SUPPLEMENTARY DATA

CHD3 and CHD4 recruitment and chromatin remodeling activity at DNA breaks is promoted by early poly(ADP-ribose)-dependent chromatin relaxation

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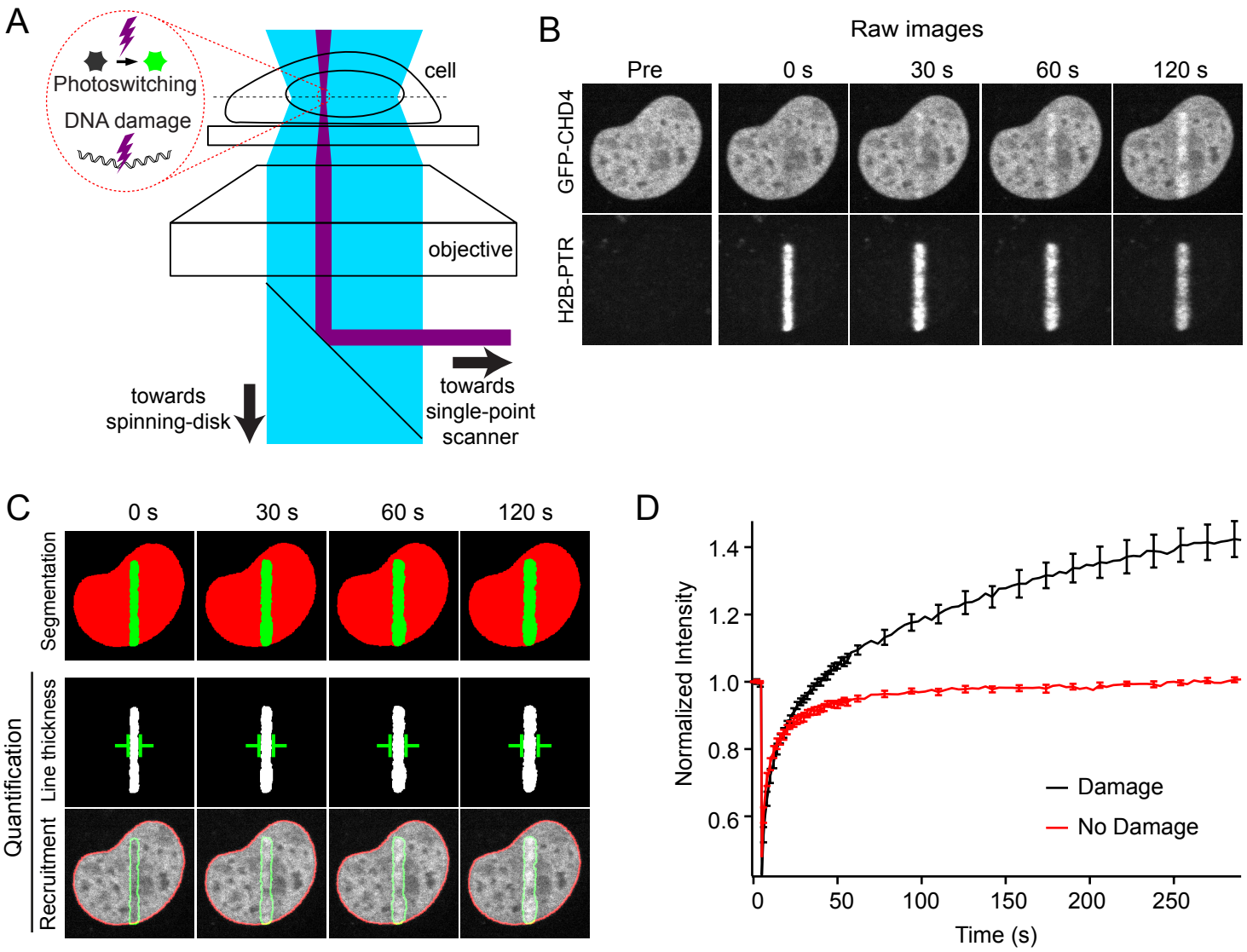
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Late recruitment of CHD4 to sites of DNA damage is not due to slow diffusion of CHD4. (**A**) Sketch of the microscope system used for live cell imaging and laser micro-irradiation. The cells are imaged using a spinning-disk confocal setup. The laser micro-irradiation and photoactivation at 405 nm is performed through an independent light path. Within this light path, the single point scanning mirrors allow to define local micro-irradiation / photoactivation areas. (**B**) Raw images collected before (Pre) and after microirradiation, showing accumulation of GFP-CHD4 and photoactivation of H2B (**C**) Overview of image analysis pipelines. Time series images of GFP-CHD4 and H2B-PTR are collected and used to segment the nucleus and area of DNA damage respectively. Quantification of the thickness over the line is measures and can be expressed directly in μm or as a ratio normalized to the 0 s time point. Mean fluorescence of the GFP signal is measured inside the damage area as defined by H2B-PTR segmentation. Fluorescence intensity is normalized to the pre-damage time point. (**D**) U2OS cells expressing GFP-CHD4 were irradiated with 405 and 488 nm light to simultaneously induce DNA damage in Hoechst sensitized nuclei and bleach GFP. Florescence intensity was normalized to pre-bleach images. Sensitized nuclei (Damage, Black) are compared to non-sensitized controls (No Damage, Red).

Supplemental Figure 2. CHD4 binding is enhanced upon chromatin relaxation and does not directly interact with Alc1. Chromatin relaxation (**A**) and CHD4 recruitment (**B**) 120 s post UV microirradiation in cells over-expressing wild-type Alc1 (WT) or the ATPase-dead (E175Q) mutant. (**C**) PARP1-mCherry or mCherry-CHD4 were co-expressed with GFP-Alc1 that was tethered to the LacO array using the LacI-GFP-trap. Images are shown before (Pre) and 120 s after (Post) DNA damage induction. Insets show magnification of the LacO array. CHD4 does not localize with LacO-tethered Alc1 either Pre or Post DNA damage induction. In contrast, PARP1 enriches at LacO-tethered

Alc1 after DNA damage induction. (**D**) GFP-CHD4 recruitment to sites of DNA damage in WT (Alc1$^{+/+}$) or Alc1 knockout (Alc1$^{-/-}$) U2OS cells. Images show representative images before (Pre) and 120 s after (Post) DNA damage induction. Scale bars are 5 μm. (**E**) FCS curves of GFP-CHD4 before (black) and after (red) UV microirradiation. (**F**) GFP-CHD4 dynamics measured by FCS pre and post DNA damage induction.

Supplemental Figure 3. CHD4 knockdown enhances DNA damage signaling and does not alter pre-damage chromatin structure. (**A**) Immunofluorescence showing knockdown efficiency of CHD4 (siCHD4 #1, siCHD4 #2) as compared to scrambled siRNA (siScr). Nuclei were visualized with Hoechst staining. Scale bar is 10 μm. (**B**) Immunoflorescence of H2AX-phosphorylation post X-ray induced DNA damage (10 Gy) with siRNA-mediated knockdown of CHD4 (siCHD4 #1, siCHD4 #2, white) compared to scrambled siRNA (siScr, grey). Statistical analysis between Scr and CHD4 knockdown is $p<1x10^{-16}$ for each time point. (**C**) Texture analysis of Hoechst stained nuclei with isotonic or hypotonic treatment. A decrease in pixel-to-pixel contrast is indicative of a more relaxed chromatin landscape. (**D**) Effect of DNA damage-induced chromatin relaxation with isotonic or hypotonic treatments. Boxplots show chromatin relaxation 120 s after microirradiation. (**E**) Texture analysis of Hoechst stained nuclei treated with scrambled (siScr) or CHD4 (siCHD4 #1, siCHD4 #2) siRNA.



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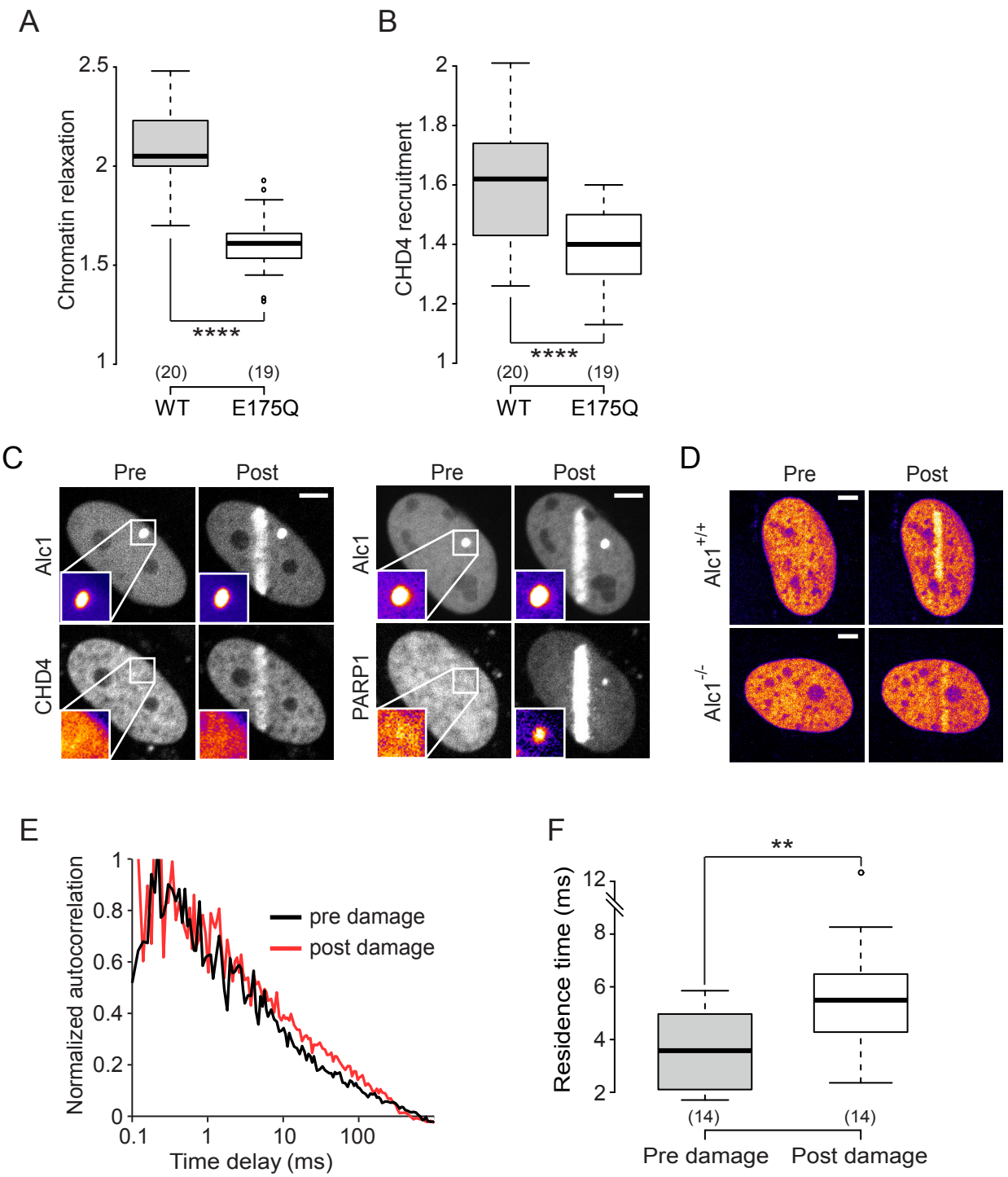

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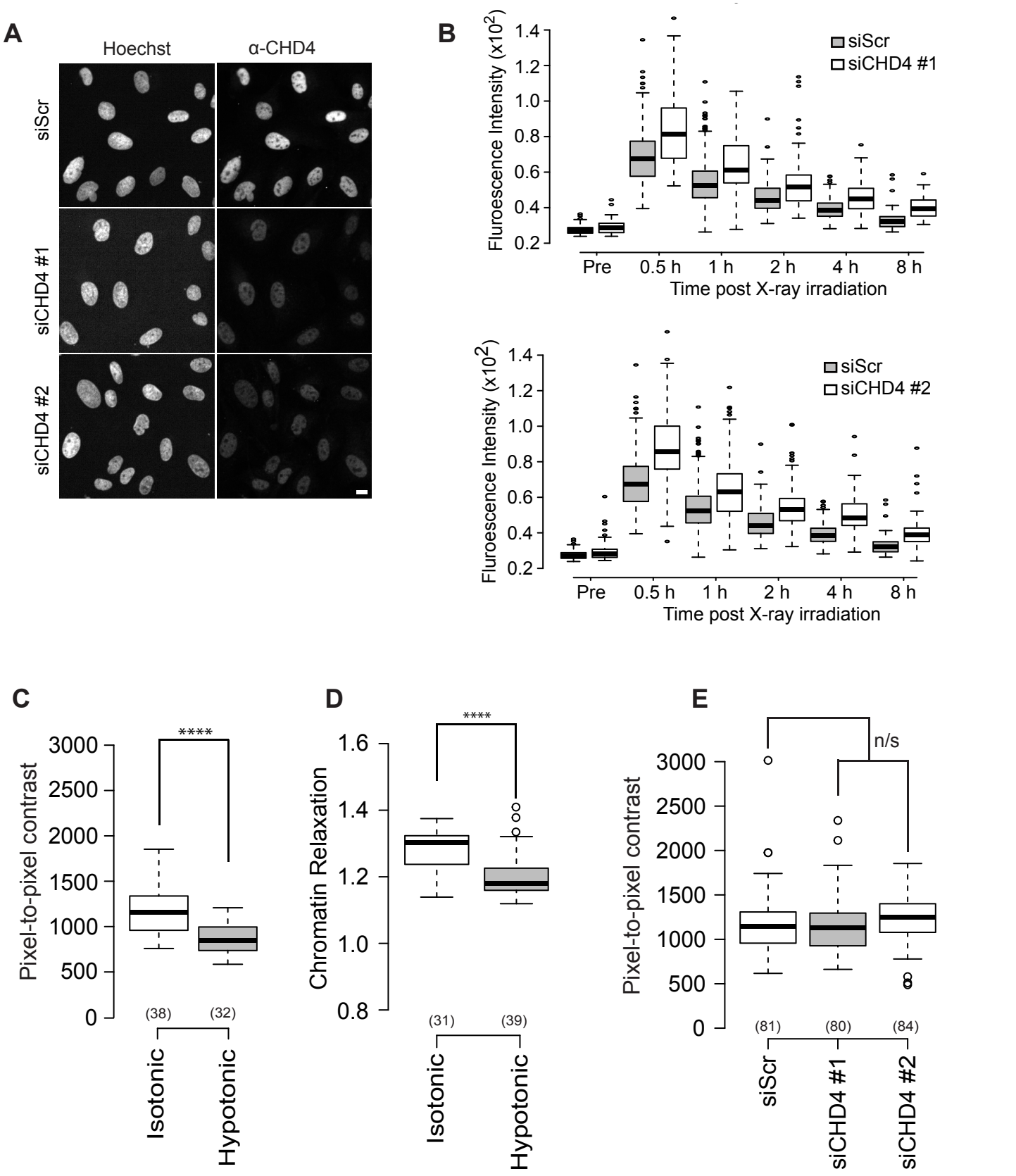

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