Expanded View Figures

Figure EV1. Cryo-EM single particle image analysis of CA-CC^{CT}, CA-CC^{CT}-CN^{NT}, and CA-CC^{pep}-CN^{NT} complexes.

A Cryo-EM density map of the chicken CA-CC^{CT} complex at a 6.78 Å resolution, colored according to the local resolution estimated by RELION in the left panel. Gold-standard Fourier shell correlation (FSC) curve of the Cryo-EM density map is displayed in the right panel. Reported resolution was based on the FSC = 0.143 criterion.

B Cryo-EM density map and FSC curve of the chicken CA-CC^{CT} complex at a 4.5 Å resolution obtained using the CENP-A nucleosome + CENPC-CT + CENPN-NT sample (major component) are shown as in (A).

C Cryo-EM density map and FSC curve of the asymmetric chicken CA-CC^{CT}-CN^{NT} complex at 7.8 Å resolution obtained using CA + CC^{CT}+CN^{NT} sample (minor component) are shown as in (A).

D Cryo-EM density map and FSC curve of the asymmetric chicken CA-CC^{pep}-CN^{NT} complex at a 4.2 Å resolution are shown as in (A).

E A representative micrograph of the CENP-A complex with CENPC-CT and CENPN-NT.

F Representative 2D class averages of the CENP-A nucleosome + CENPC-CT + CENPN-NT sample.

G Flow chart showing the image processing pipeline for the cryo-EM single particle image analysis of the chicken CA-CC^{CT} complex and asymmetric chicken CA-CC^{CT}-CN^{NT} complex.

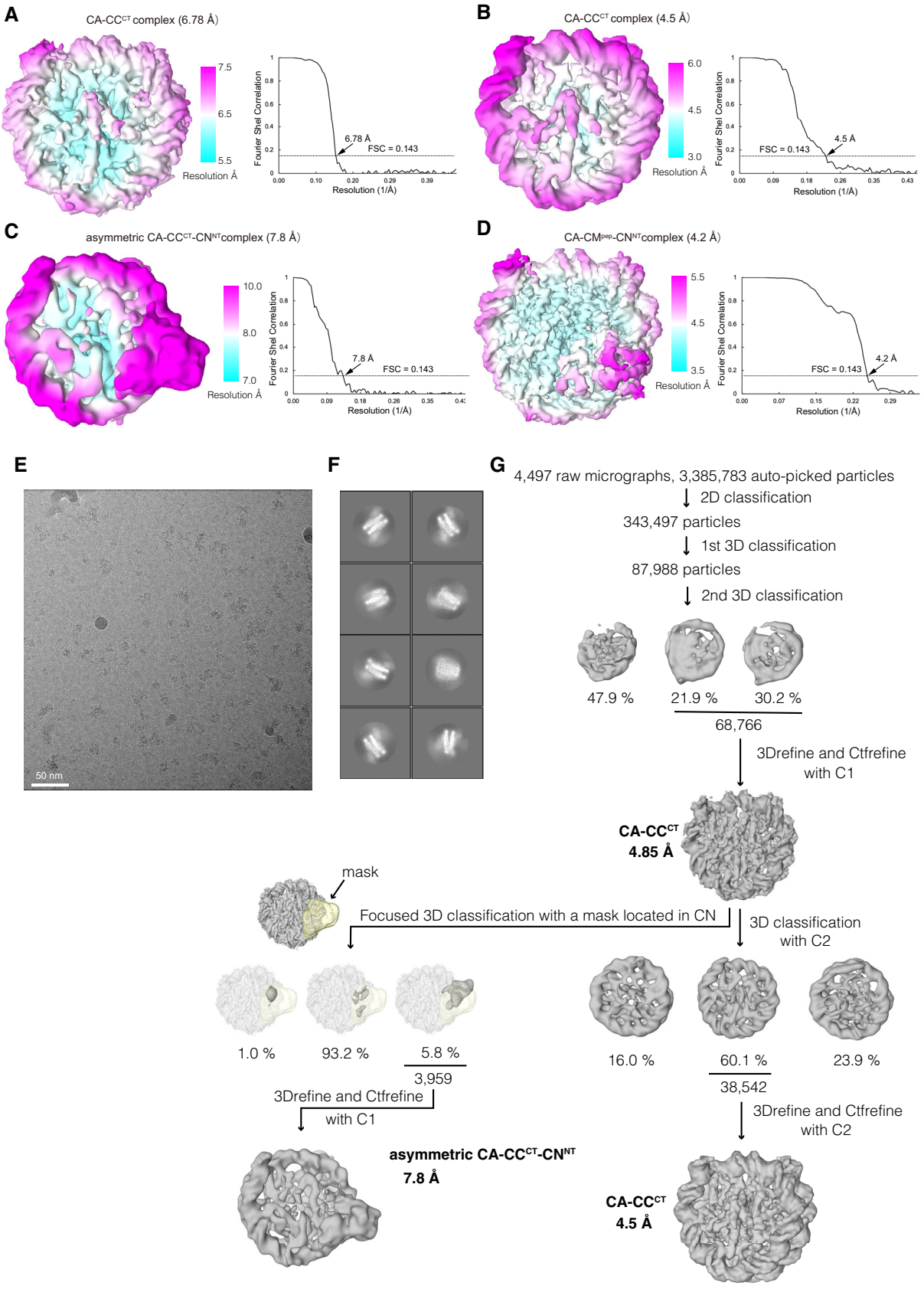


Figure EV1.

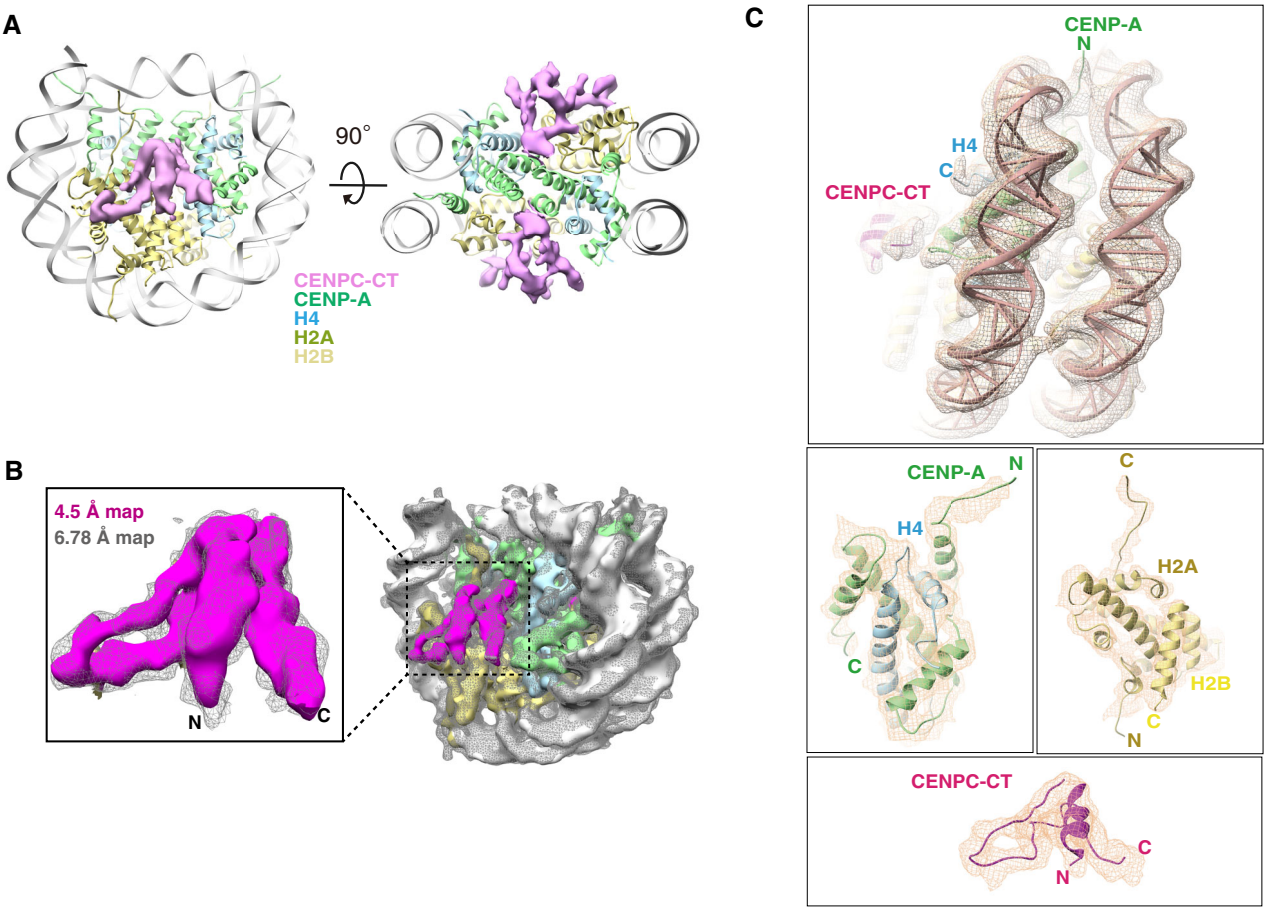
Figure EV2. Cryo-EM structure of the CA-CC^{CT} complex.

A Cryo-EM density map of the CENPC-CT bound to the CENP-A nucleosome at a 6.78 Å resolution. The EM map for CENPC-CT is shown in a pink surface representation together with the ribbon representation of the CENP-A nucleosome. The molecules in the complex are color-coded as indicated in the figure. The right panel shows a slice view along the two-fold axis. The two CENPC-CT fragments symmetrically bind to the CENP-A nucleosome.

B Comparison of lower (6.78 Å) and higher (4.5 Å) resolution EM densities of the CA-CC^{CT} complex. The map at 4.5 Å resolution is depicted in a surface representation and superposed on the 6.78 Å resolution map shown as a mesh representation (light gray). The superposed maps corresponding to the CENPC-CT are enlarged in a left panel.

C Detailed views of the cryo-EM density map of the CA-CC^{CT} complex at a 4.5 Å resolution. The map is shown as a mesh representation with the ribbon model of the final cryo-EM structure.

D Protease sensitivity of CENPC-CT is altered by the presence of the CENP-A nucleosome. Schematic diagram showing Factor Xa cleavage sites in MBP-fused chicken CENPC-CT with its functional domain organization. The possible minor cleavage sites (Gly-Arg sequence) are indicated as cutting site 2 (between residues 642 and 643) and 3 (between residues 682 and 683), in addition to a major cleavage site between CENPC-CT and MBP (site 1). The amino acid sequence of the folded region in CENPC-CT is indicated below. The lower left panel shows the result of SDS–PAGE analysis of limited proteolysis product of CENPC-CT in the absence or presence of the CENP-A nucleosome. Possible fragments generated by Factor Xa digestion are shown in the lower right panel. Bands corresponding to each fragment are indicated in the gel. In the absence of the CENP-A nucleosome, bands of the limited proteolysis products (b, c, e, f, and g) were observed. These bands were not observed in the presence of CENP-A nucleosome.



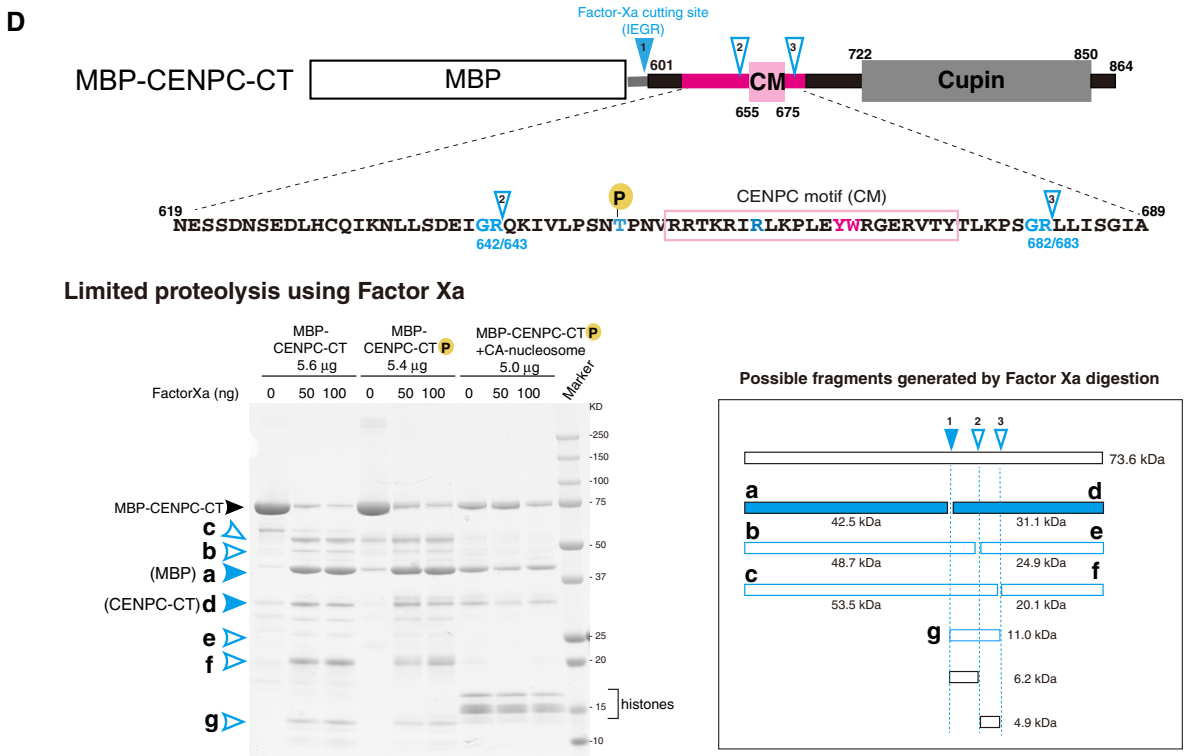


Figure EV2.

Figure EV3. Conserved CENP-A nucleosome-binding sites in the chicken CENPC-CT fragment.

A Schematic diagram showing functional elements in chicken CENPC-CT. The amino acid sequence of CENPC motif (CM) is enclosed in a pink box. The aligned sequences of rat CENPC motif and human central domain (CD), which were used for previous structural studies, are shown at the bottom. Key residues for CENP-A nucleosome binding, R659, Y667, and W668 in chicken CENP-C, are colored in blue (R659) and magenta (Y667 and W668). Corresponding residues in rat and human CENP-C are also colored.

B Magnified views of the binding sites for the CENP-A C-terminal region and the H2A/2B acidic patch are presented in the cryo-EM map. Side chains of the key residues are indicated as a stick model.

C Cryo-EM structure of CENPC-CT bound to the CENP-A nucleosome. The cryo-EM density for CENPC-CT is shown in a mesh representation. The crystal structure of the CENPC motif in complex with the nucleosome (PDB ID: 4X23) is superimposed. The entire backbone structures are well superimposed.

D Structural comparison between the chicken CA-CC^{CT} complex and the human CD structures (PDB ID: 6MUO and 6SE6) on the CENP-A nucleosome. The structures of CD bound to the CENP-A nucleosome superimposed to that of the CENPC motif in the CA-CC^{CT} complex.

E CENP-A nucleosome-binding assays with WT or R659A mutant of CENPC-CT. The substitution of R659 residue with alanine (R659A) caused a loss of the CENP-A nucleosome-binding ability.

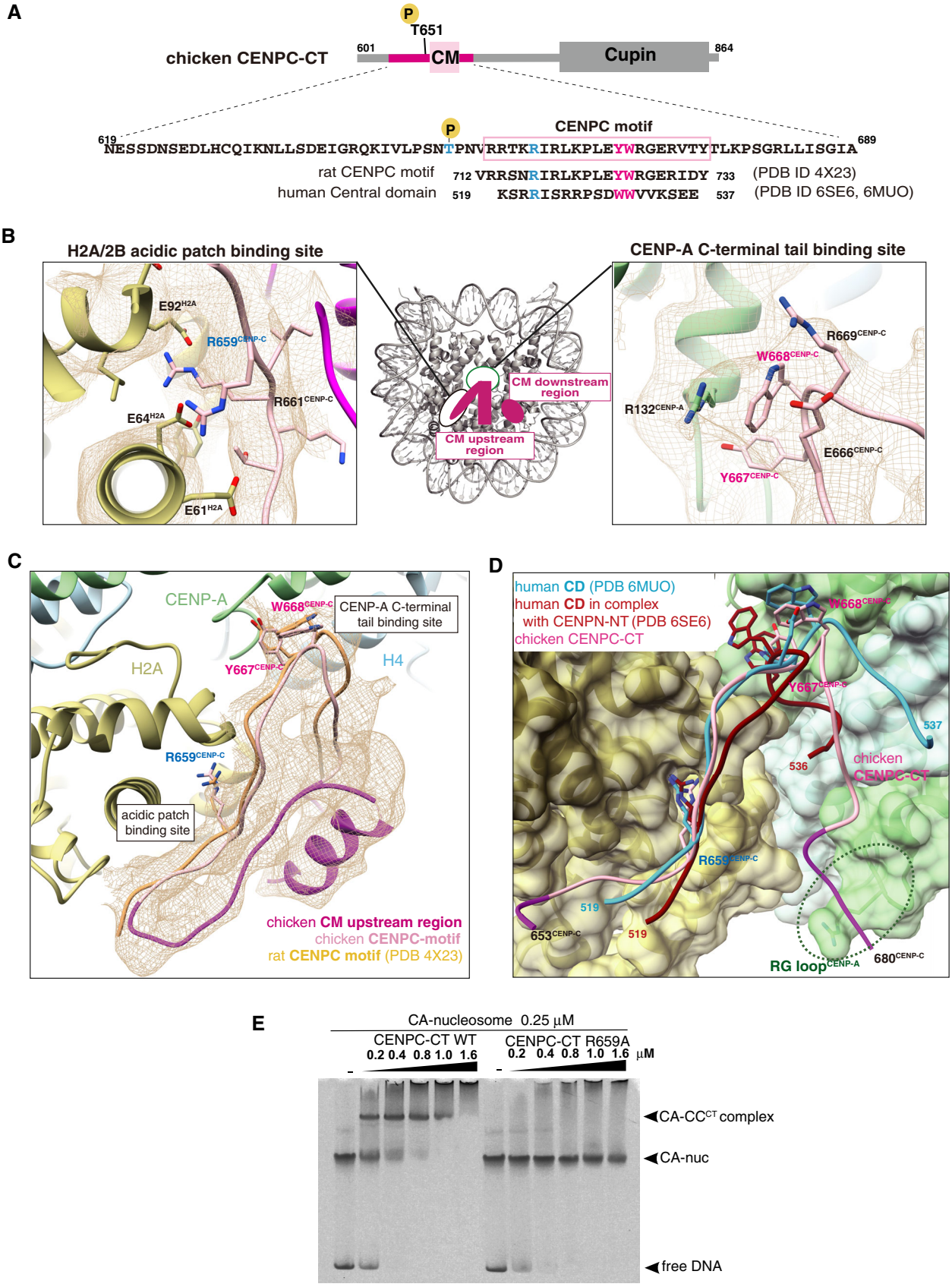


Figure EV3.

Figure EV4. Association of CENPC-CT with RG loop^{CENP-A}.

A Secondary structure prediction of CENPC-CT. A diagram of chicken CENPC-CT is shown. The secondary structure of the putative CENP-A binding region of CENPC-CT was analyzed by six different programs using a HHpred server https://toolkit.tuebingen.mpg.de/tools/hhpred. Predicted secondary structure elements are indicated by H for helix, E for strand, and D for disordered region. The canonical CENPC motif, with previously determined structure in homologues, is highlighted in pink. A schematic diagram of the cryo-EM structure of CENPC-CT is shown at the bottom.

B Crosslinking mass spectroscopy (XL-MS) interactions depicted in relation to CENPC-CT and histones, including CENP-A. Color bars represent protein sequences. Black and purple lines show inter- and intra-protein links, respectively. In the right panel, the crosslinked sites between CENP-C and histones are indicated on the CA-CC^{CT} complex structure in which K678^{CENP-C} was linked with K108^{H2B} and K79^{H4}. Detailed XL-MS data are presented in Appendix Fig S2.

C Alignment of sequences around the CENPC motif region in various species: Gg, chicken; Hs, human; Mm, mouse, and Xl; frog. The CENPC motif and the CM downstream region are depicted by pink and purple boxes, respectively, in the sequence alignment. The residue numbers of human CENP-C are indicated. Schematic diagram of human CENPC-CT wild-type (CENPC-CT WT: aa 687-943) corresponding to chicken CNEPC-CT used for the cryo-EM analysis is depicted. The conserved PSG residues (aa 762-764) in the CM downstream region were substituted with AAA (CENPC-CT 3A$^{762-764}$) and CENPC-CT in which six residues were deleted (CENPC-CT$^{\Delta 761-766}$) are shown.

D Localization analysis of GFP-fused human CENPC-CT WT and mutants shown in (C) on the mitotic chromosomes in CENP-C knock out human RPE-1 cells. CENP-A was used as a centromere marker. Scale bar indicates 10 μm.

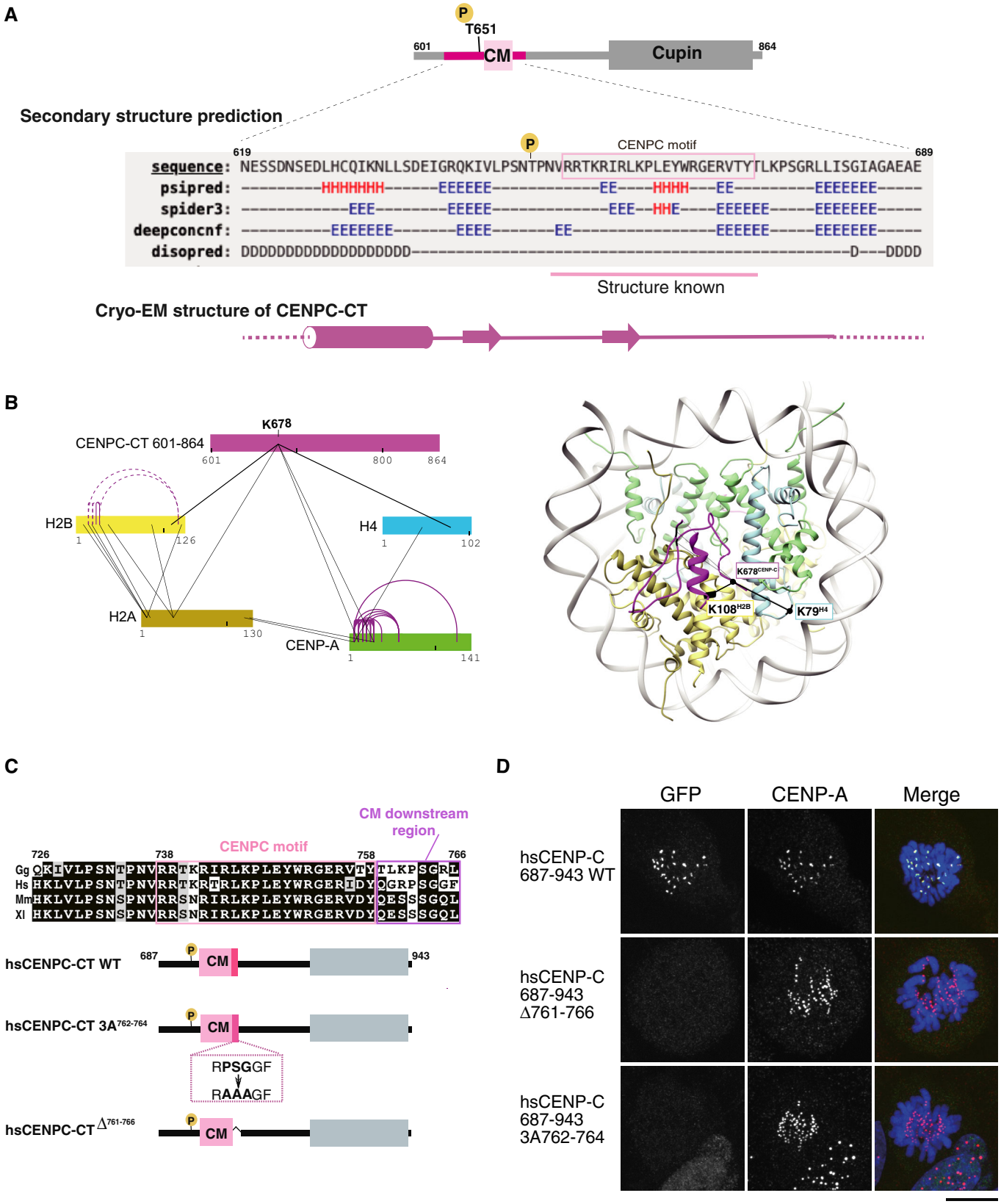


Figure EV4.

Figure EV5. Stabilization of the CENP-A nucleosome binding of CENPC-CT via phosphorylation of T651$^{CENP\text{-}C}$.

A EMSA was performed to examine the binding affinities of phosphorylated or nonphosphorylated CENPC-CT (CENPC-CT[CDK1] or CENPC-CT). The signal intensities of upper binds (bound fractions) were measured. The graph indicates mean with SD (n = 3).

B Left panel displays a magnified view of the interface between CENPC-CT and histone H2A in the CENP-A nucleosome. Arginine 71 of histone H2A (R71^{H2A}) was situated close to the phosphorylated T651$^{CENP\text{-}C}$ in the range of 3.5 to 4.5 Å. Side chains of R71^{H2A} and phosphorylated T651 of CENP-C (ph-T651$^{CENP\text{-}C}$) are indicated in stick model. The middle panel displays results of EMSA performed to examine the significance of the interaction between R71^{H2A} and ph-T651$^{CENP\text{-}C}$. Right panel displays quantification of the EMSA results. The graph indicates mean with SD (n = 3).

C Left panel displays the stable expression of GFP-fused CENPC-CT or the CDK1 phosphorylation site mutant (CENPC-CT T651A) in CENP-C knockout chicken DT40 cells was confirmed by immunoblot analysis. α-Tubulin (Tub) was probed as a loading control. Parental CENP-C knockout cells were also analyzed (parental). Right panel displays the results of localization analysis of GFP-fused CENPC-CT WT and T651A mutant in CENP-C knockout chicken DT40 cells. CENP-T was used as a centromere marker. Scale bar indicates 10 μm.

D Localization analysis of GFP-fused human CENPC-CT (687-943) WT and T734A mutant (equivalent to T651A of chicken CENP-C) in CENP-C knockout human RPE1 cells. CENP-A was used as a centromere marker. Scale bar indicates 10 μm.

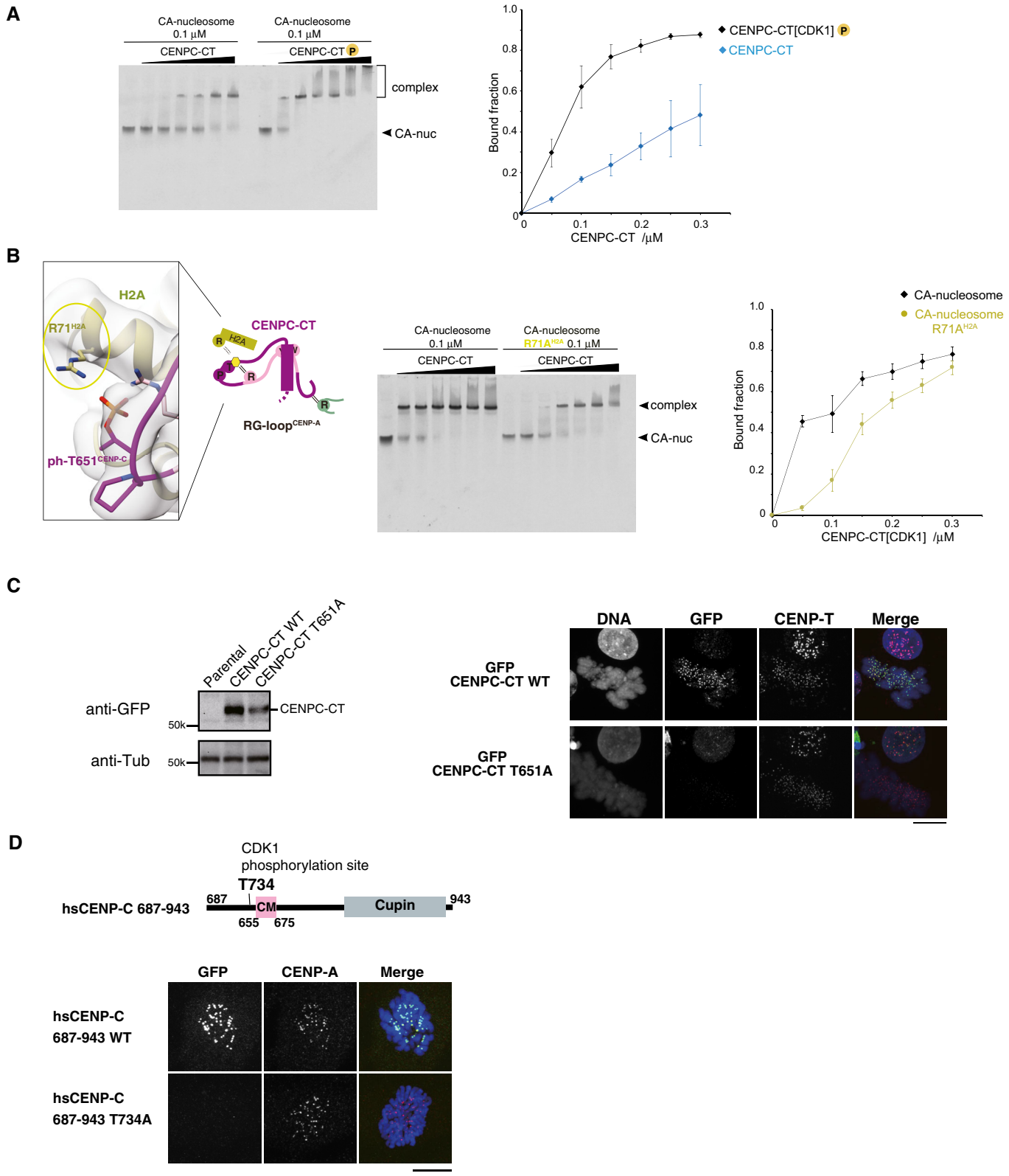


Figure EV5.