BrdU-positive cells were quantified by CellProfiler

(C) SA-Bgal staining. HCA2 cells were treated as in (A), allowed to senesce for 7-10 d, fixed, and stained for SA-Bgal.



Supplemental Figure 2

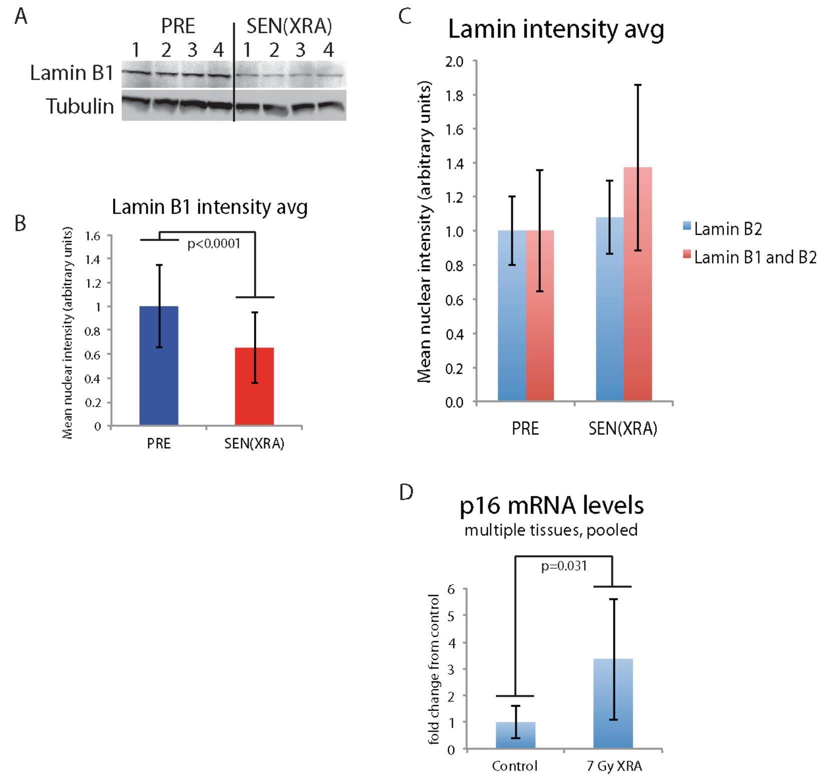
(A) p38MAPK inhibition by SB203580 effectively reduces senescence-associated extracellular IL-6. HCA2 cells (PRE) were irradiated (XRA), infected with a lentivirus expressing oncogenic RASV12 (RAS) or MKK6EE (MKK6) and allowed to senesce. Conditioned media (CM) were then collected and analyzed by ELISA. SB: cells were cultured in 10 uM SB203580 for 48h prior to CM collection.

(B) RelA depletion effectively reduces senescence-associated extracellular IL-6. HCA2 cells were infected with lentiviruses expressing either of two shRNAs against RelA (shRNA #1, #2) or GFP (shGFP; control) and selected. Cells were then mock-irradiated (PRE) or irradiated and allowed to senesce (SEN(XRA)). CM were collected and analyzed by ELISA.

(C) ATM depletion effectively reduces senescence-associated extracellular IL-6. HCA2 cells were infected with lentiviruses expressing an shRNAs

against ATM (shATM) or GFP (shGFP; control) and selected. Cells were then mock-irradiated (PRE) or irradiated and allowed to senesce (SEN(XRA)). CM were collected and analyzed by ELISA.

(D) N-acetylcysteine (NAC) effectively reduces reactive oxygen species (ROS) in senescent cells. Cells were infected with a lentivirus lacking an insert (PRE) or expressing MKK6EE (MKK6EE) and selected. 8 d after infection, cells were collected and H2O2 levels were measured by flow cytometry. NAC: ROS signaling was inhibited with 10 mM NAC for 48 h before collection.



Supplemental Figure 3

(A) Lamin B1 declines at senescence in mouse embryonic fibroblasts (MEFs). MEFs were isolated from 4 different mice. MEFs were mock irradiated (PRE) or irradiated (10 Gy X-ray) and allowed to senescence (SEN(XRA)). Whole cell lysates were analyzed by western blotting.

(B) Validation of lamin B1 quantitation via immunofluorescence: average lamin B1 intensity. Data from Figure 4B is presented as average intensity. $p<0.0001$

(C) An antibody specifically targeting lamin B2 and an antibody that targets both lamin B1 and B2 do not show a decrease at senescence by immunofluorescence. HCA2 cells were mock irradiated (PRE) or irradiated (XRA) and allowed to senesce (SEN(XRA)). Cells were then fixed and

immunostained. Lamin B intensity was quantitated with Cell Profiler and average intensity for each condition was calculated.

$p16^{INK4A}$ mRNA is increased *in vivo* after irradiation. Mice were treated and mRNA was collected and analyzed as described in Figure 4F.