Figure S1

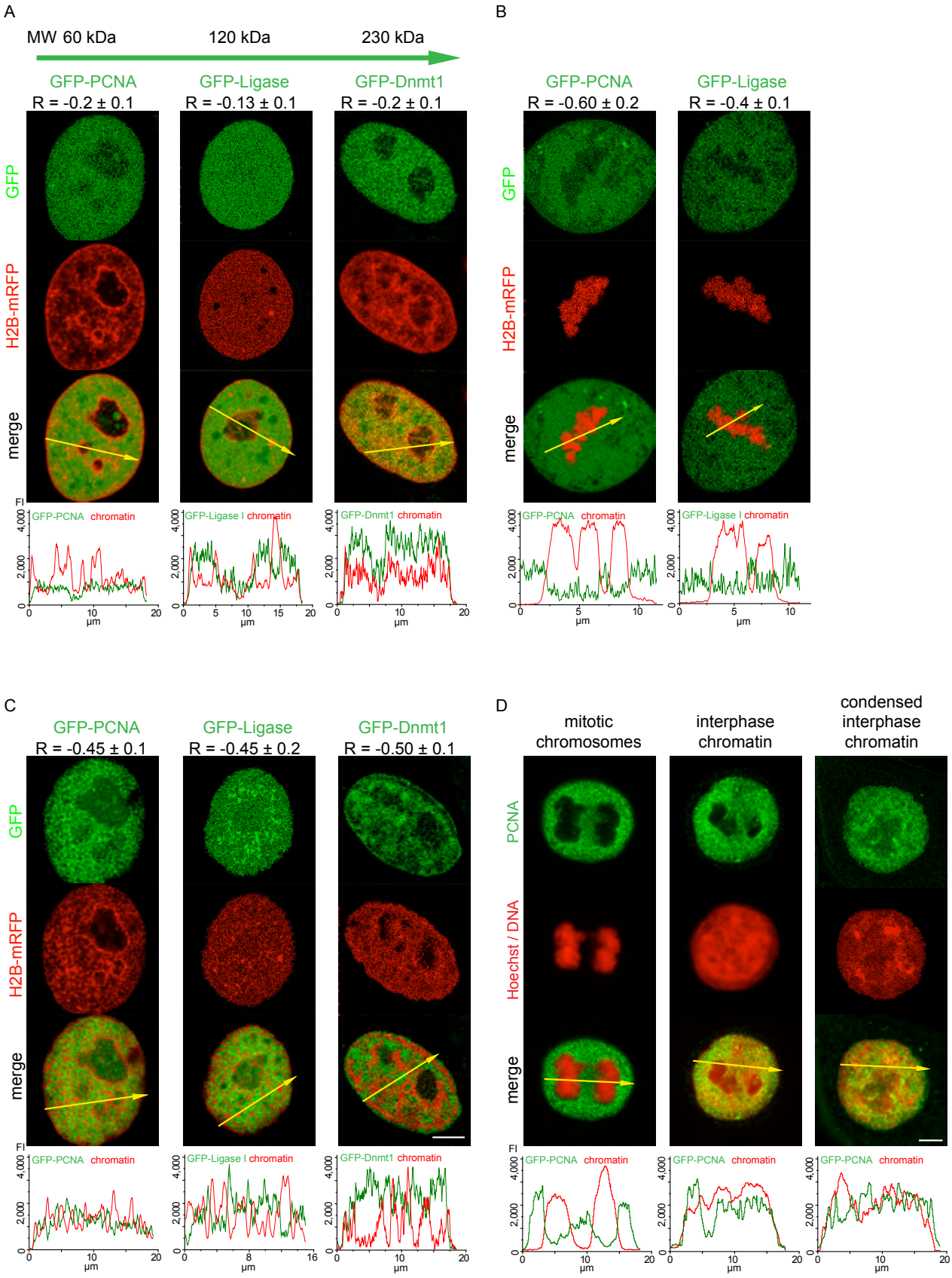
A
MW 60 kDa
120 kDa
230 kDa
GFP-PCNA
R = -0.2 ± 0.1
GFP-Ligase
R = -0.13 ± 0.1
GFP-Dnmt1
R = -0.2 ± 0.1
GFP
H2B-mRFP
merge
FI
GFP-PCNA chromatin
GFP-Ligase I chromatin
GFP-Dnmt1 chromatin
µm
B
GFP-PCNA
R = -0.60 ± 0.2
GFP-Ligase
R = -0.4 ± 0.1
C
GFP-PCNA
R = -0.45 ± 0.1
GFP-Ligase
R = -0.45 ± 0.2
GFP-Dnmt1
R = -0.50 ± 0.1
D
mitotic chromosomes
interphase chromatin
condensed interphase chromatin
PCNA
Hoechst / DNA

Fig S1 Effect of chromatin condensation state on accessibility of nucleoplasmic proteins of different size

Distribution of fusion proteins of different size GFP-DNA Ligase I, GFP-Dnmt1 and GFP-PCNA in representative optical sections of live HeLa cells relative to chromatin labeled with H2B-mRFP in interphase A), mitosis B) and in C) the same cells as in A) upon hyperosmolar treatment yielding hypercondensation of chromatin to the level of mitotic chromosomes. The Pearson's correlation coefficients (R) given above the images were calculated excluding the nucleoli (mean of 10 cells). The linescans below the images show the pixel intensity distribution along the direction of the arrow in the merged images.

In A) as with GFP alone the proteins were homogeneously distributed in the nucleoplasm as described before (20,21,22). The lowest level of the fusion proteins was found in nucleoli and a slight reduction could be measured in large heterochromatin regions as displayed in the images and linescans. The increasing size of the proteins had no influence on their chromatin access in interphase nuclei as demonstrated by the correlation analysis (nucleoli excluded) with R-values between -0.13 and -0.2 indicating almost no correlation.

In B) cells in mitosis are shown with the chromosomes mostly excluding the proteins, supported by the drop of the protein fluorescence intensity in the area locating the condensed chromosomes in the linescans. The strong negative correlation coefficient with R-values of -0.6 and -0.4, respectively, further confirms the exclusion of non chromatin nuclear proteins from chromosomes. The GFP-Dnmt1 was not included in the mitosis analysis since it associates with constitutive heterochromatin from late S-phase to early G1 (22).

The hypercondensation of interphase chromatin in C) again leads to an exclusion of the fusion proteins from the condensed chromatin structures and an accumulation in the enlarged interchromatin space, This is also visible in the linescans by the inverse correlation of the protein and chromatin labeling intensity curves. Importantly, for interphase nuclei with induced hypercondensation of chromatin the Pearson coefficient for GFP fusion proteins drops to a level similar to mitotic cells R= -0.45 and -0.5 respectively.

In D) optical sections of fixed HeLa cells stained with an antibody to PCNA in mitosis, interphase and with interphase chromatin condensed to the mitotic chromosome volume are shown. As with the GFP fusions, in mitosis no protein labeling was visible in the chromosome area labeled with the DNA dye Hoechst 33258 whereas in interphase PCNA was distributed in the whole nucleus with some reduction in the nucleoli. Upon hyperosmolar condensation of interphase chromatin redistribution of the proteins could be observed with a concentration of protein in the enlarged interchromatin regions.

Scale bars 5 µm.