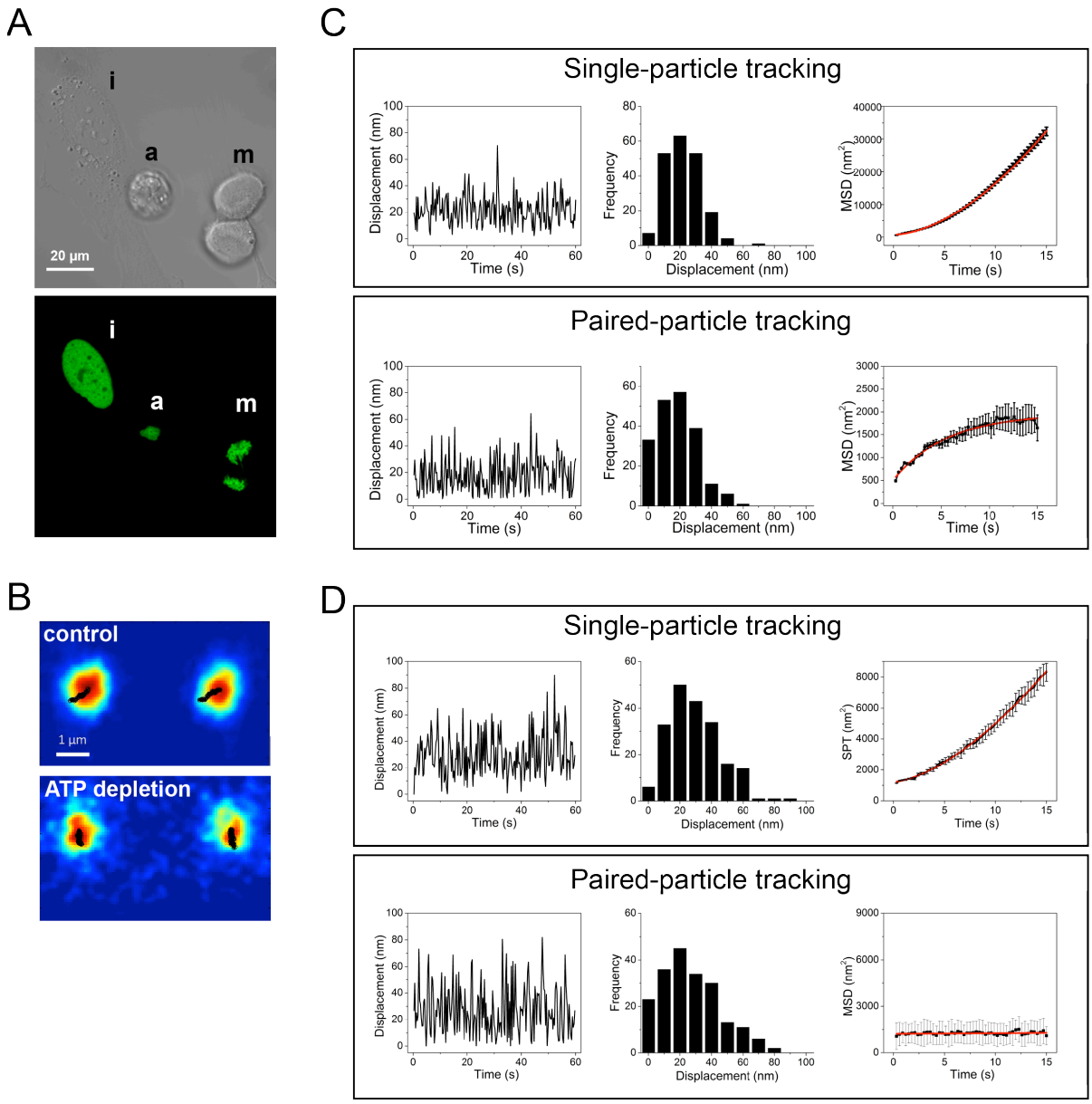

Fig. S1. Validation of the PAGFP-PPT method. (A) Labeling of the chromatin with PAGFP-H2A. An entire microscopic field containing cells with interphase nuclei (i), mitotic cells (m), and apoptotic cells (a) was photoactivated before imaging. (B) Projection of PAGFP-H2A time-course confocal datasets (untreated or ATP-depleted cells). Pixel intensities are presented in pseudocolors and the localization coordinates are in black. Both PAGFP-H2A spots have a similar projection pattern, indicating translational movement of the cell nucleus. (C) Comparison between single- and paired-particle tracking of PAGFP-H2A spots. Time-course displacements (left), displacement histograms (center), and MSD plots (right) are shown that correspond to a live, untreated cell. (D) SPT and PPT measurements as in (C), but from a cell after ATP depletion, a treatment used to validate the method. The flat PPT MSD curve reflects the expected decreased chromatin mobility in ATP-depleted cells compared to control. In contrast, SPT measurements, influenced by global cell motions, do not reveal differences in chromatin mobility in the two treatments. MSD data in C-D show the mean ± s.e.m.

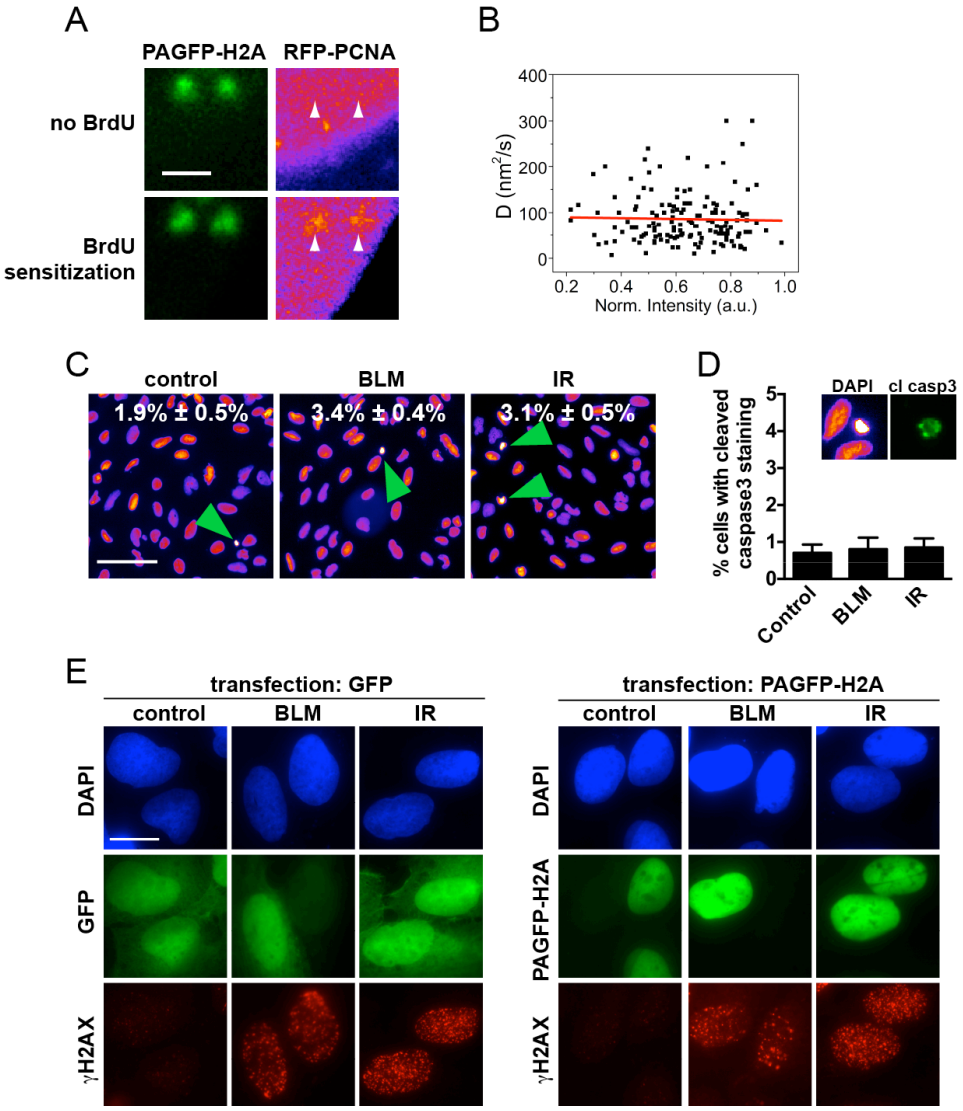

Fig. S2. Photoactivation of PAGFP-H2A does not induce DNA damage, the intensity of photoactivated spots does not influence diffusion measurements, and minimal cell death is induced by the DNA-damaging treatments. (A) Cells coexpressing PAGFP-H2A and RFP-PCNA as reporter of DNA damage were illuminated with the 405 nm laser line to photoactivate GFP (green spots). Confocal images were recorded two minutes after photoactivation. To validate DNA damage detection by PCNA, cells were incubated with BrdU (10 µM; 48 h) to sensitize DNA. RFP-PCNA fluorescent signals are displayed as heat maps. The arrowheads indicate the location of the photoactivated spots. Scale bar, 3 µm (B) Scatter plot of chromatin diffusion (*D*) and fluorescence intensity of photoactivated PAGFP-H2A spots. The red line represents the best linear fit and denotes absence of correlation between *D* and PAGFP intensity. (C) Illustration of pyknotic nuclei (green arrowheads) in untreated cells (control) and in cells treated with BLM (20 mU/ml, 1h) or exposed to IR (10 Gy followed by 1h recovery). DAPI signals are shown using heat map colors. The percentages of pyknotic nuclei are indicated. Scale bar, 100 µm (D) Fraction of cells positive for cleaved caspase 3 (mean ± s.e.m. from eight microscopic fields [>1500 cells] from two biological replicates). Representative staining of a positive cell is shown in the inset. (E) Immunostaining for γH2AX in cells expressing GFP or PAGFP-H2A and treated as in (C). Scale bar, 20 µm

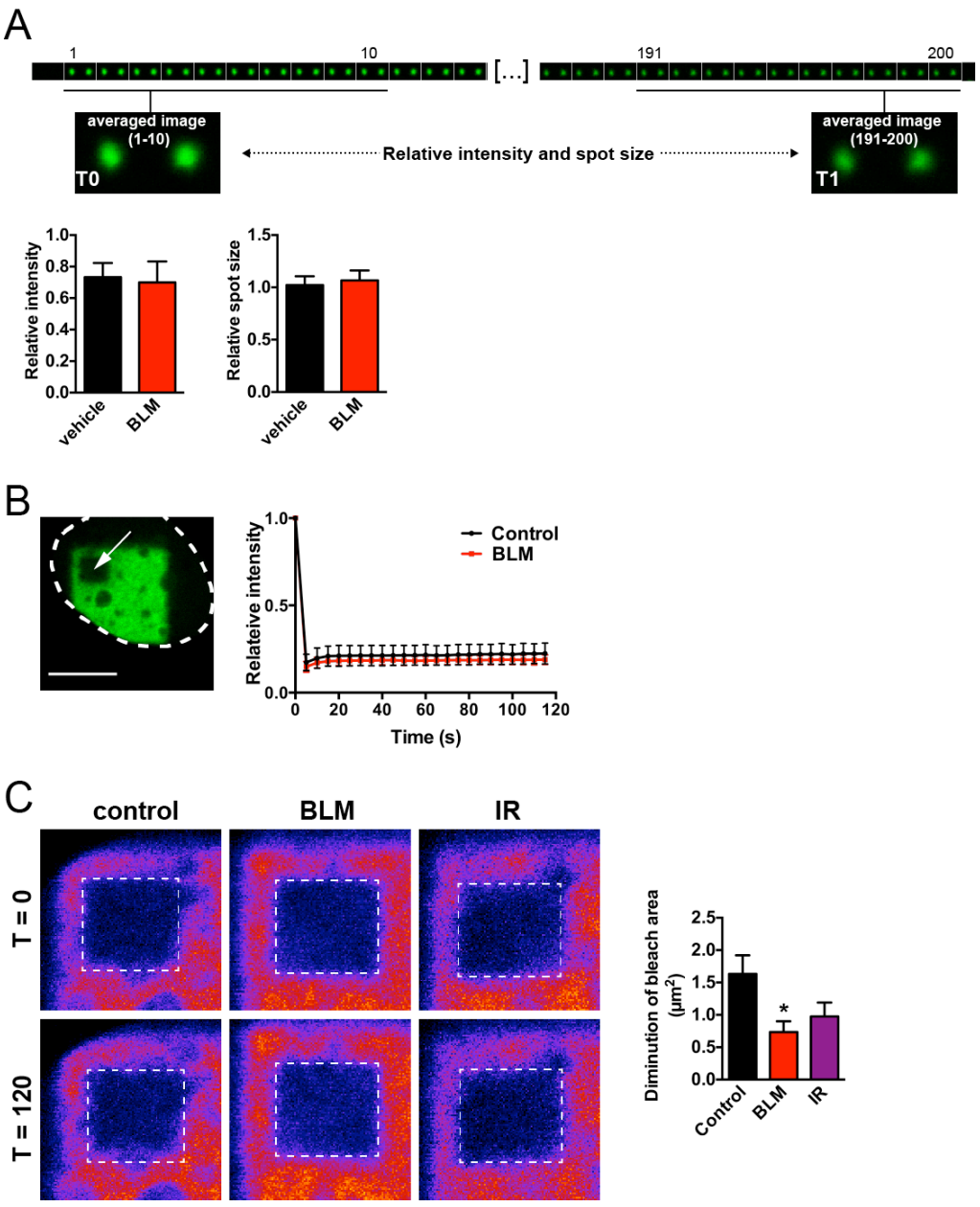

Fig. S3. DNA damage does not alter the rate of PAGFP-H2A histone exchange. (A) Relative size and intensity of photoactivated PAGFP-H2A spots in cells treated with vehicle or with BLM. Ten images were averaged at the beginning (T0) and at the end (T1) of PPT time courses to reduce the impact of instrumental noise on the morphometric and intensity measurements (top). Area and mean intensity values measured using ImageJ (Rasband, W.S.; http://imagej.nih.gov/ij/) were expressed relative to T0. Bar graphs represent mean values ± s.e.m. (10-20 cells). (B) FRAP analysis after PAGFP-H2A photoactivation in cells treated with vehicle (control) or with BLM. A representative post-bleach image is shown on the left (scale bar, 10 μm). Averaged intensities of the bleached regions, corrected for background and photobleaching during imaging, are shown in the graph. Error bars represent s.e.m. (C) Area of bleached regions in the presence or absence of DNA damage induced by BLM or IR. Bleached squares were measured immediately after bleaching (T = 0) and after 120 seconds (T = 120). Representative images are shown on the left with heat map colors. The graph represents averaged differences in size and s.e.m. ($n \geq 15$ cells; *, $P < 0.05$, Tukey, compared to control).

Movie 1 Time-course imaging of photoactivated PAGF-H2A spots in live, untreated cells. The spots were followed for one minute, collecting approx. three frames per second. For each frame, the centers of the spots, determined by fitting with a PSF, are shown as blue dots.

Movie 2 Time-course imaging of PAGFP-H2A spots in fixed cells.