Fig. S1

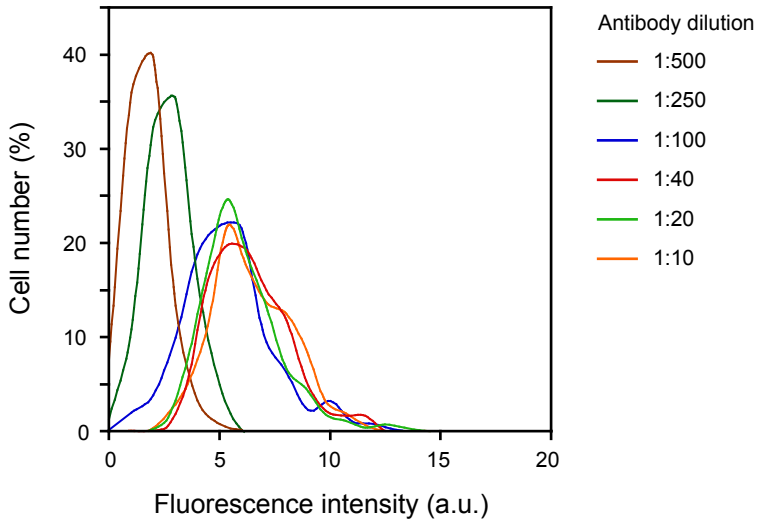

Fig. S1 Titration of antibody dilutions to determine saturating concentrations for quantitative signal detection. Titration of HP1β antibody on early passage HDF is shown as an example. Cells were stained and images acquired as described in Experimental Procedures.

Fig. S2

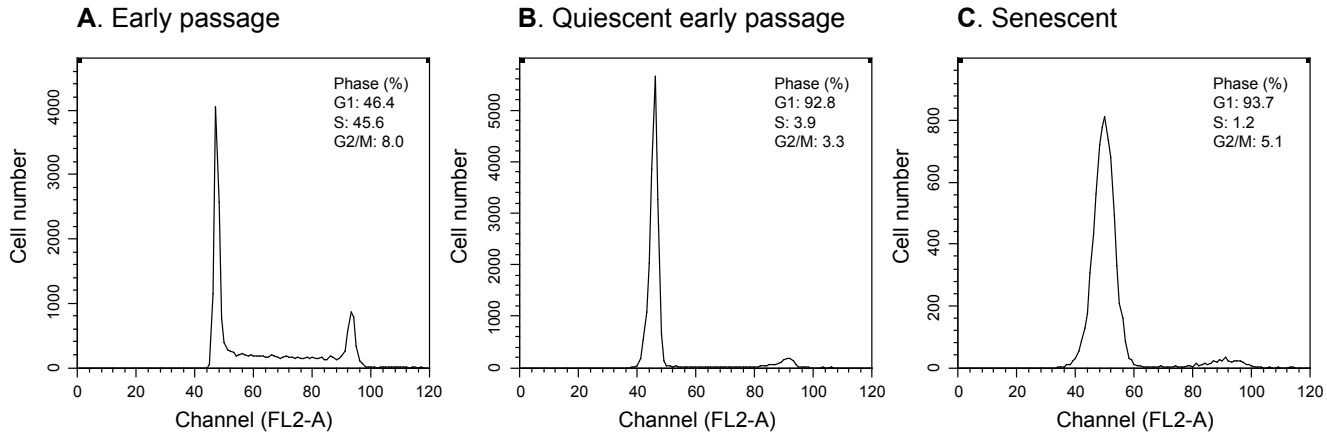

Fig. S2 Flow cytometric analysis of the DNA content of early passage growing, quiescent, and late passage senescent HDF cells. Cells were harvested by trypsinization, fixed with 70 % ethanol at 4°C, incubated with 1 µg /ml RNaseA in PBS for 30 min. at room temperature, and stained with 50 µg/ml propidium iodide for 1 hr. at room temperature. Analysis was performed using a BD Biosciences FACSCalibur flow cytometer and CellQuest and Modfit software.

Fig. S3

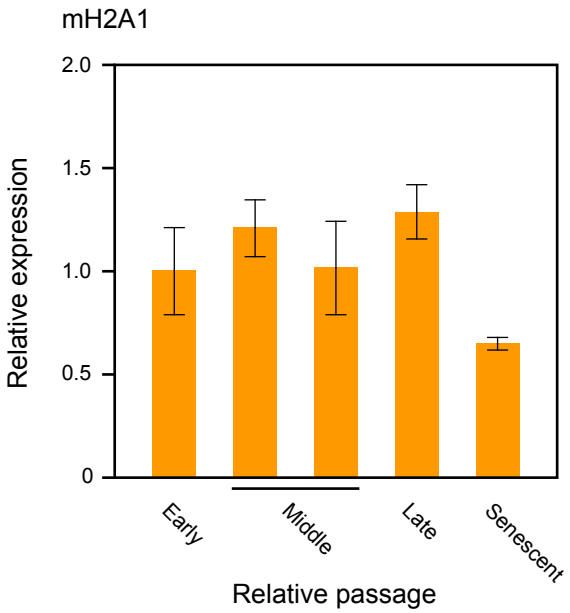

Fig. S3 Assessment of mH2A1 mRNA expression in HDF and murine tissues.
RNA was extracted from HDF at the indicated relative passages, and mH2A1 (H2AFY) mRNA levels were measured by quantitative real-time PCR (qPCR). Normalization was to GAPDH. The modest rise during passage was not statistically significant; the decrease in senescent cells (0.65 relative to early passage cells) was statistically significant in this experiment. Early and senescent cells were also examined by expression profiling using Affymetrix U133 human microarrays. The mH2A1 (H2AFY) and HP1β (CBX1) genes are represented by 6 and 1 probe sets, respectively, on this array. All probe sets reported expression at a moderate level. None of the probe sets reported a statistically significant change in expression above a 1.5-fold cutoff. Finally, we also examined young and old mouse liver and skeletal muscle using Affymetrix GeneST 2.0 mouse microarrays. The H2afy and Cbx1 genes are represented by 3 and 8 probe sets, respectively, on this array. All probe sets reported expression at a moderate level. None of the probe sets reported a statistically significant change in expression above a 1.5-fold cutoff.

Fig. S4

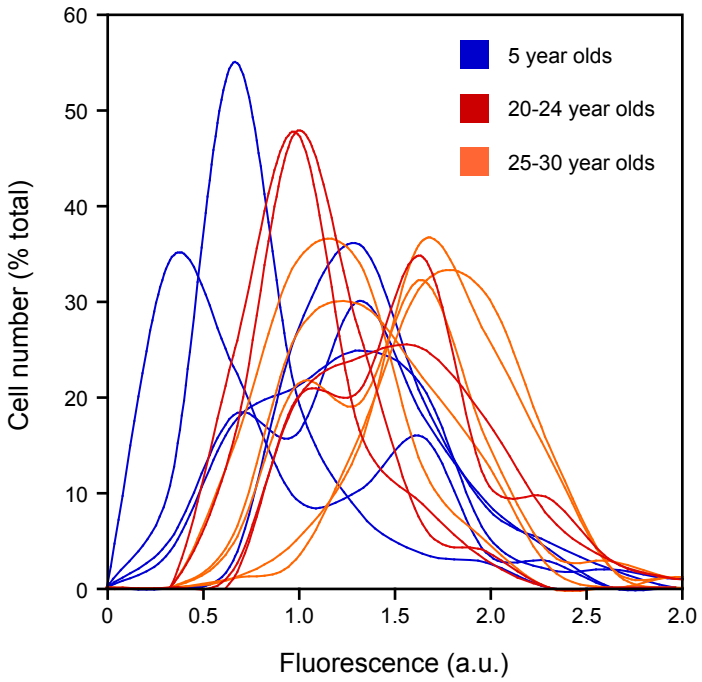

Fig. S3 Quantitative single cell measurements of mH2A protein levels in baboon skin. Five 5 year old (blue) animals, four 20-24 year old animals (red) and five 25-30 year old animals (orange) were included in the experiment shown. Full thickness skin biopsies were obtained as described (Herbig *et al*., 2006) from the medial forearm to provide samples from a relatively protected area and with a minimum of hair follicles. Animals of both sexes were included. Only fibroblasts in the dermal layer were scored. Immunofluorescent images were collected and quantified as indicated in Experimental procedures, expressed as ratios of mH2A and DAPI intensities for each nucleus, and plotted as histograms of cell number (% of total) against fluorescence intensity in arbitrary units (a.u.). Note the biphasic profile of some of the histograms. The average of the mean fluorescence intensities of the old group (9 animals) was 1.3-fold higher than the young group (5 animals), and was significant at $p = 0.065$ calculated using a two-tailed student's test. The experiment was repeated three times with consistent results.

Fig. S5

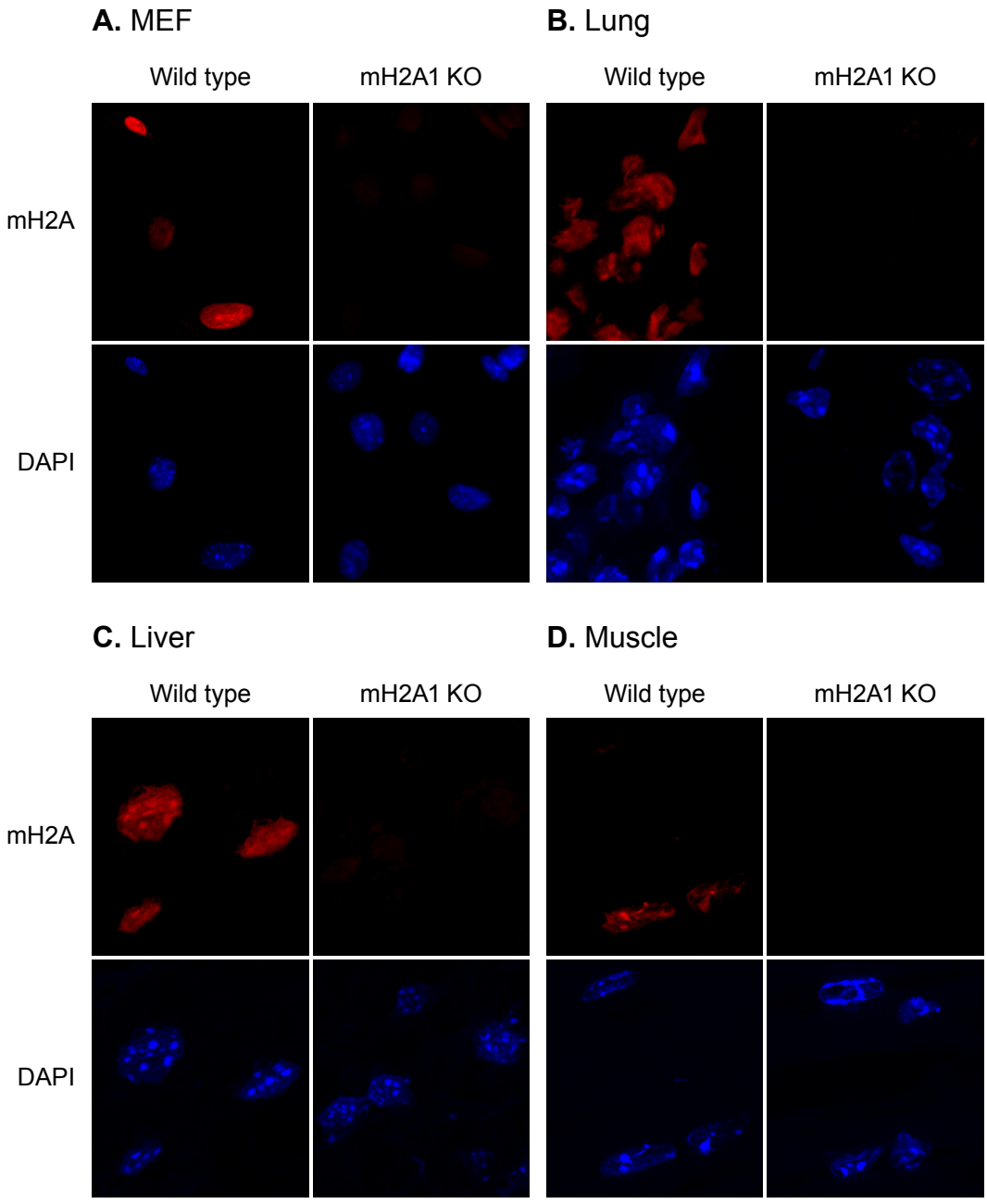

Fig. S5 Quantitative single cell masurements of mH2A protein levels in mouse embryo fibroblasts (MEF) and tissues of mH2A1 knockout mice. Representative images are shown for (A) MEF, (B) lung, (C) liver, and (D) skeletal muscle. Samples were processed and images were collected and analyzed as described in the Experimental Procedures. The mean intensities of the nuclear mH2A signals in the knockout samples were approximately 10-fold lower than the corresponding signals in the wild type samples.

Table S1: Saturating concentrations of antibodies used in this study

Antibody target	Dilution	Type	Source
mH2A	20 µg/ml	Rabbit polyclonal	Raised at Fox Chase Cancer Center
mH2A-647	20 µg/ml	Rabbit polyclonal	As above, labeled with AlexaFluor 647
HP1β	1:40	Mouse monoclonal ascites	Millipore
Anti-rabbit-Cy5	10 µg/ml	Donkey polyclonal	Jackson ImmunoResearch
Anti-mouse-Cy3	10 µg/ml	Donkey polyclonal	Jackson ImmunoResearch