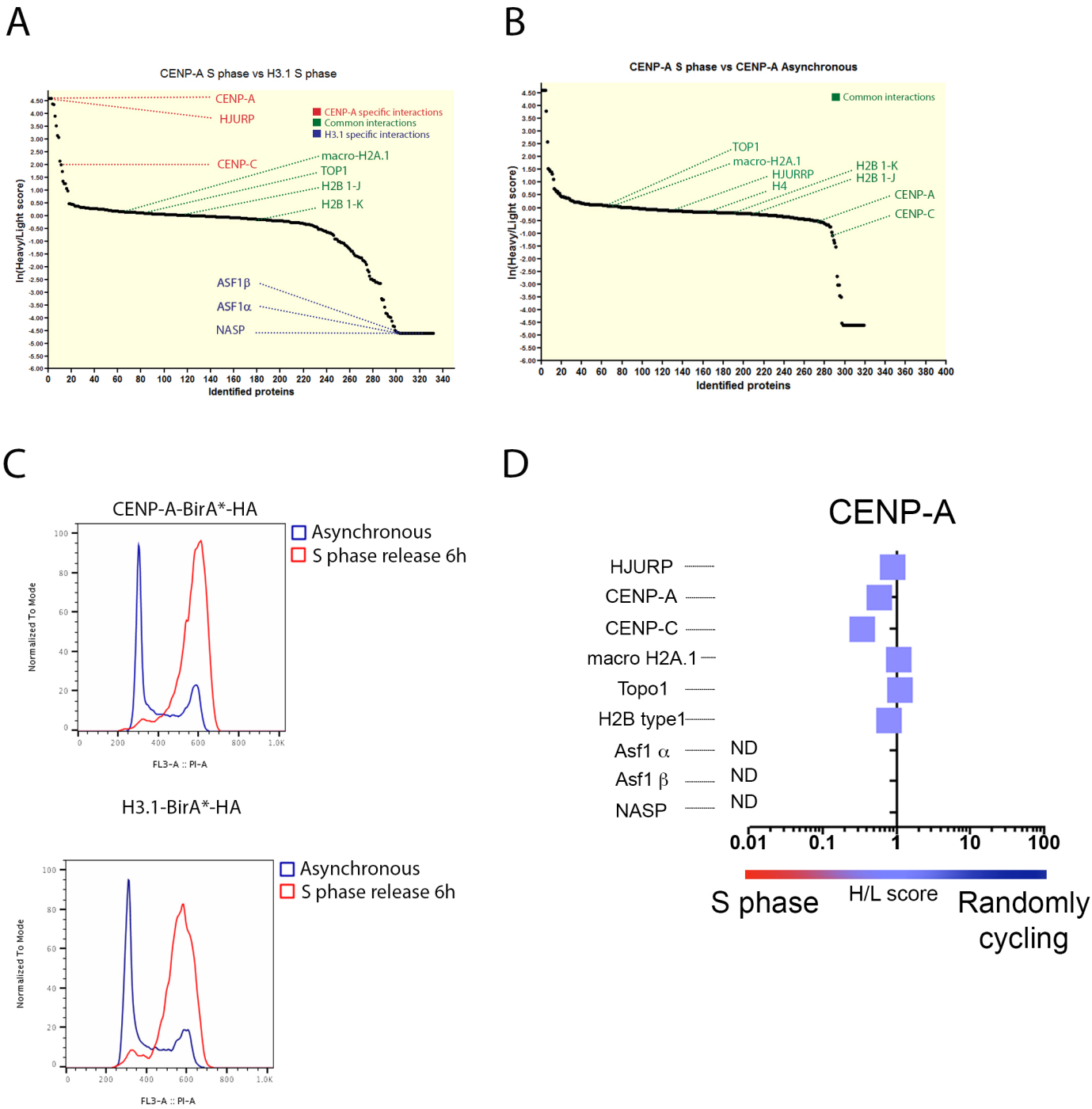

Figure S1. Related to figure 1. (A) Graphs demonstrating proteins identified in indicated samples and their log2 transformed H/L score. SILAC analysis of lysates prepared from cells expressing either CENP-A-BirA*-HA (heavy component) or H3.1-BirA*-HA (light component) released into S phase in the presence of biotin. (B) SILAC analysis of lysates prepared from cells expressing CENP-A-BirA*-HA either released into S phase (heavy component) or randomly cycling (light component) and treated with biotin. The heavy and light components were mixed at 1:1 ratio. The Heavy/Light ratio (H/L) was calculated for each protein detected in the sample. Arrowheads are pointing proteins identified in the screen and known to be physically associated with CENP-A or histone H3.1, or both histone proteins. (C) FACS profiles of indicated cell lines used as an input for Mass Spec experiments. (D) Graph demonstrating SILAC comparison between CENP-A-BirA*-HA biotinylation profile in cells undergoing S phase (heavy component) versus biotinylation profile of CENP-A in asynchronous cells (light component). Selected proteins identified in the sample and their corresponding H/L scores are displayed. The specificity of interaction is demonstrated by the heat map.

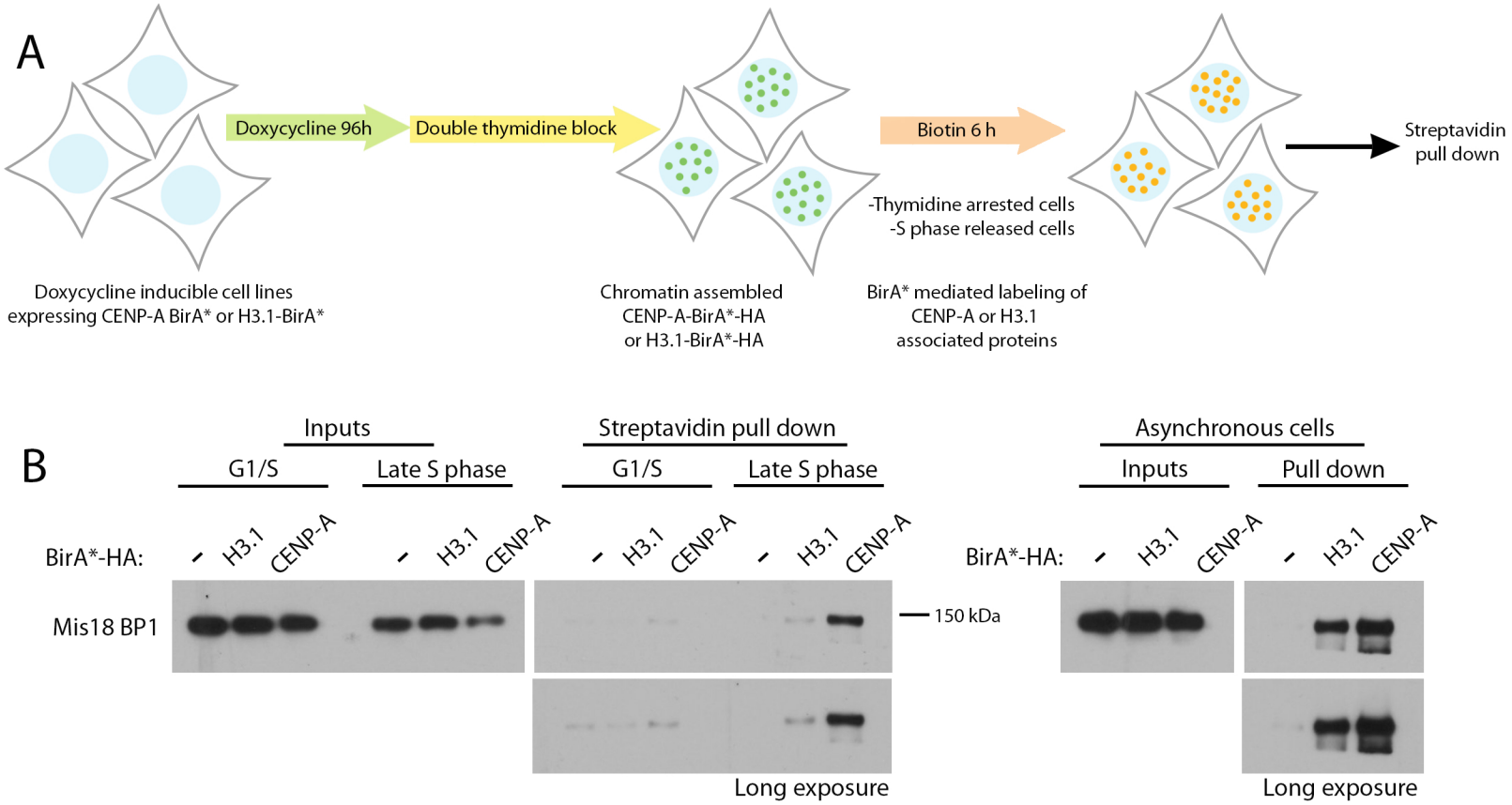

Figure S2. Related to figure 2 and 3. (A) Schematic representation of the experimental approach used in B. Cells were blocked at the G1/S boundary or early S phase by addition of thymidine, and allowed to under S phase following thymidine removal. (B) Cells expressing BirA* fused proteins under doxycycline inducible promoter were treated as shown in A. Biotinylated proteins were isolated by streptavidin purification following by immunoblot analysis with use of Mis18BP1 antibody. The experiment was conducted at least twice. The blots were separated in two sections because all input and all pull down fractions included in the experiment were run on independent gels.

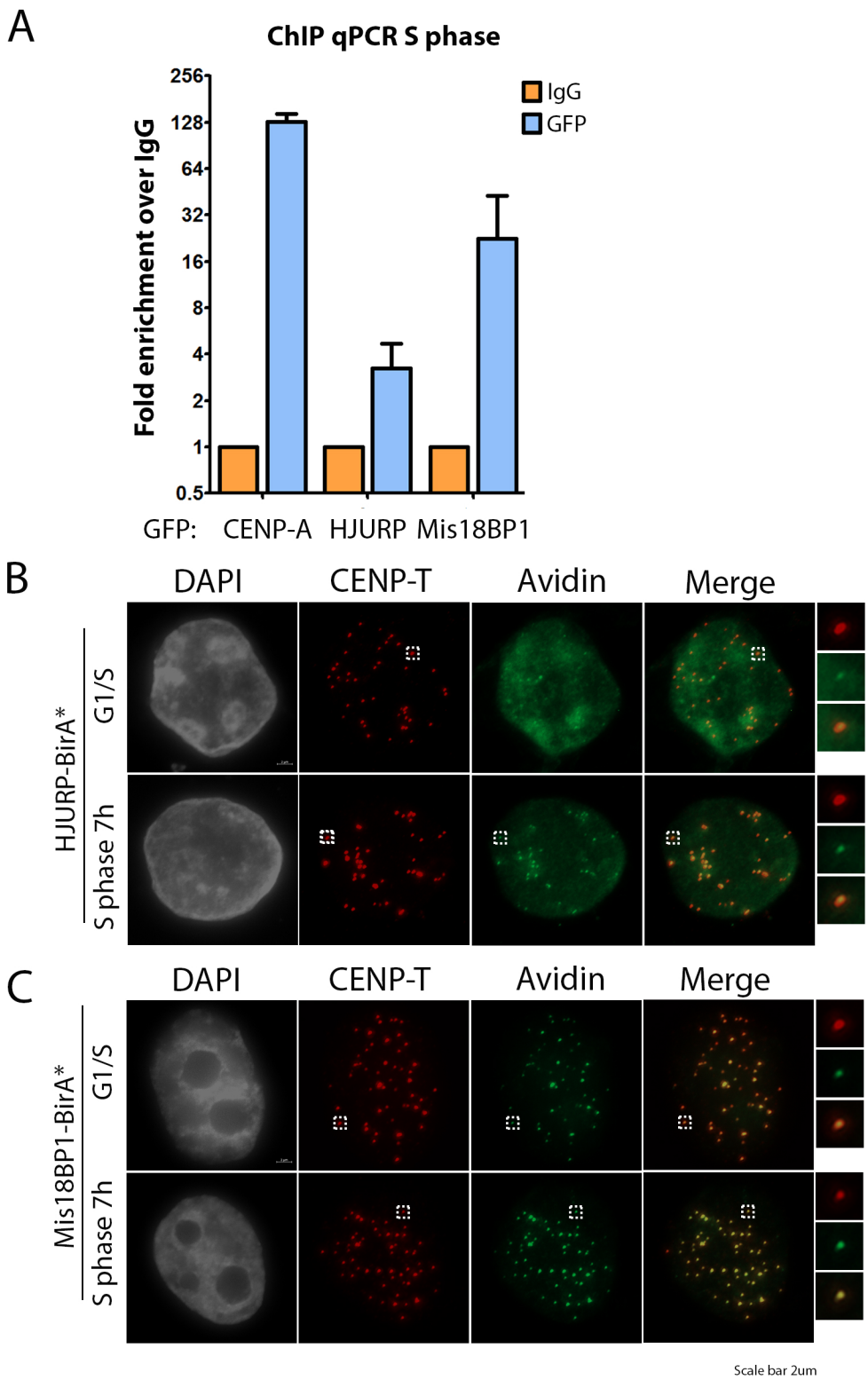

Figure S3. Related to figure 2. (A) Chromatin Immunoprecipitation from cells expressing indicated GFP-tagged proteins in S phase. ChIP was performed using anti GFP antibody and normal rabbit IgG. RT-PCR was performed using primers against α-satellite DNA specific for chromosome 7. The graph represents an average of two independently performed experiments, data are represented as mean +/- SEM. (B)(C) Biotinylation pattern mediated by HJURP (B) or Mis18BP1 (C) during thymidine arrest and S phase release. Cells were treated with biotin for 7 hours at indicated cell cycle stages. Scale bar is 2μm.

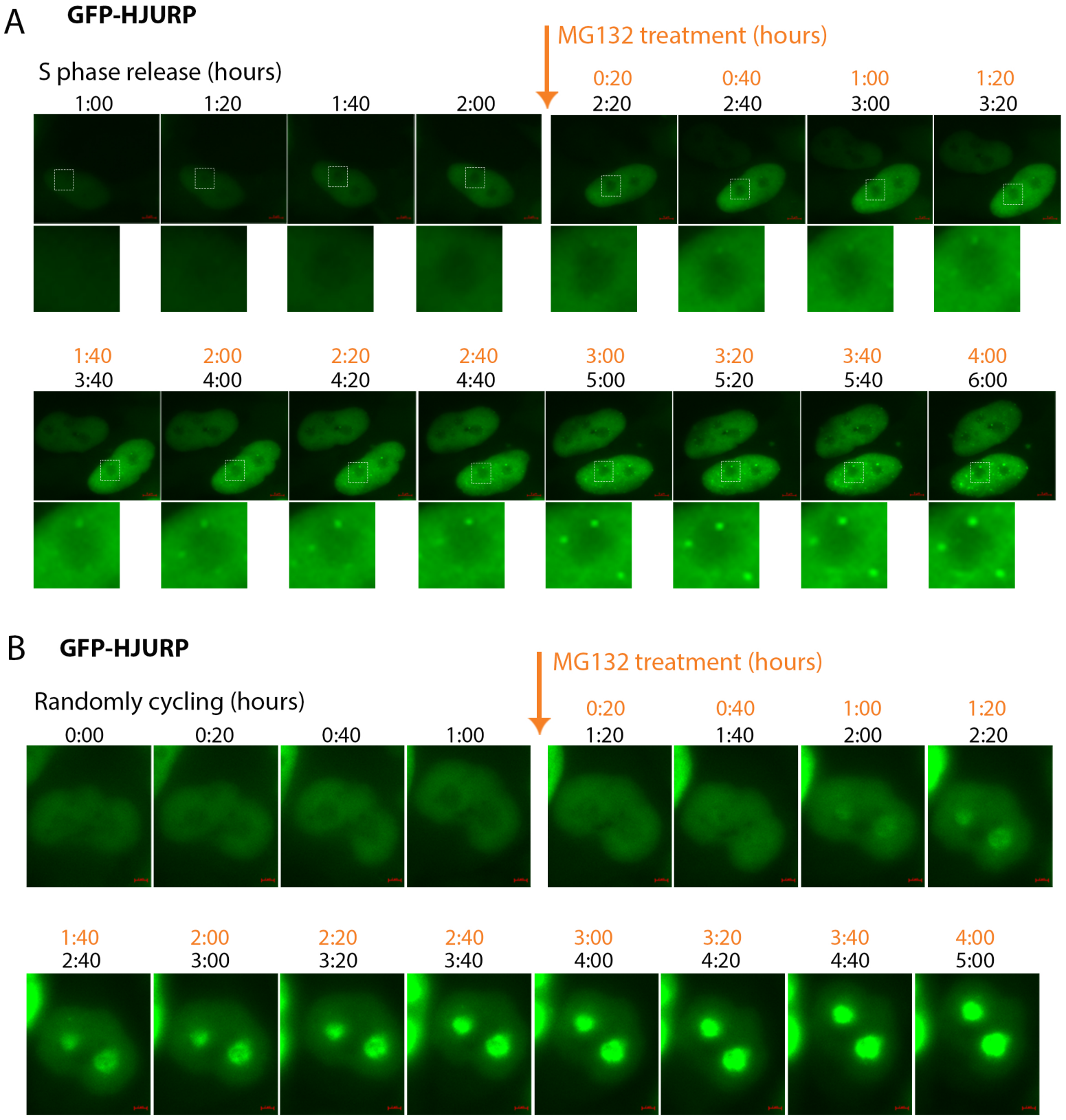

Figure S4. Related to figure 3. (A)(B) Montage of live cell movies of cells expressing GFP-HJURP in S phase released (A) or in asynchronous (B) cell populations. Cells were treated with MG132 during the imaging and the treatment started from the time points indicated by the arrow. Scale bar is 2μm.

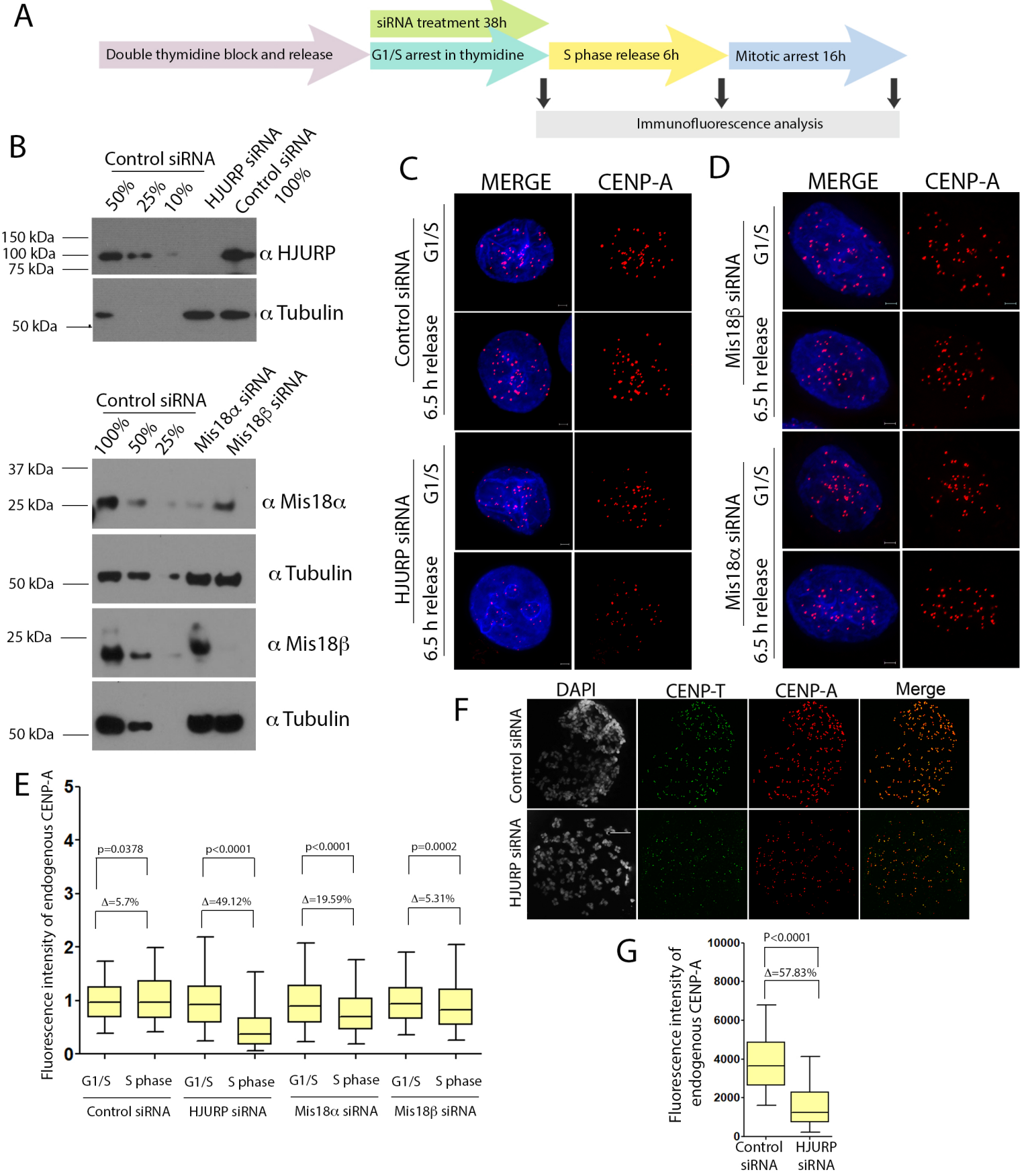

Figure S5. Related to figure 4. (A) Schematic representation of the experiment in C,D and F. (B)Immunoblot analysis of the efficiency of siRNA mediated depletion of HJURP and Mis18 complex subunits, demonstrated by staining with indicated antibodies. Tubulin staining was used as a loading control. (C)(D)(F) Representative images of cells at indicated time points and treated as shown in panel A. DNA was visualized by DAPI, CENP-T is shown in green and CENP-A is shown in red. Scale bar is 2μm and 5μm in C,D and F, respectively. (E)(G) Quantification of C and D. Fluorescent intensity of Centromeric CENP-A was plotted using box-and-whisker plot: 5-95 percentiles. The statistical significance was calculated using unpaired t-test and the p values are indicated.

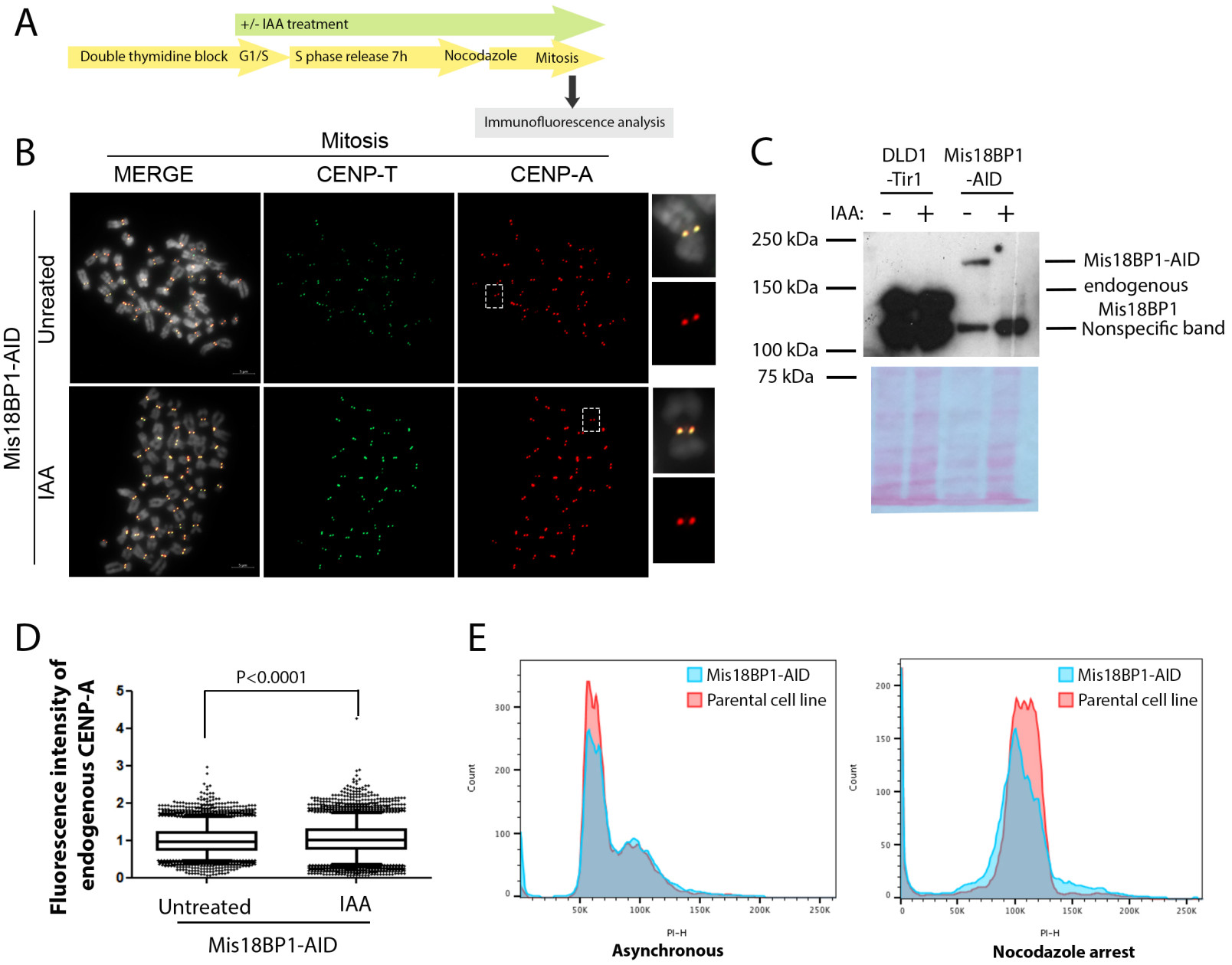

Figure S6. Related to figure 4 (A) Schematic representation of the experiment in B-E using cells where Mis18BP1 was endogenously tagged with AID. (B) Representative images of cells in mitosis and treated as shown in A. DNA was visualized by DAPI, CENP-T is shown in green and CENP-A is shown in red. Scale bar is 5μm. (C) Immunoblot analysis of the efficiency of IAA dependent Mis18BP1 degradation demonstrated by staining with Mis18BP1 antibody. Ponceau was used as a loading control. (D) Quantification of B. The data are normalized to untreated condition. Normalized data from two independent experiments were plotted using box-and-whisker plot: 5-95 percentile, n > 5680 centromeres. The statistical significance was calculated using unpaired t-test and the p values are indicated. (E) FACS profiles of Mis18BP1-AID and DLD1-Tir1 cell lines at indicated cell cycle stages.

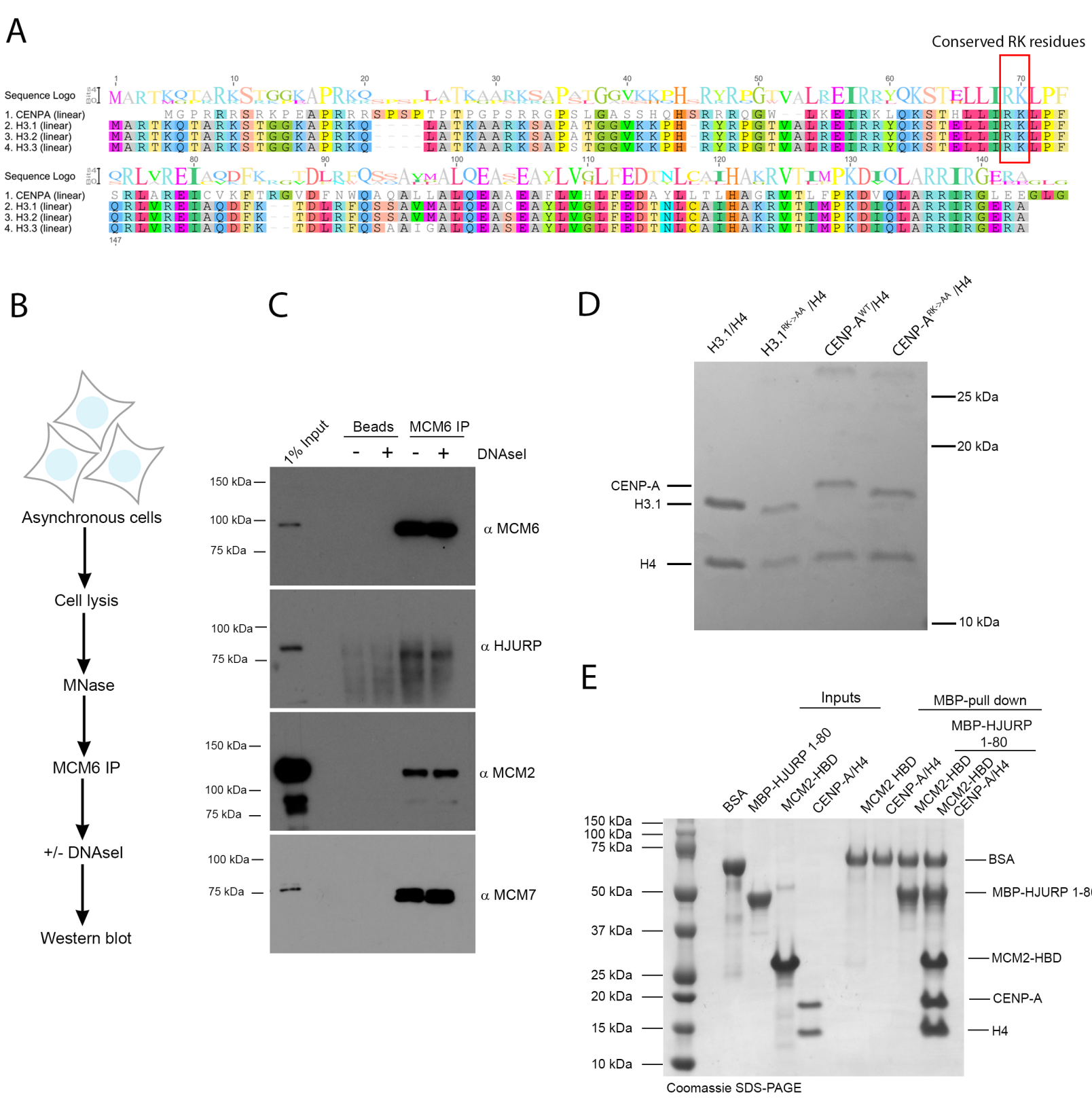

Figure S7. Related to figure 6. (A) Sequence alignment of all human histone H3 variants demonstrating the conserved nature of RK motif implicated in mediating the interaction with MCM2 chaperone. (B) Schematic representation of the immunoprecipitation experiment shown in C. (C) Immunoblot analysis of MCM6 IP experiment performed from HEK293-derived cell lysates treated as indicated in panel B, demonstrating that the HJURP and MCM6 interaction is independent of DNA. Samples were analyzed with indicated antibodies. (D) Coomassie stained SDS-PAGE gel demonstrating the expression of recombinant histone H3 variants in complex with histone H4 used for SPR experiments shown in Figure 7C. (E) Coomassie gel of MBP-HJURP1-60 in vitro pull down demonstrating the interaction with MCM2-HBD only in the presence of CENP-A/H4 heterodimer.