Supporting Information

Jenness et al. 10.1073/pnas.1717509115

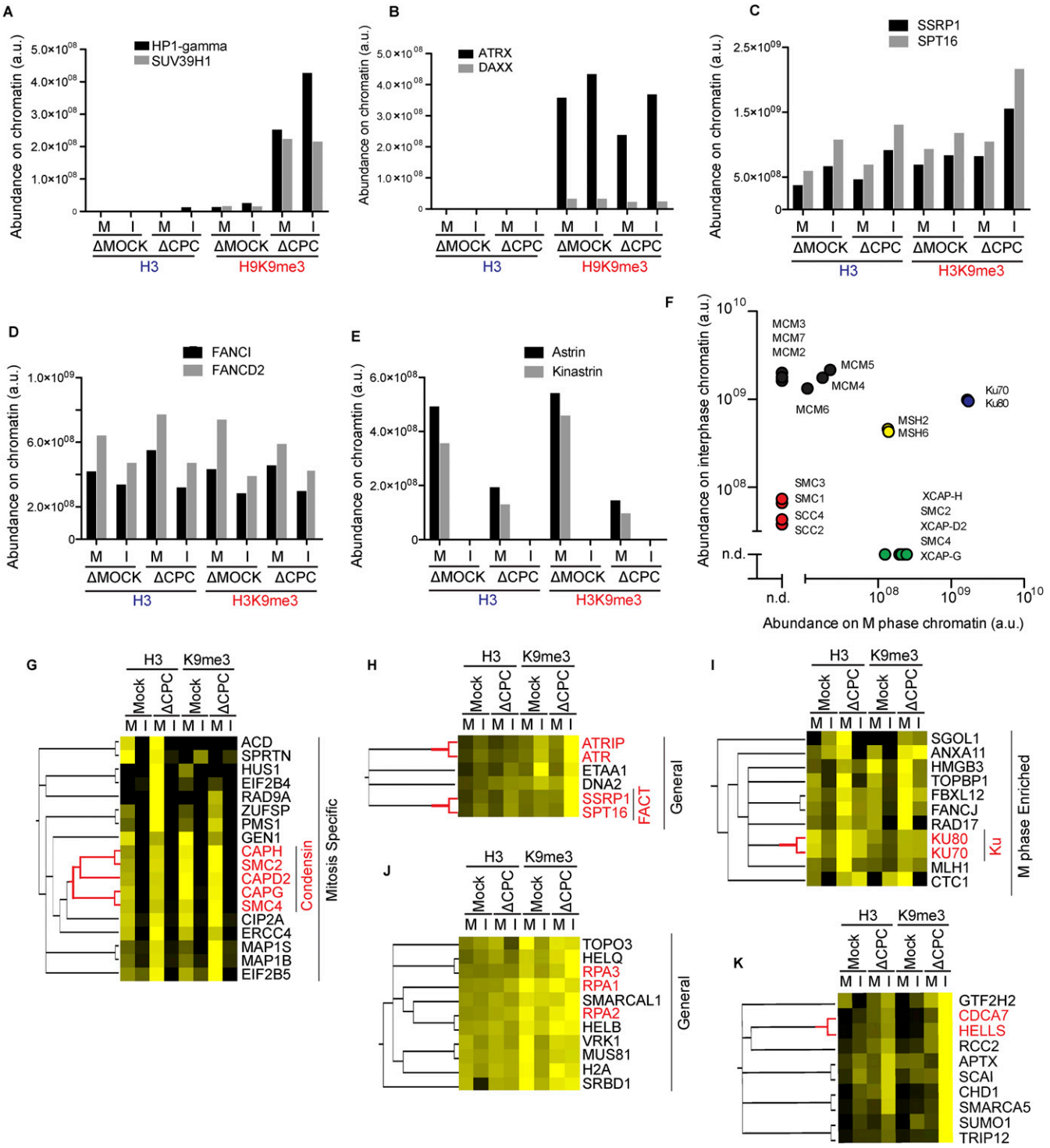

Fig. S1. Proteomic profiling of chromatin regulation by the cell cycle, CPC, and H3K9me3 to identify how protein complexes are regulated. (*A–E*) Abundance of HP1-gamma-SUV39H1 (*A*), ATRX–DAXX (*B*), SSRP1–SPT16 (*C*), FANCI–D2 (*D*), and Astrin–SKAP (*E*) identified by LC-MS/MS on the indicated chromatin beads. Abundance (arbitrary units) was calculated by integrating LC-MS signals for each peptide, as described in *Methods*. (*F*) Abundance of the indicated protein complexes on M phase and interphase chromatin identified by LC-MS/MS. (*G–K*) Dendrogram of the mitosis-specific branch (*F*), general branch (*H* and *J*), M phase enriched branch (*I*), or HELLS–CDCA7 branch (*K*) of hierarchical clustering shown in Fig. 2*A*. A heatmap of each protein's normalized profile is displayed, with proteins enriched on a given chromatin sample colored yellow. Note the condensin subunits cluster within the larger mitosis-specific cluster.

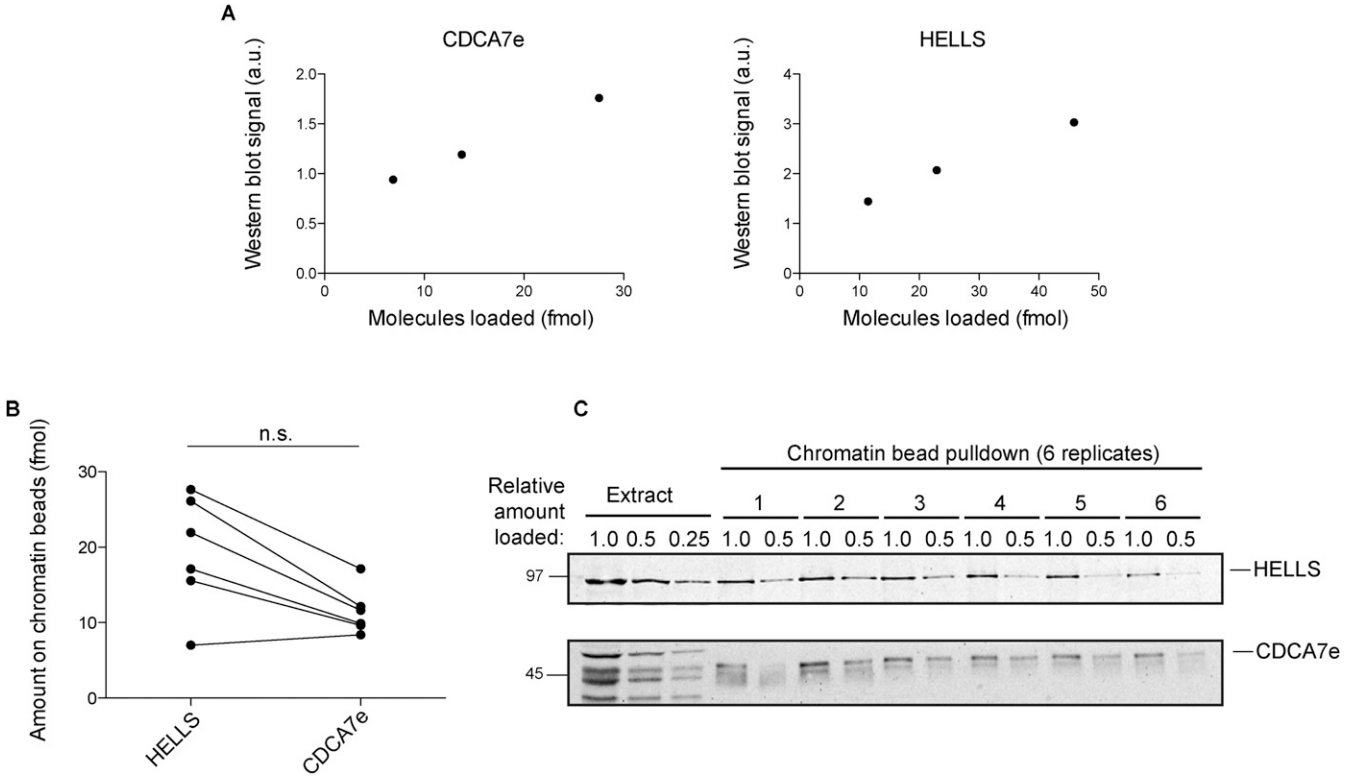

Fig. S2. HELLS and CDCA7e are stoichiometrically equivalent on chromatin beads. (*A*) Quantification of Western blot bands of CDCA7e and HELLS in *Xenopus* egg extract. The amount loaded of each protein was calculated from their concentration in extract (34). (*B*) Western blot quantification of the amount of HELLS and CDCA7e on nucleosome beads recovered from interphase extract. Each connected pair of dots is an independent incubation of chromatin beads in extract. Wilcoxon matched pairs test was used to determine significance. (*C*) Western blots used to quantify *A* and *B*.

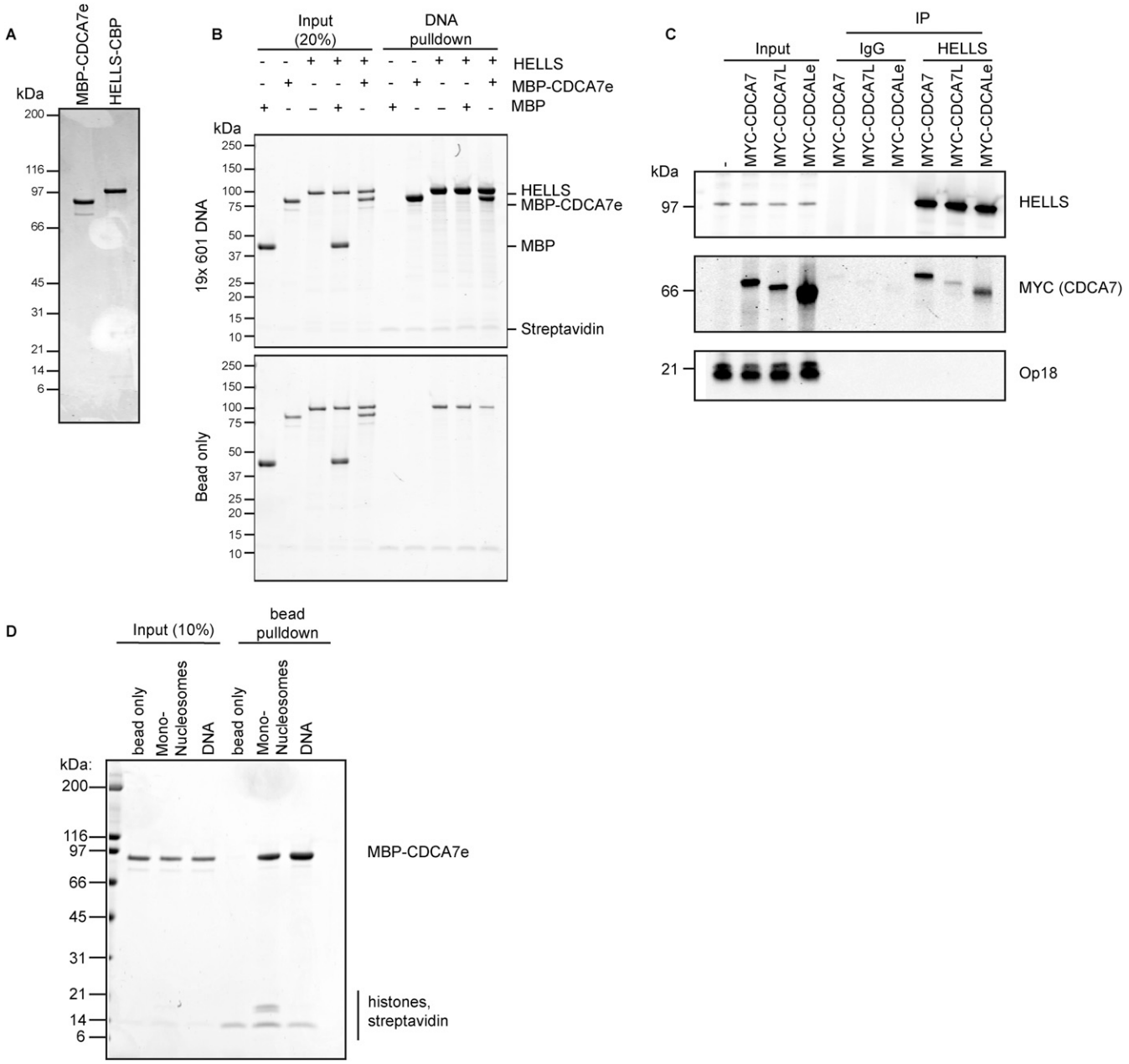

Fig. S3. Characterization of HELLS and CDCA7 interaction and chromatin binding. (*A*) Coomassie-stained gel of purified MBP–CDCA7e and HELLS–CBP used in this study. (*B*) Coomassie-stained gel of a pulldown of 19 × 601 DNA beads incubated with MBP, MBP–CDCA7e, or HELLS–CBP. Uncoupled beads were used to control for nonspecific binding. (*C*) Western blot analysis of HELLS coimmunoprecipitation with CDCA7 paralogs. MYC–CDCA7 paralogs were expressed from mRNA in interphase *Xenopus* egg extract. Immunoprecipitation was performed using beads coupled with anti-HELLS antibodies or control IgG. Representative of $n = 2$ independent experiments. (*D*) Coomassie-stained gel of a pulldown of mononucleosomes with 5 bp 5′ linker or the naked DNA equivalent incubated with MBP–CDCA7e. Uncoupled beads were used to control for nonspecific binding.

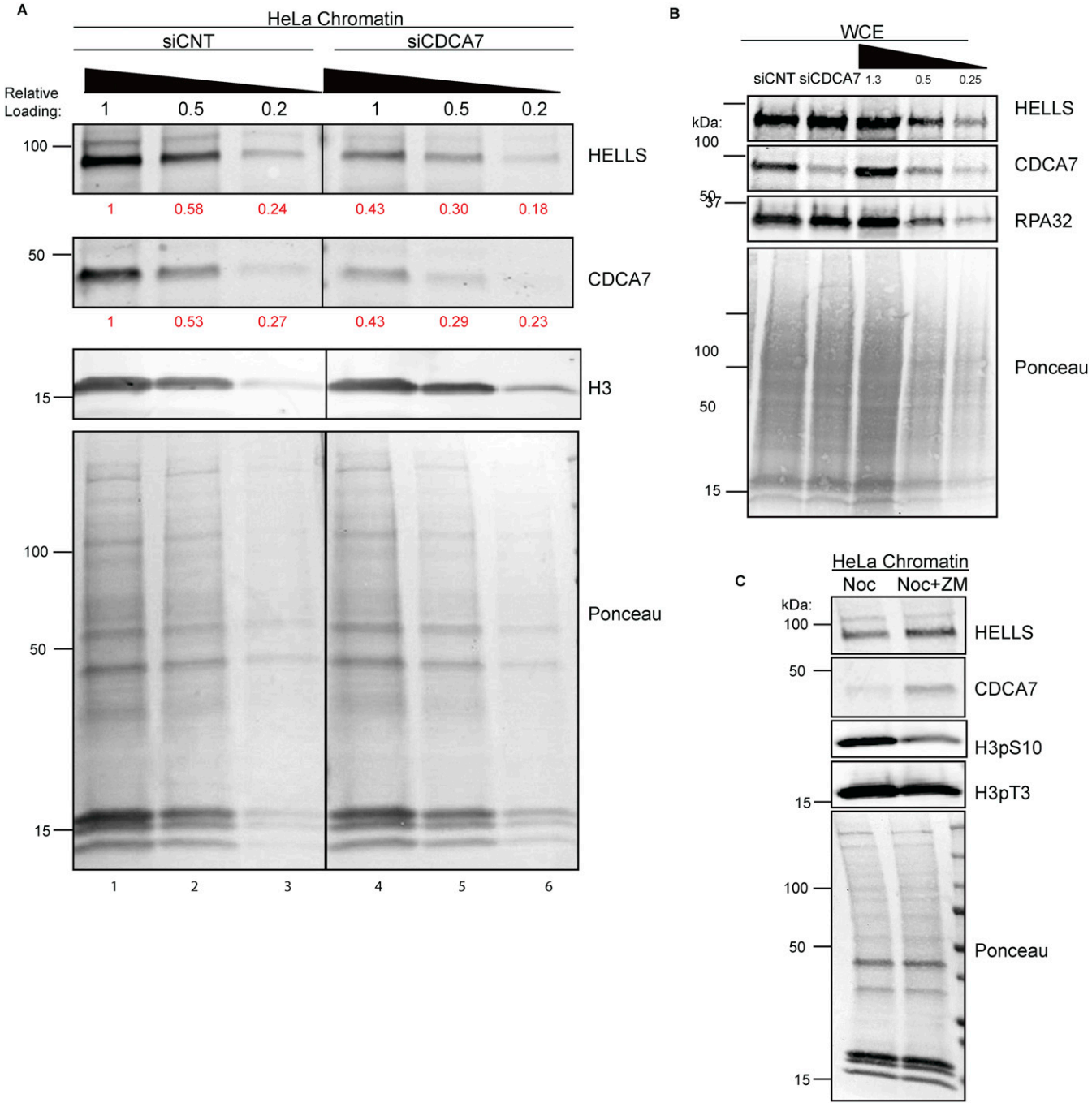

Fig. S4. Human HELLS chromatin binding is reduced upon CDCA7 depletion. (*A*) Western blot of chromatin from HeLa cells after control (siCNT) and CDCA7 (siCDCA7) RNAi, blotted as indicated. Numbers indicate signal intensity normalized to the loading and to lane 1 (red). (*B*) Whole cell extract of control (siCNT) and CDCA7 (siCDCA7) RNAi, and 130% (1.3), 50% (0.5), and 25% (0.25) of the control sample, blotted as indicated. (*C*) Western blot of chromatin fraction from HeLa cells treated with nocodazole (Noc) alone, or in combination with ZM 447439 (ZM) for 24 h, and blotted as indicated.

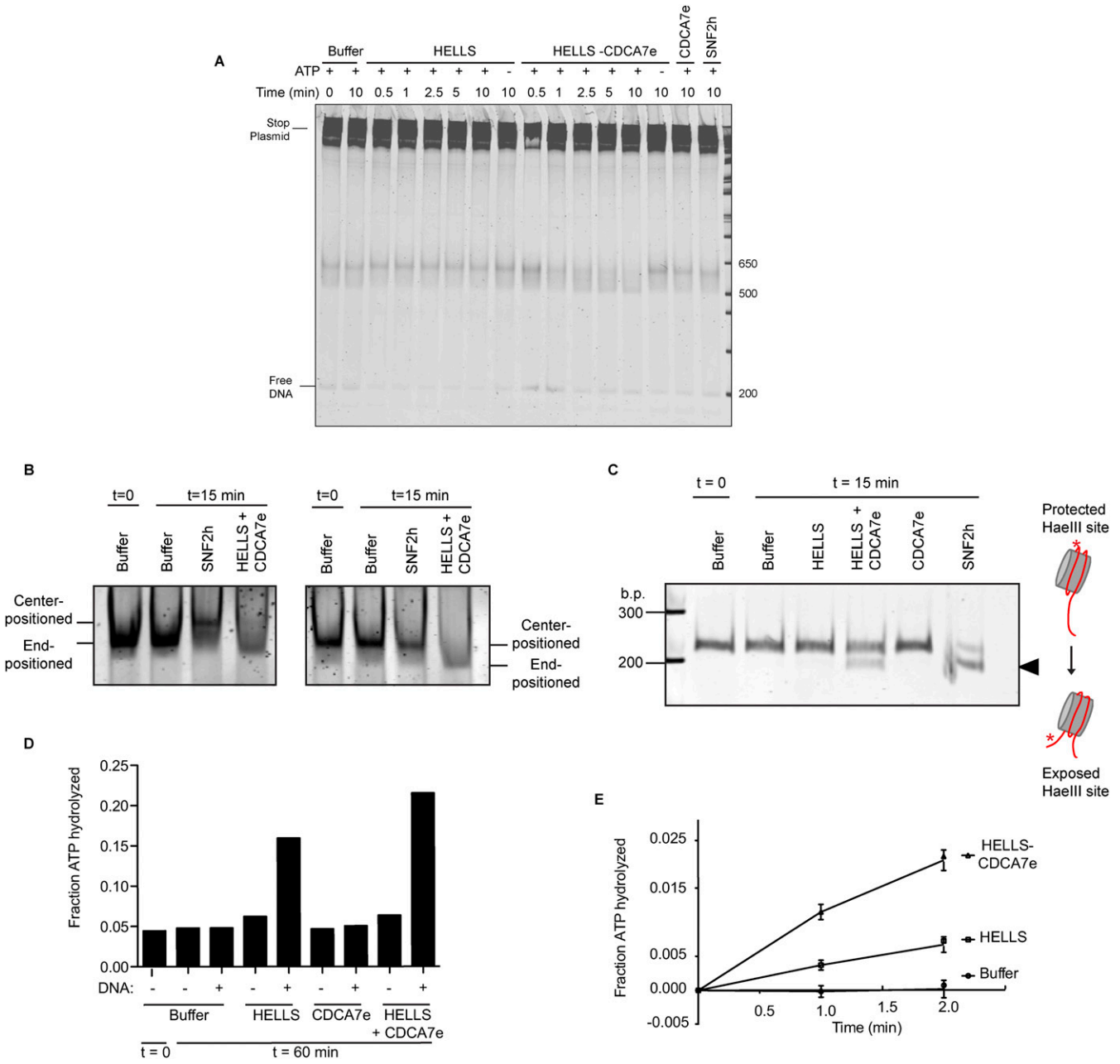

Fig. S5. Characterization of HELLS–CDCA7e ATPase and remodeling activity. (*A*) Entire gel from Fig. 4*B*. (*B*) Native gel nucleosome remodeling assay. End-positioned (*Left*) or center-positioned mononucleosomes (*Right*) were incubated with the indicated remodeling proteins. Reactions were stopped, resolved on a 5% polyacrylamide gel, and visualized with SYBR Gold. Representative of $n = 2$ independent experiments. (*C*) Restriction enzyme accessibility nucleosome remodeling assay. The 601-positioned mononucleosomes with a HaeIII site 11 bp into the nucleosome with 60 bp flanking DNA on the 3′ end were incubated with the indicated remodeling proteins and HaeIII. Productive nucleosome sliding exposes the HaeIII site, resulting in cleaved DNA (arrow). DNA was resolved on a 10% polyacrylamide gel and visualized with SYBR Gold. (*D* and *E*) Quantification of ATPase activity. The indicated proteins were incubated with gamma-33P ATP for the indicated times at 37 °C (*D*) or 16 °C (*E*) for the indicated time. Reaction was separated by TLC, exposed to a PhosphorStorage screen, and the fraction of hydrolyzed ATP was quantified. In *E*, mean and SD from $n = 3$ distinct replicates are displayed.

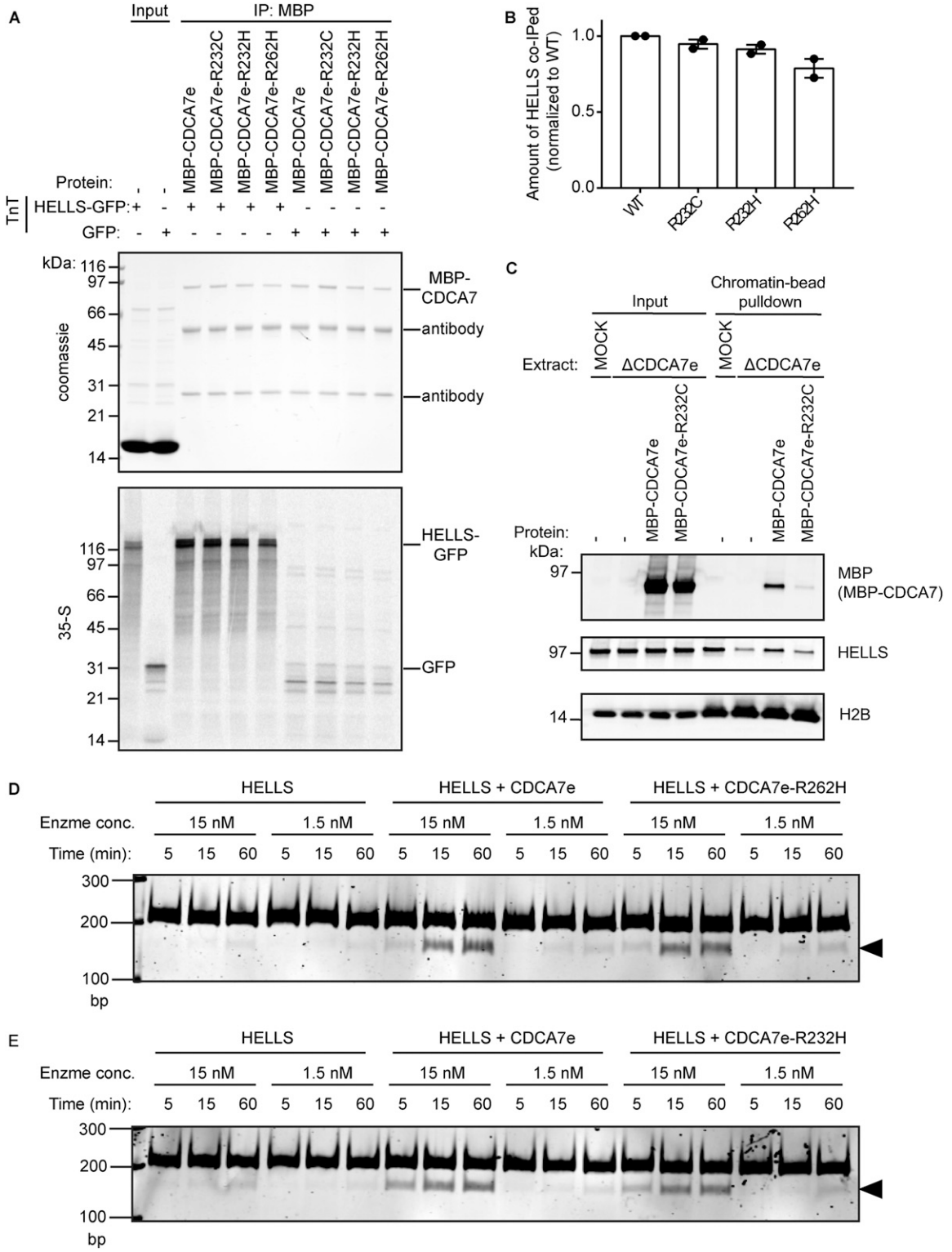

Fig. S6. Characterization of CDCA7e ICF mutations. (*A*) Coomassie staining (*Top*) and autography (*Bottom*) of HELLS–CDCA7e ICF mutant immunoprecipitation. Recombinant MBP–CDCA7e harboring the indicated ICF mutations was immunoprecipitated from reticulocyte lysate expressing 35S-labeled HELLS–GFP or GFP alone. Representative of $n = 2$ independent experiments. (*B*) Quantification of *A*. Mean and range from two independent experiments are plotted. (*C*) Western blot analyses of proteins copurified with chromatin beads recovered from interphase extracts mock depleted or depleted of CDCA7e. Beads coated with 19×601 naked DNA were chromatinized in interphase extract for 90 min before addition of 1 μM recombinant MBP–CDCA7e harboring the indicated ICF mutation. Following an additional 60-min incubation, chromatin beads were recovered. (*D* and *E*) Restriction enzyme accessibility nucleosome remodeling assay. The 601-positioned mononucleosomes (15 nM) with a 34 and 15 bp flanking DNA on the 5′ and 3′ end, respectively, incubated with the indicated remodeling proteins at their indicated concentration and MspI endonuclease. Productive nucleosome sliding exposes an MspI site, resulting in cleaved DNA (arrow). DNA was resolved on a 10% polyacrylamide gel and visualized with SYBR Gold. Representative of $n = 2$ (*D*) or $n = 1$ (*E*) independent experiments.

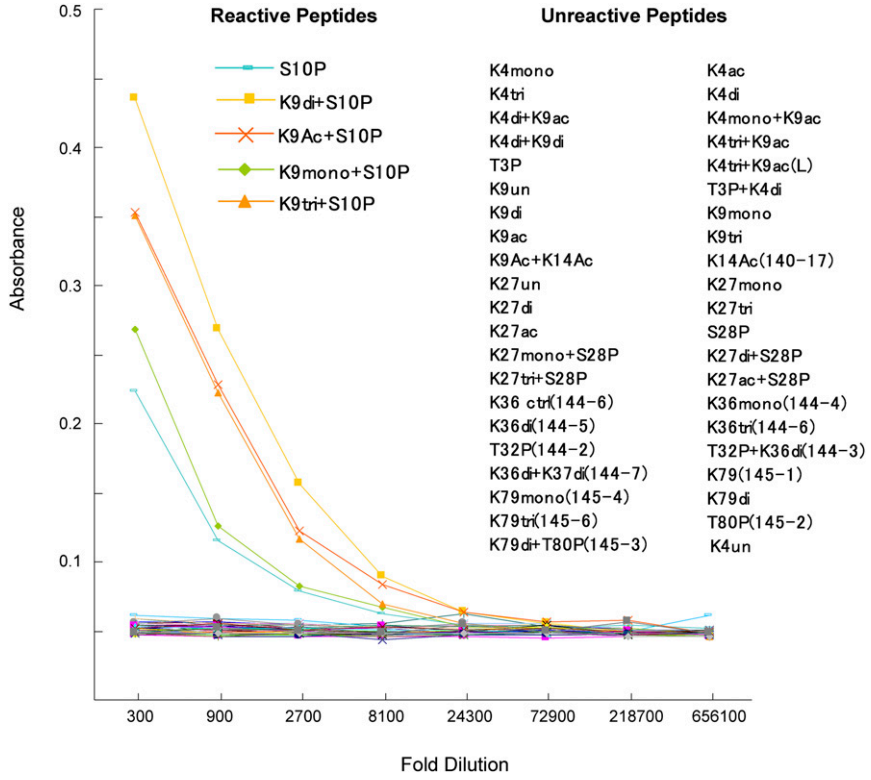

Fig. S7. The specificity of CMA314 evaluated by ELISA. A serial dilution series of CMA314 hybridoma culture supernatant was incubated with plates coated with different peptides. CMA314 reacted with peptides containing H3S10ph. Details of peptides can be found in ref. 64.

Dataset S1. Proteomic analysis of nucleosome interacting proteins

Dataset S1

The dataset contains all proteins identified as copurifying with H3 or H3K9me3 nucleosome beads from the indicated extract by LC-MS/MS. Each protein contains a protein ID, name, gene symbol, the total peptide spectral matches (PSMs) identified, the unique peptides identified, the sequence coverage, a MASCOT score, and an area value for each condition (*A*–*H*). Area values (calculated as described in *Methods*) are used as a measure of protein abundance. Samples are as follows: (*A*) H3 nucleosomes in mock-depleted M phase extract, (*B*) H3 nucleosomes in mock-depleted interphase extract, (*C*) H3 nucleosomes in CPC-depleted M phase extract, (*D*) H3 nucleosomes in CPC-depleted interphase extract, (*E*) H3K9me3 nucleosomes in mock-depleted M phase extract, (*F*) H3K9me3 nucleosomes in mock-depleted interphase extract, (*G*) H3K9me3 nucleosomes in CPC-depleted M phase extract, and (*H*) H3K9me3 nucleosomes in CPC-depleted interphase extract.