Ivanov et al., http://www.jcb.org/cgi/content/full/jcb.201212110/DC1

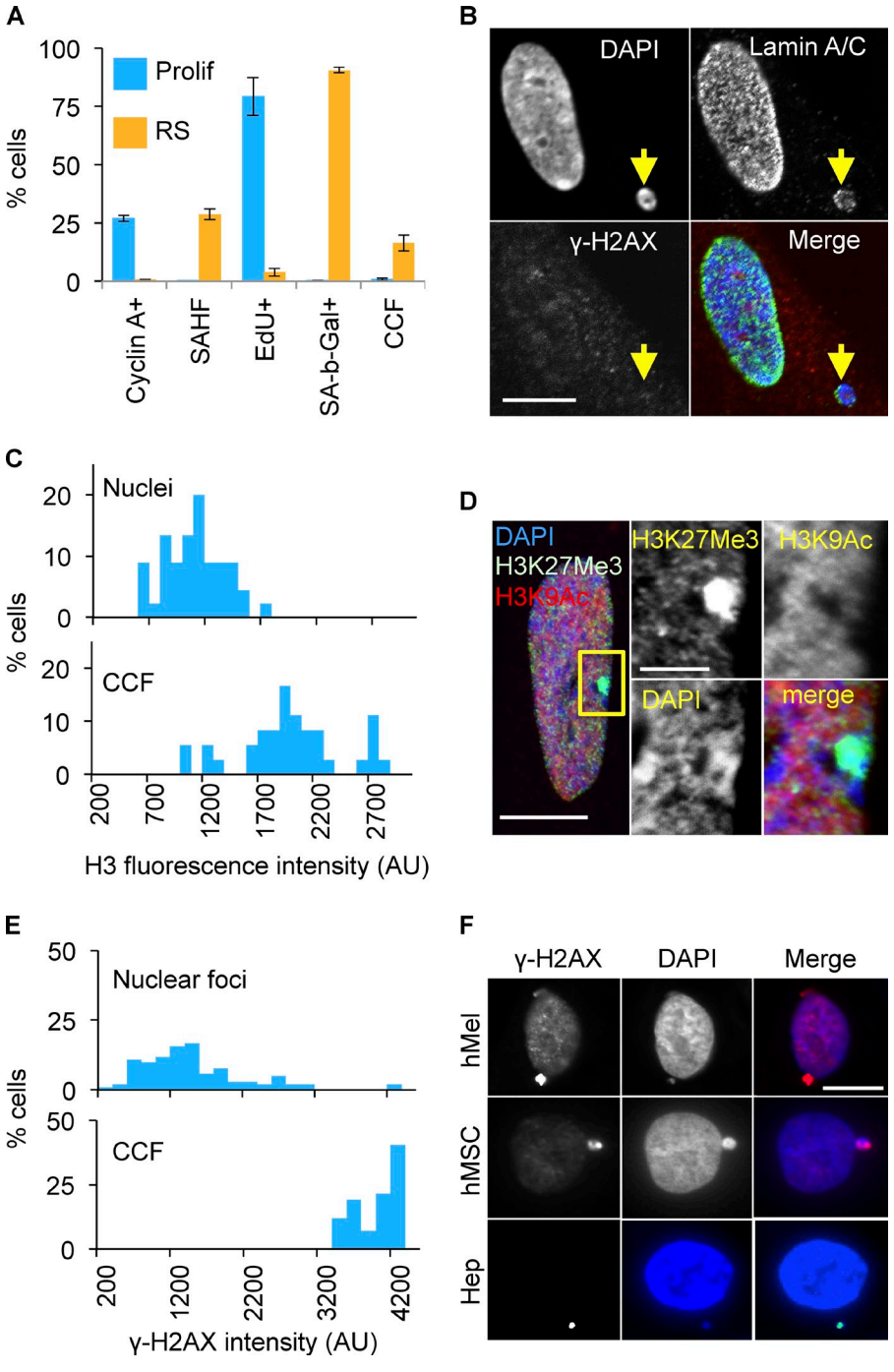


Figure S1. **Cytoplasmic chromatin fragments in senescent cells.** (A) Comparison of indicated markers of cellular senescence with the proportion of CCF^+ cells in RS IMR90 cells. Average ± SEM $n = 3$, $P < 0.0015$. (B) Conventional micronuclei (yellow arrow) generated by Eg5 inhibitor III are lamin A/C positive and do not contain γ-H2AX. IMR90 cells were treated with Eg5 inhibitor III (1 µM) for 16 h, followed by a 24-h chase in drug-free medium. Cells were stained for lamin A/C and γ-H2AX. Bar, 10 µm. (C) Quantitative immunofluorescence to quantitate histone H3 in nuclei and CCFs of senescent cells. At least 40 CCFs and 40–100 randomly selected nuclei were scored. The result shown is representative of two independent experiments that were scored, and the phenomenon was visually apparent in at least two additional experiments. (D) In proliferating cells, H3K27me3 is enriched in the presumptive inactive X-chromosome, whereas H3K9ac is excluded from this region, confirming specificity of H3K27me3 and H3K9ac immunofluorescence staining. Yellow boxed area in left merge panel is magnified in right four panels. Bars: (left) 10 µm; (right) 2 µm. (E) Quantitative immunofluorescence to quantitate γ-H2AX in intranuclear foci and CCFs of senescent cells. At least 50 CCFs and 100 randomly selected cells with γ-H2AX foci were assessed; $n = 3$. (F) CCFs in RS primary human melanocytes (hMel) and RS human mesenchymal stem cells (hMSC) in vitro, and in mouse hepatocytes (Hep) in vivo are γ-H2AX positive. Bars, 10 µm.

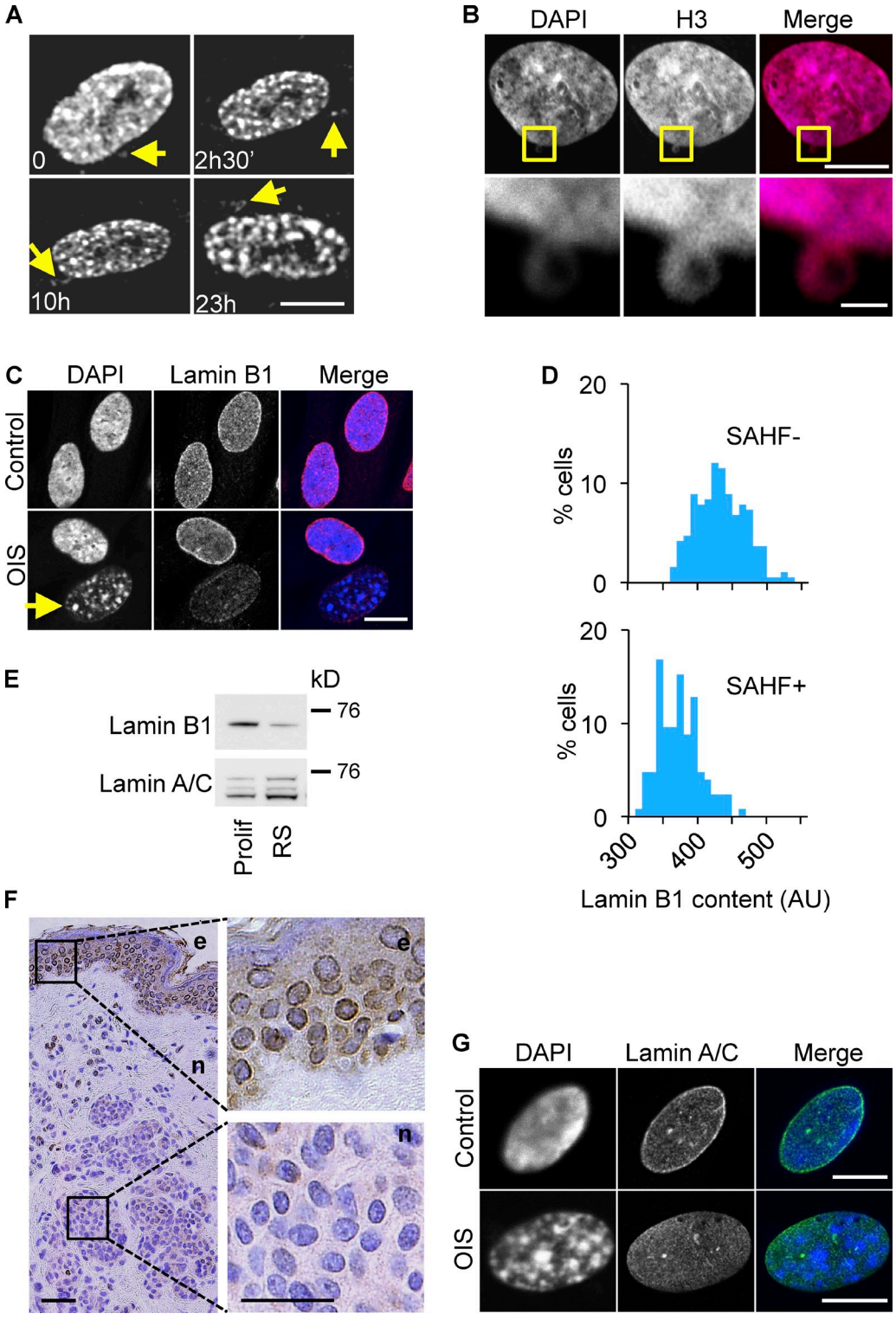


Figure S2. **Nuclear-to-cytoplasm chromatin blebbing in senescent cells associated with depletion of lamin B1.** (A) Dispersal of chromatin fragments (yellow arrowheads) from nuclei of senescent cells. Confocal time-lapse imaging of transiently transfected GFP-H2B–labeled senescent (RS) IMR90 cell. 60× original magnification; time points in hours. Bar, 10 µm. See also Video 1. (B) Blebbing of histone H3 from the nucleus of an OIS IMR90 cell. Yellow boxed area in top panels is expanded in bottom panels. Bars: (top panels) 10 µm; (bottom panels) 2 µm. (C) Lamin B1–deficient OIS cells are enriched in SAHF (e.g., cell with yellow arrow). Bar, 10 µm. (D) Quantitation of results from C. Cells were designated $SAHF^+$ or $SAHF^-$ in DAPI channel and then scored by quantitative immunofluorescence for lamin B1 content. At least 100 cells were scored per sample. Data shown are from a single representative experiment out of three repeats. (E) Decreased lamin B1 content in RS melanocytes in vitro. (F) Immunohistochemistry for lamin B1 shows decreased lamin B1 content in senescent nevus melanocytes (n) as compared with epidermal keratinocytes (e). Boxed regions from left image panels are magnified in right panels. Bars: (left) 100 µm; (right) 50 µm. (G) Immunofluorescence for lamin A/C in control and OIS IMR90 cells. Bars, 10 µm.

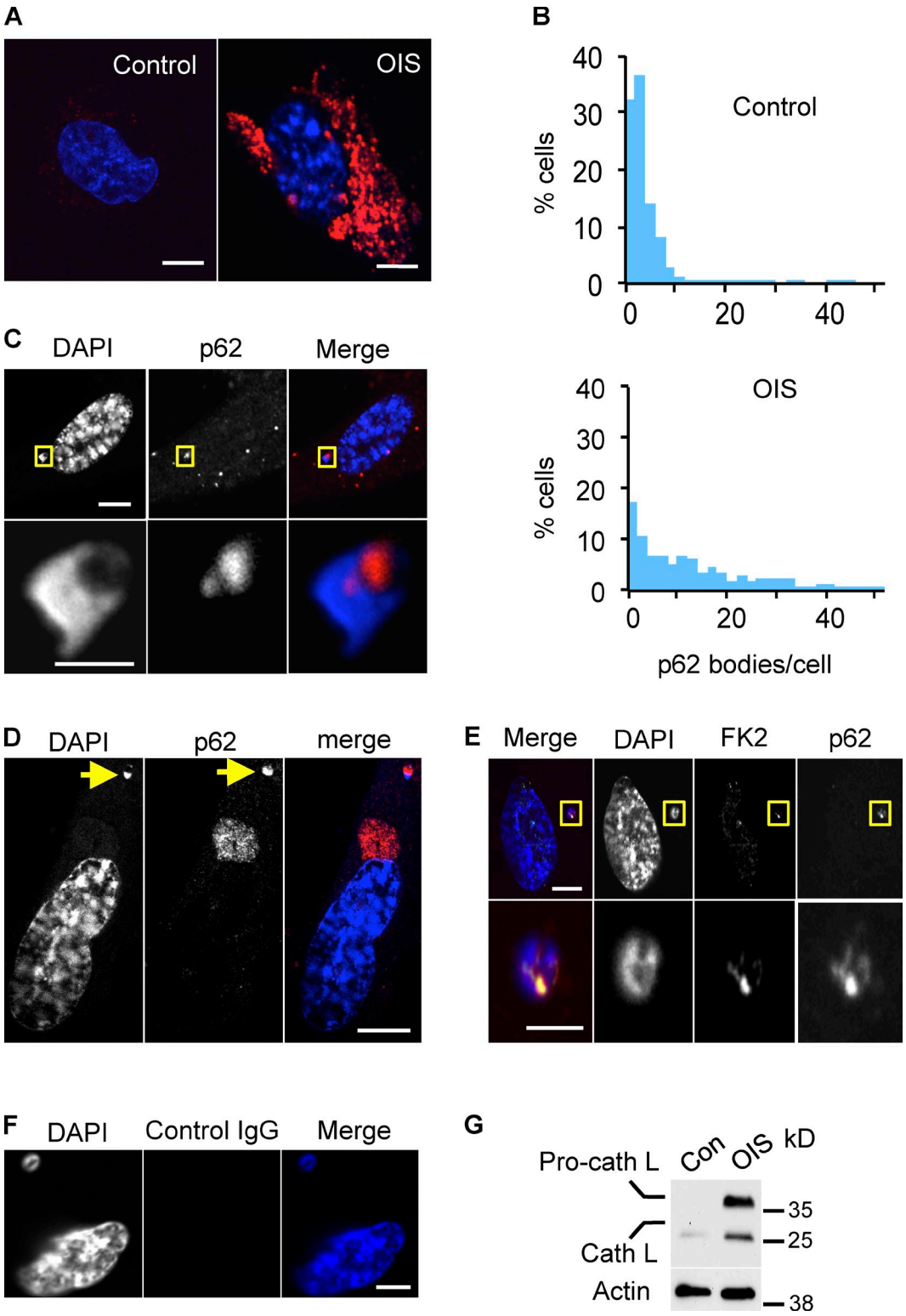


Figure S3. **Cytoplasmic histone is processed by a lysosomal/autophagy pathway.** (A) Control and OIS cell stained with DND99 (Lysotracker red) and Hoechst (blue) to show the increased acidic (presumed lysosomal) compartment in senescence. Bar, 10 µm. (B) Histograms showing increased proportion of OIS cells containing larger numbers of cytoplasmic p62 bodies, compared with control cells. 400 and 900 cells were assessed for control and OIS samples, respectively. (C) Close juxtaposition of CCF to a p62 body in OIS. Yellow boxed area in top panels is magnified in bottom panels to show p62 nestled within invagination in chromatin. Bars: (top) 10 µm; (bottom) 2 µm. (D) Colocalization of CCF and p62 (arrow) in OIS to show CCFs are distinct from TASCC (large p62-positive domain adjacent to nucleus). Bars, 10 µm. (E) p62 and ubiquitinated proteins (stained with FK2) partially colocalize in CCFs in OIS. Yellow boxed areas in top panels are magnified in bottom panels. Bars: (top) 10 µm; (bottom) 2 µm. (F) Classed matched IgG negative control (mouse anti–HA-tag IgG1) for FK2 antibody (E). Bar, 10 µm. (G) Up-regulation of cathepsin L in OIS IMR90 cells.

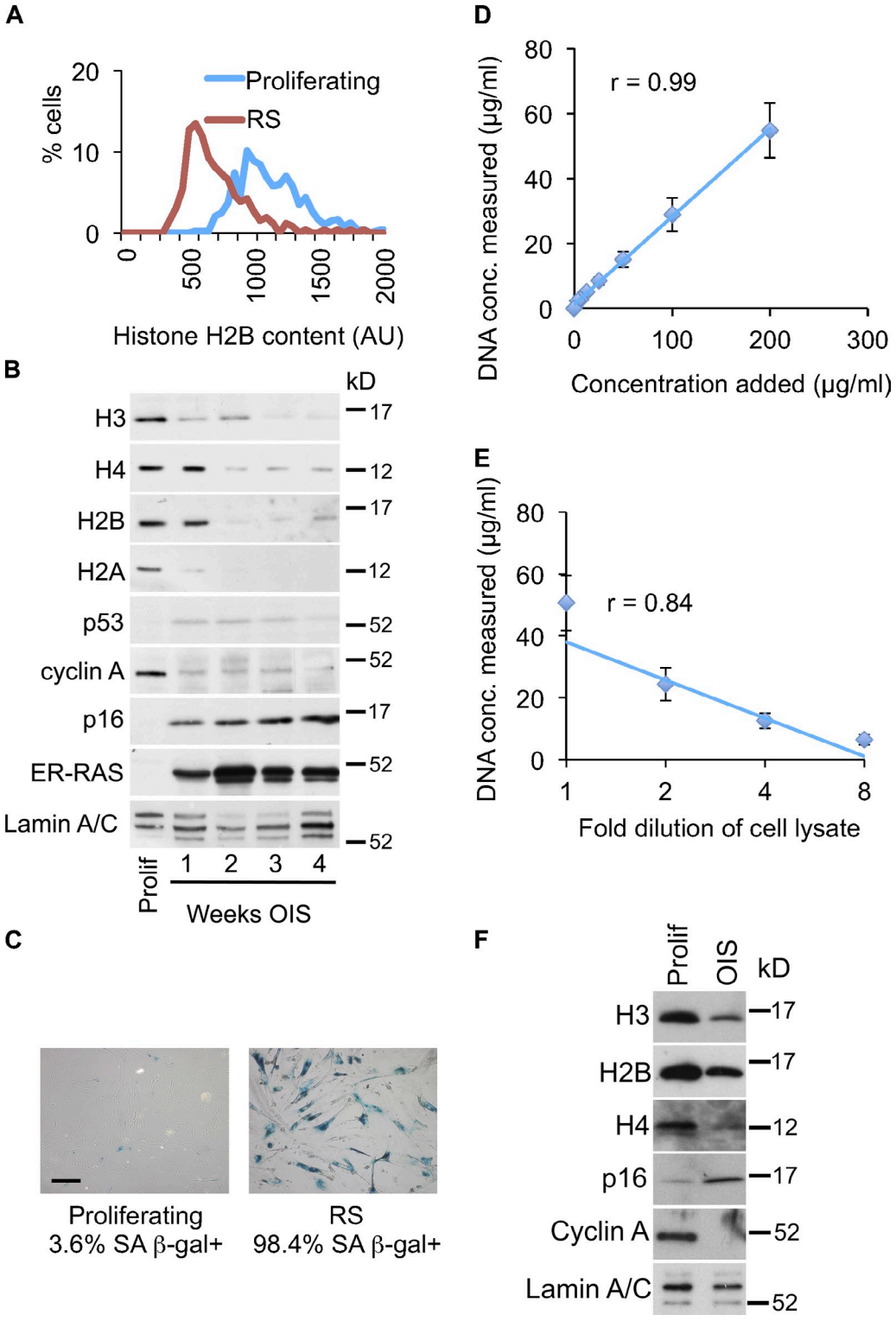


Figure S4. **Senescent cells in vitro contain reduced histone content.** (A) Quantitative immunofluorescence showing reduced histone H2B content in RS cells. Single representative experiment out of three repeats. (B) Progressive loss of core histones in senescent IMR90 cells in OIS. Whole-cell lysates were assessed by Western blot at weekly intervals after RASG12V induction by tamoxifen (tamoxifen added 1 week before week 1 harvest). Western blotting was performed using the same cellular lysates showing accumulation of H3cs.1 in Fig. 4 F, and the same lamin A/C panel is shown in both figures. See Materials and methods for details of time course. (C) SA β-gal staining of cells from week 1 of Fig. 5 C. Even at week 1, the cells are close to 100% SA β-gal positive. (D) Relationship between actual concentration of DNA (x-axis) and measured DNA concentration (y-axis); DNA diluted in 1× Laemmli sample buffer. (E) Relationship between fold dilution of whole-cell lysate (x-axis) and measured DNA concentration (y-axis); cell lysate prepared and diluted in 1× Laemmli sample buffer. (F) Western immunoblotting for control and OIS cells after loading was normalized by DNA content. Equivalent of 50 ng of measured DNA was added per well. Total DNA content was measured by the Qubit fluorometer (Invitrogen) in 1 µl of cellular lysate in 1× Laemmli sample buffer.

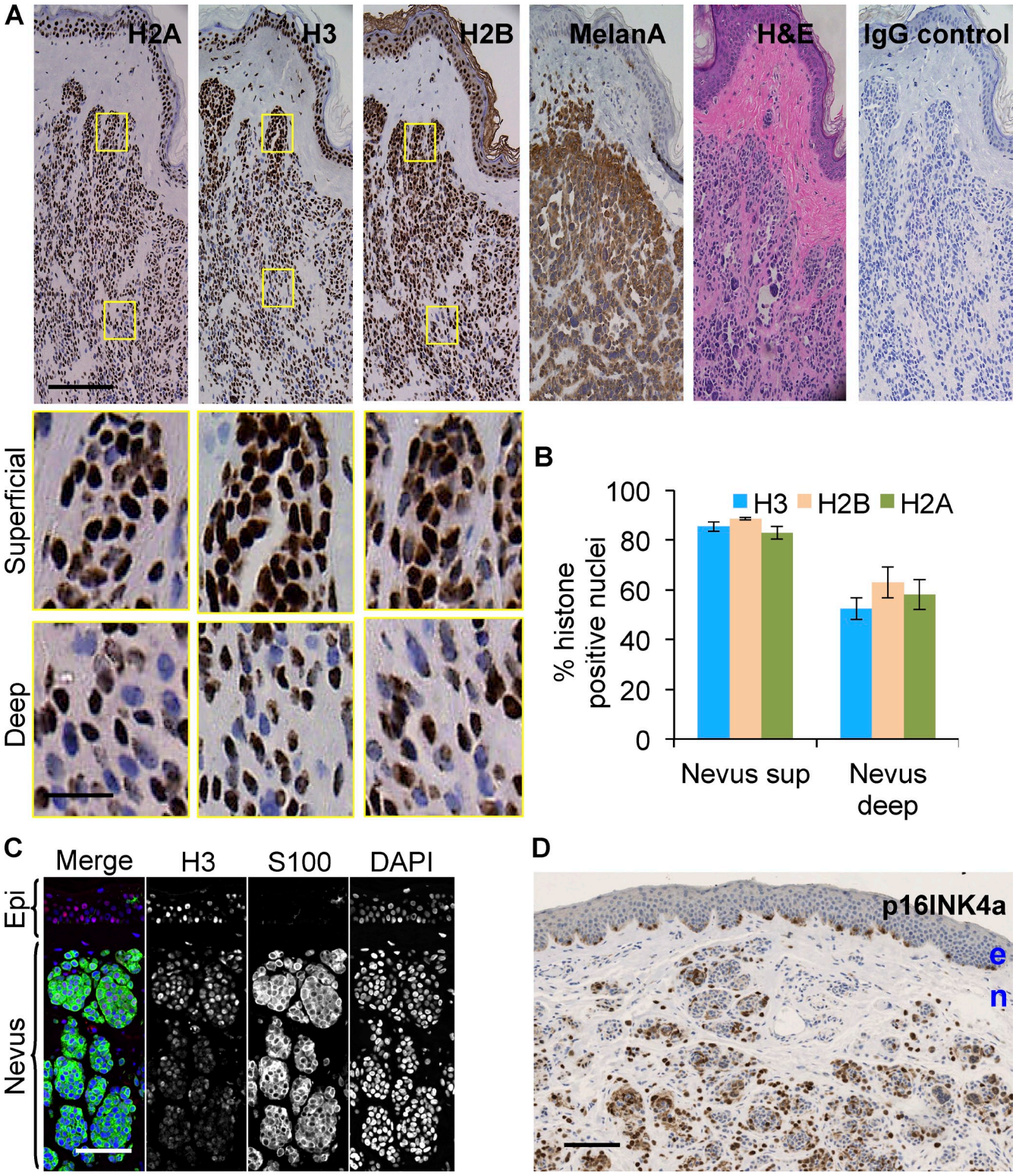
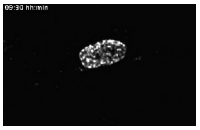


Figure S5. **Senescent cells in vivo contain reduced histone content.** (A) Immunohistochemistry of histones H3, H2A, and H2B in serial sections of a melaNa$^+$ human benign nevus shows histone-deficient nuclei in the deeper parts of the nevus. Yellow boxed regions from top panels are magnified in bottom panels. We were unable to identify a reliable antibody for histone H4 immunohistochemistry. Bars: (top) 200 μm; (bottom) 50 μm. (B) Quantitation of histone$^+$ nuclei in superficial (sup) vs. deep parts of the nevus. Bars represent average values for six different nevi ± SEM. $P < 0.0001$ for H3, $P < 0.04$ for H2B, $P < 0.01$ for H2A. (C) Deeper parts of the nevus contain reduced histone H3 content. Immunofluorescent staining for DAPI (blue), S100 (green), and H3 (red). (D) Non-uniform nature of p16 staining in a benign nevus. There is no consistent trend to p16 staining across superficial and deep regions of the nevus. Bars, 200 μm.



Video 1. **Blebbing of RFP-labeled histone H2B from the nucleus of a senescence cell.** Pre-senescent IMR90 cells were transfected with pPA-H2B-TagRFP vector (Evrogen) followed by confocal time-lapse microscopy using a laser-scanning confocal microscope (model A1R; Nikon). Images were collected every 30 min for 1,350 min in total.