Appendix:

Hoffmann et al, "A genetic memory initiates the epigenetic loop necessary to preserve centromere position"

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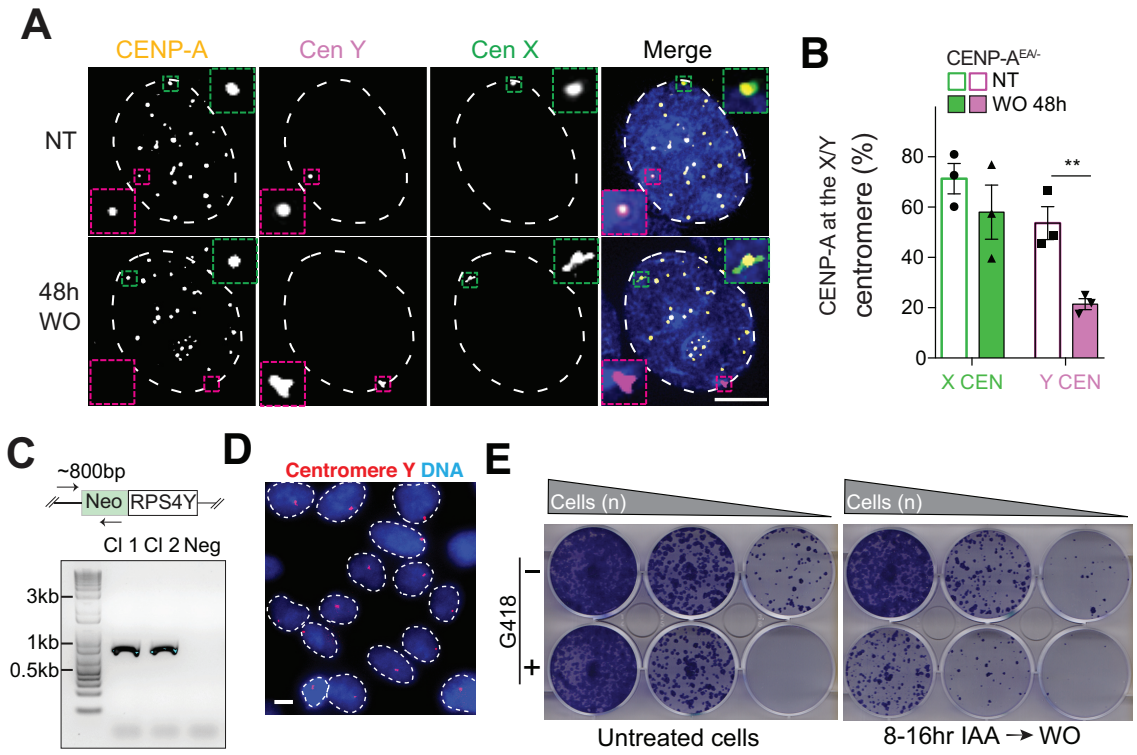


Appendix Figure S1 (related to Fig. 4). Impaired centromere formation at the Y chromosome after a CENP-A$^{OFF/ON}$ cycle.

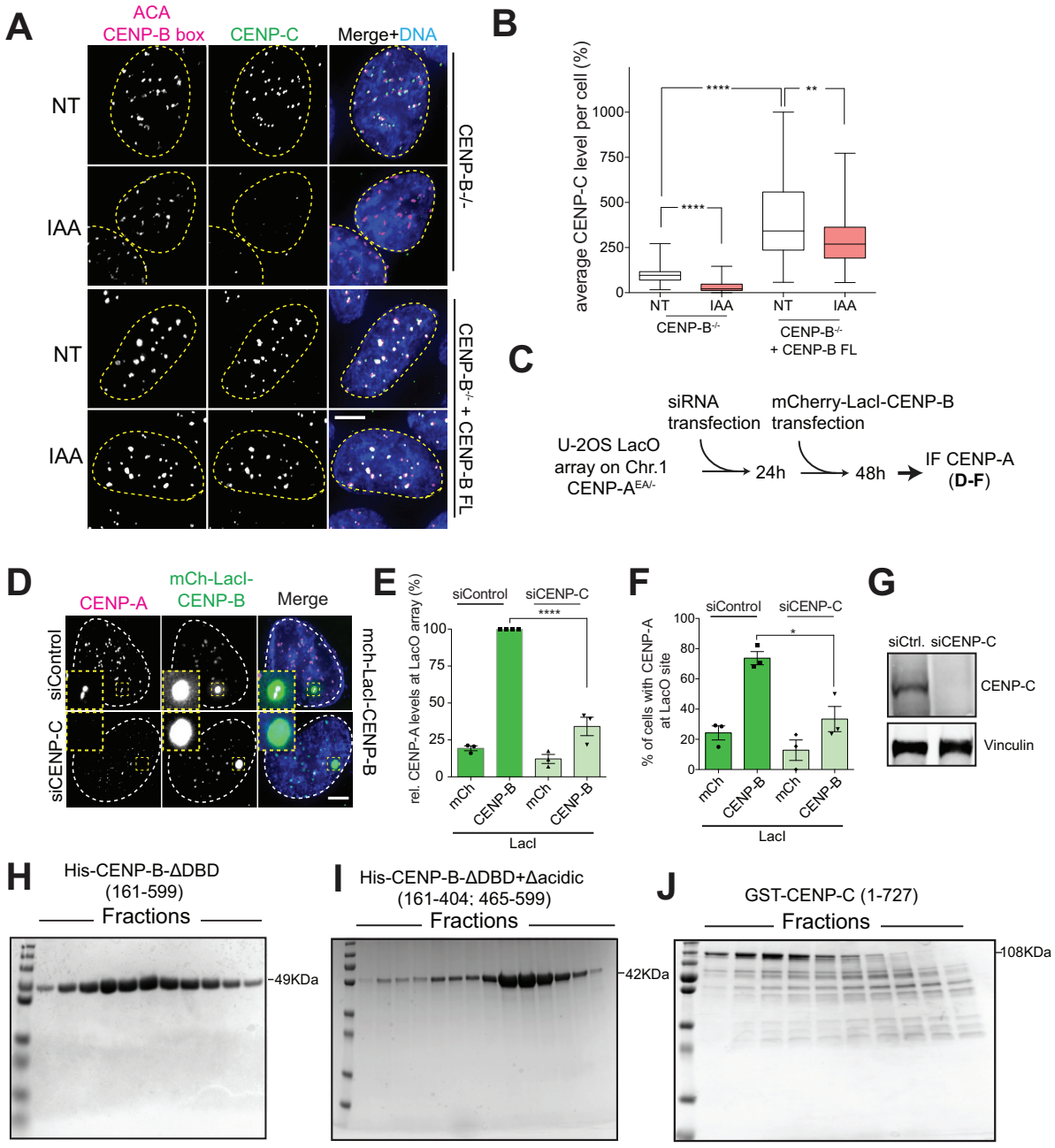
(**A**) Representative IF-FISH images on interphase cells (nucleus highlighted by a white dashed line) showing *de novo* CENP-A deposition at the X but not at the Y centromere after a CENP-A$^{OFF/ON}$ cycle (48 h after IAA wash-out (WO). Scale bar, 5 µm.

(**B**) Bar plot showing frequency of CENP-A presence at the X (green) or Y (magenta) centromere in non-treated cells or following one CENP-A$^{OFF/ON}$ cycle. Unpaired t test, **p=0.0096. Error bars represent SEM of 3 independent experiments.

(**C**) Agarose gel of a PCR run confirming successful integration of the Neomycin resistance cassette at the Y chromosome of DLD-1 cells.

(**D**) Representative FISH using a Y centromere probe in Neomycin selected DLD-1 cells (nuclei are highlighted by white dashed contour lines). Scale bar, 5 µm.

(**E**) Colony formation assay under selective pressure (Neomycin, G418) in untreated conditions or following a CENP-A$^{OFF/ON}$ cycle.



Appendix Figure S2 (related to Fig. 5). CENP-C is required for the recruitment of CENP-A at ectopically tethered CENP-B loci.

(**A**) Representative IF-FISH images of non-treated and IAA treated (48 h) CENP-B$^{-/-}$ +/- CENP-B^{FL} rescue DLD-1 cells (nuclei are contoured by yellow dashed lines). Scale bar, 5 µm.

(**B**) Box plots of centromeric CENP-C levels in the indicated conditions (>70 centromeres per conditions). Box plot shows median and 25th and 75th percentiles, whiskers show minimum and maximum values. Unpaired t-test, ****p<0.0001, **p=0.0049.

(**C**) Timeline of experiments shown in D-G.

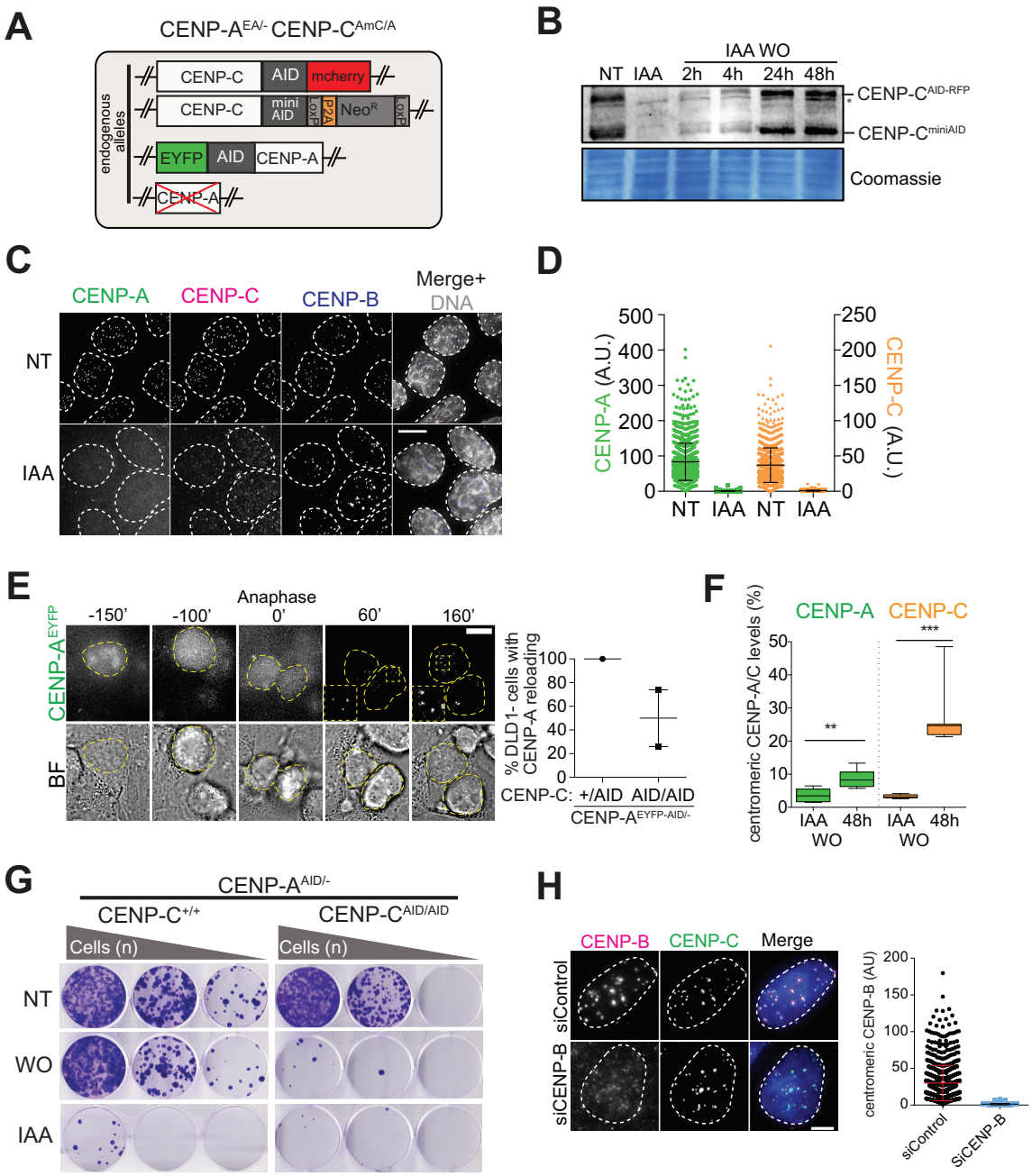
(**D**) Representative immunofluorescence images showing CENP-B (bait) mediated CENP-A (prey) recruitment in the indicated conditions. Nuclei are marked by white dashed contour lines. Inset shows LacO array. Scale bar, 5μm.

(**E**) Bar graph showing CENP-A intensities at the LacO array in the indicated conditions normalized to CENP-A intensities in siRNA control conditions at the mCherry-LacI-CENP-B-LacO array. Each dot represents one independent experiment (>20 cell analyzed for experiment). Unpaired t test, ****$p<0.0001$, error bars show SD of 3 independent experiments.

(**F**) Frequency of CENP-A recruitment to the LacO array in the indicated conditions. Each dot represents one independent experiment. Unpaired t test, *$p=0.0128$, error bars show SD of 3 independent experiments.

(**G**) Immunoblot showing CENP-C knock-down after siRNA treatment.

(**H-J**) SDS PAGE analysis of fractions with an unique peak containing the protein of interest after a final purification step by size exclusion chromatography of the indicated constructs.



Appendix Figure S3 (related to Fig. 6). Partial *de novo* CENP-A and CENP-C reloading at CENP-A/C-depleted centromeres is dependent on CENP-B.

(**A**) Schematic representation of the genomic make-up of DLD-1 cells used for CENP-A/C$^{OFF/ON}$ assays.

(**B**) Immunoblot following CENP-C depletion and re-expression in DLD-1 cells using the AID system. Asterisk marks an unspecific band.

(**C**) Representative immunofluorescence images showing CENP-A and CENP-C depletion after IAA treatment. Nuclei are contoured by white dashed lines. Scale bar, 5 µm.

(**D**) Quantification of centromeric CENP-A (green) and CENP-C (orange) level in the indicated conditions in cells with genomic make-up shown in A. Each dot represents one centromere. Error bars show SD.

(**E**) Left: stills of live cell imaging to monitor *de novo* CENP-A^{EA} reloading in DLD-1 cells after one CENP-A/$C^{OFF/ON}$ cycle. Images were taken every 10 min. Nucleus/mitotic cell is highlighted by yellow dashed contour line based on bright field (BF) images. Scale bar, 10 μm. Right: Plot to show the frequency of CENP-A reloading observed in cells that underwent mitosis. Each dot represents one experiment with at least 10 dividing cells. Error bars show SD of 2 independent experiments.

(**F**) Quantification of centromeric CENP-A (green) and CENP-C (orange) levels normalized to non-treated level using CENP-A/EYFP or CENP-C antibody. Box plot shows median and 25^{th} and 75^{th} percentiles, whiskers show minimum and maximum values. Average centromeric CENP-A/C intensities from five independent experiments (with at least 30 cells per condition) were used to generate the box plots. Unpaired t test: **p=0.0048, ***p=0.0003.

(**G**) Colony formation assay to assess cell viability in the indicated conditions and cell lines.

(**H**) Left: representative immunofluorescence image showing CENP-B knock-down during a CENP-A/$C^{OFF/ON}$ assay. White dashed lines highlight nuclei. Scale bar, 5 μm. Right: quantification of centromeric CENP-B level after RNAi treatment. Each dot represents one centromere (n>700 centromeres). Error bars show standard deviation.