Figure S1

related to Figure 1

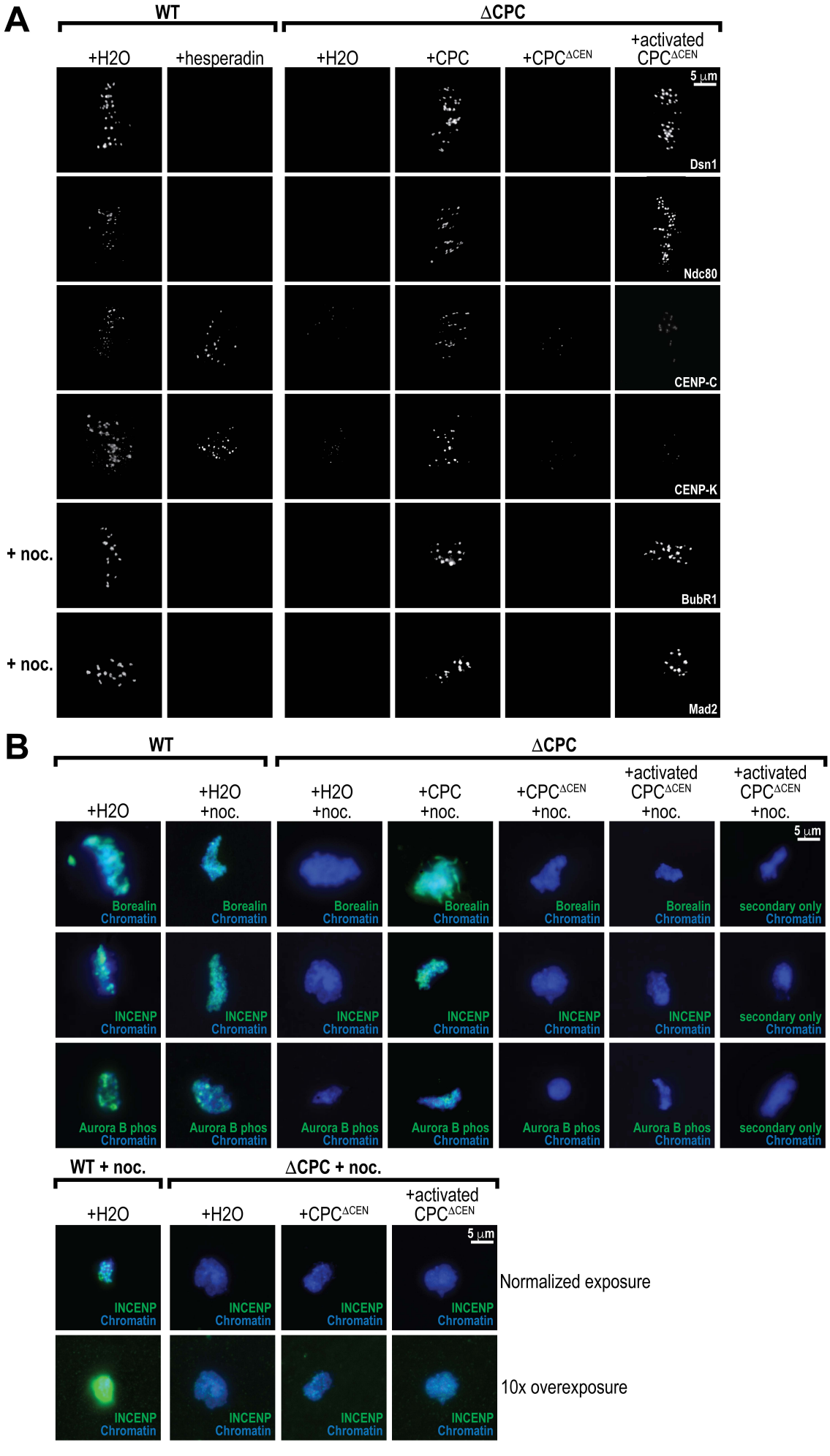


Figure Legends:

Figure S1. The CPC Regulates the Assembly of the Kinetochore; Related to Figure 1

(**A**) Representative IF images of replicated chromosomes in metaphase spindles in *Xenopus laevis* WT and ΔCPC extracts with indicated CPC conditions. Hesperadin added when indicated. Some images are also shown in Figures 1 and 5 and are shown here for comparison purposes. Spindles were stained for the indicated kinetochore components (white).

(**B**) Representative IF images of indicated CPC component localization on metaphase chromosomes in WT and ΔCPC extracts with indicated CPC conditions. Far right column shows undetectable contribution to background fluorescence from presence of α-INCENP.

Figure S2

related to Figure 1

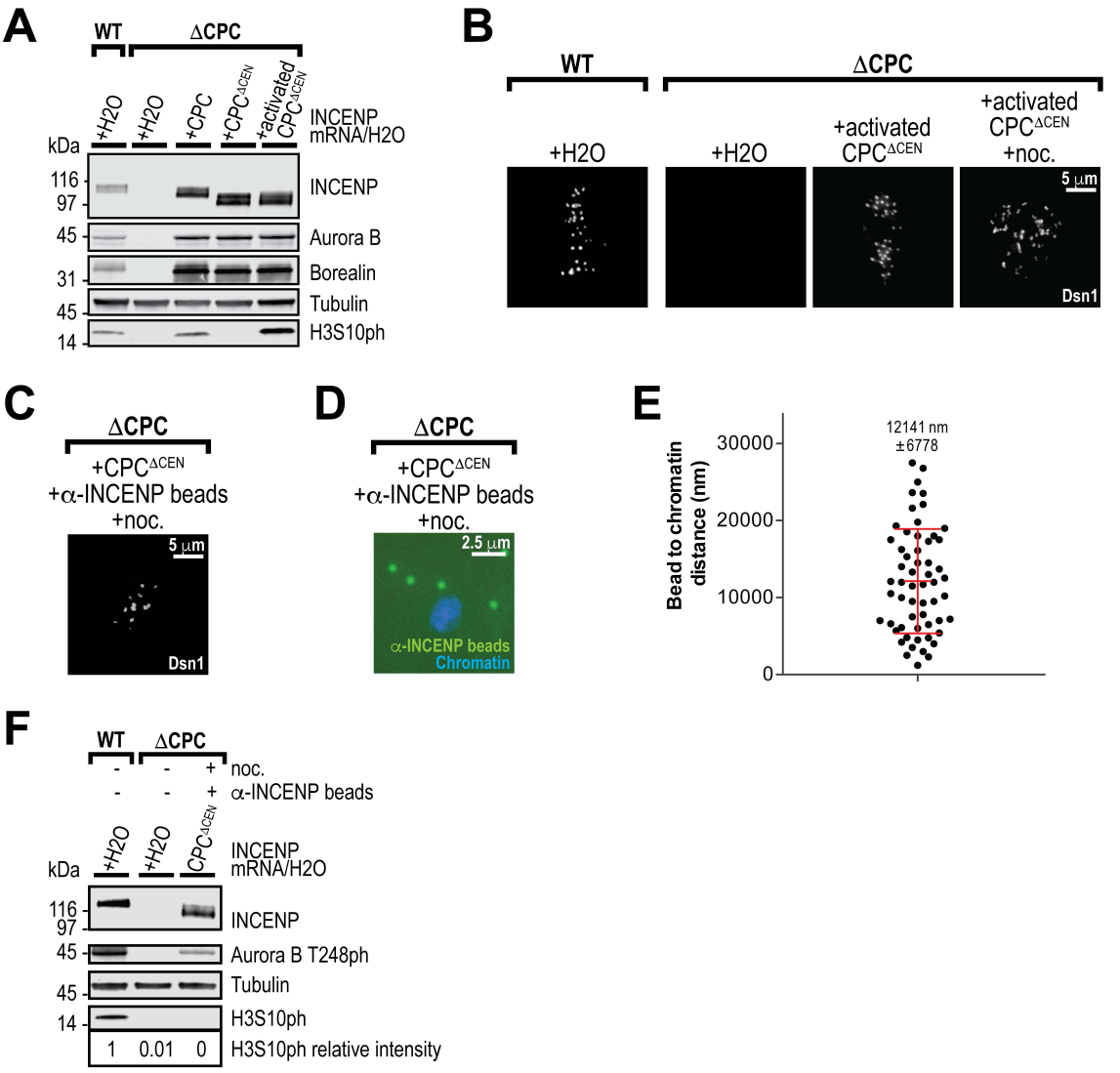


Figure S2. Outer Kinetochore Assembly Does Not Require the Interaction of the CPC with Microtubules or Chromatin; Related to Figure 1

(**A**) Western blots for the CPC components, Histone H3 phosphorylation (H3S10ph) and tubulin for samples in (B) and Figures 1 and 2 and Figures S1 and S2B.

(**B**) Representative IF images of replicated chromosomes in metaphase in *Xenopus laevis* WT and activated $CPC^{\Delta CEN}$ extracts +/- nocodazole. Nocodazole was added to depolymerize microtubules. Spindles were stained for Dsn1 (white).

(**C**) Representative IF image of replicated chromosomes in metaphase in *Xenopus laevis* ΔCPC extracts in the presence of $CPC^{\Delta CEN}$ clustered on the surface of anti-INCENP Protein A Dynabeads. Nocodazole was added to depolymerize microtubules. Spindles were stained for Dsn1 (white).

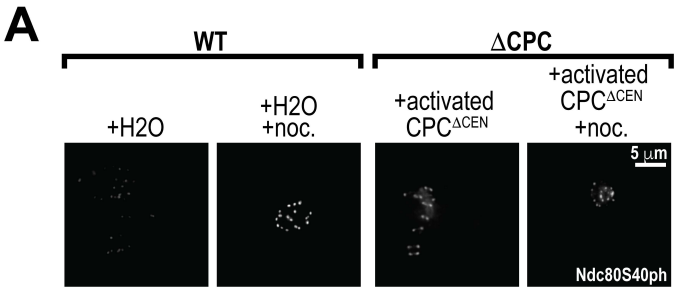
(**D**) Example images showing the proximity of anti-INCENP Protein A beads to chromosomes in *Xenopus laevis* extracts.

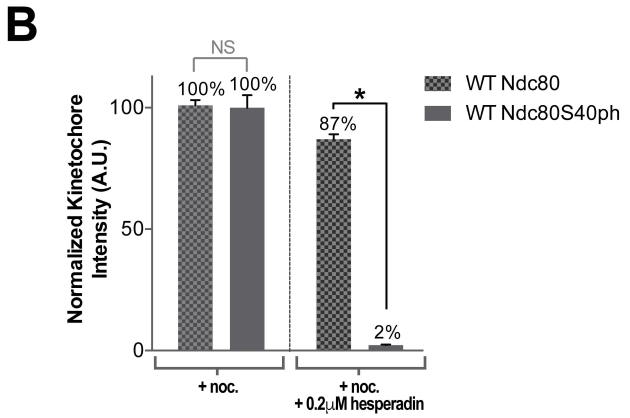
(**E**) Mean distance of anti-INCENP Protein A beads to chromosomes. Error bars represent the standard deviation. n=50

(**F**) Western blots for the CPC components, Histone H3 phosphorylation (H3S10ph) and tubulin for samples in (C-E) where $CPC^{\Delta CEN}$ is activated with anti-INCENP Protein A beads. Note the low levels of Aurora B activity which are sufficient for kinetochore assembly.

Figure S3

related to Figure 2





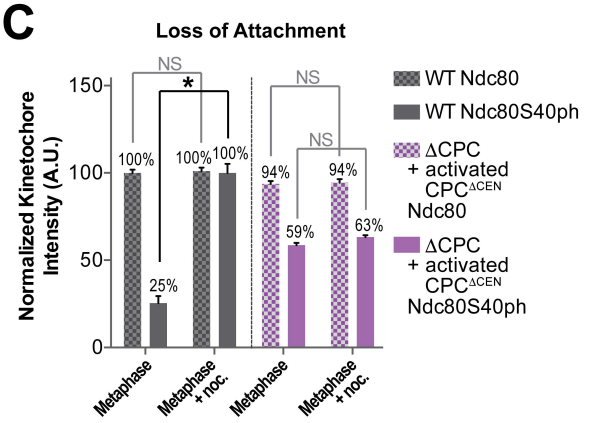


Figure S3. Aurora B-Dependent Phosphorylation of Ndc80 is Dysregulated in Activated CPC$^{\Delta CEN}$ Extracts; Related to Figures 1 and 2

(**A**) Representative IF images of replicated chromosomes in metaphase spindles in *Xenopus laevis* WT and activated CPC$^{\Delta CEN}$ extracts +/- nocodazole. Spindles were stained for Ndc80S40ph (white).

(**B**) Antibodies specific to phosphorylated human Ndc80 at Ser44 can be used to detect phosphorylated Xenopus Ndc80 at Ser40 (epitopes are 73% identical, 82% similar over an 11 aa window). Shown here are mean integrated fluorescence intensities of Ndc80 and Ndc80S40ph in WT *Xenopus laevis* extracts treated with nocodazole during metaphase +/- hesperadin normalized to the nocodazole treated condition. Phosphorylation of Ndc80 was reduced almost to zero by the addition of 0.2μM hesperadin, demonstrating specificity for the phosphorylated form of Xenopus Ndc80. At this dose hesperadin does not inhibit kinetochore assembly. Error bars represent the SEM of 2 independent experiments; n = 90 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$).

(**C**) Mean integrated fluorescence intensities of Ndc80 and Ndc80S40ph in WT and activated CPC$^{\Delta CEN}$ extracts +/- nocodazole. Samples were treated with nocodazole to generate unattached kinetochores. All values are normalized to the nocodazole treated control samples. Error bars represent the SEM of 3 independent experiments; n = 96 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$); NS indicates no significant difference.

Figure S4

related to Figure 3

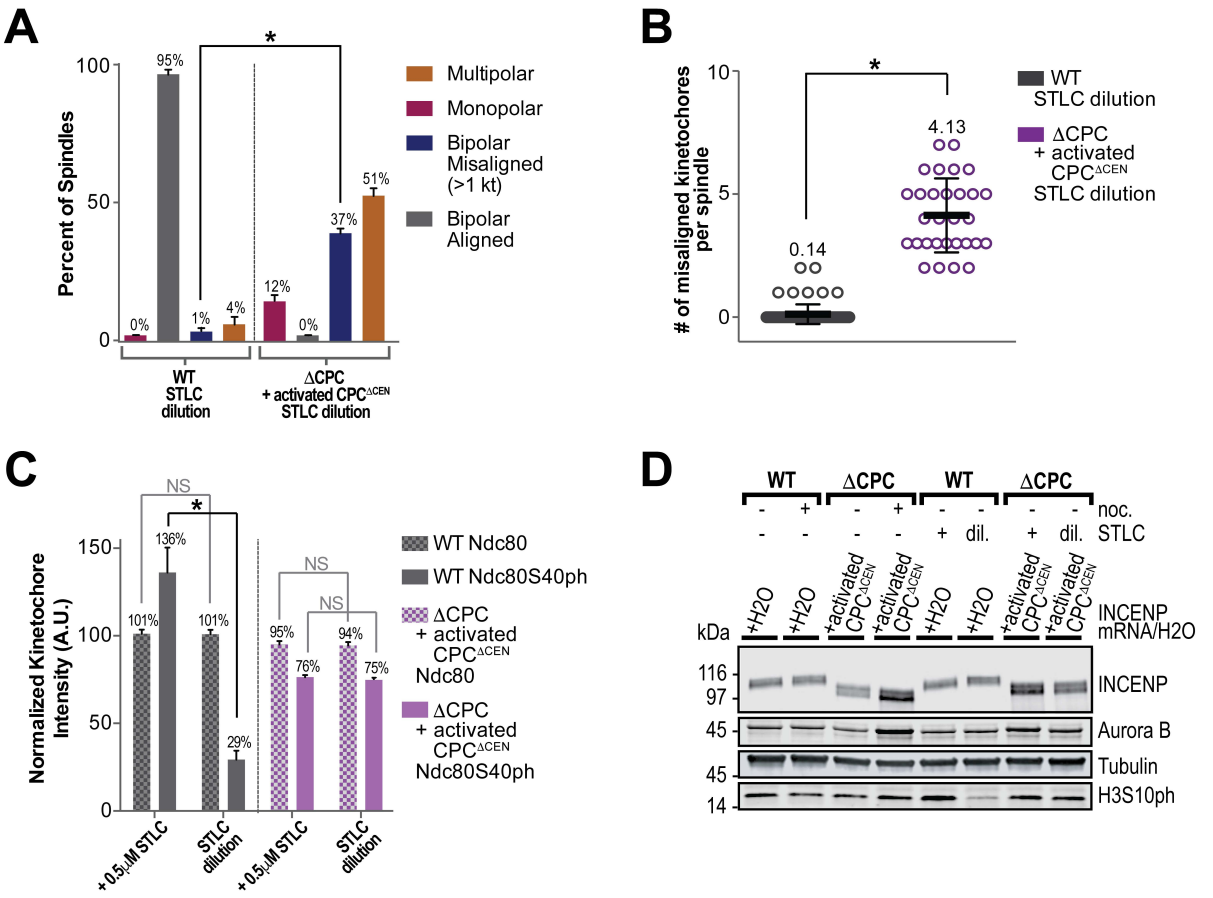


Figure S4. Error Correction in the Absence of Centromeric CPC; Related to Figure 3

(**A**) Mean percent of types of spindles observed after STLC dilution in WT and activated $CPC^{\Delta CEN}$ metaphase extracts. n = 75 spindles.

(**B**) Scatter plot of the number of misaligned kinetochores present in bipolar spindles in WT and activated $CPC^{\Delta CEN}$ metaphase extracts in Figure 3E. The mean number of misaligned kinetochores per spindle is shown above each data set. Error bars represent the SD of 3 independent experiments; n = 75 total spindle structures per condition.

(**C**) Mean integrated fluorescence intensities of Ndc80 and Ndc80S40ph in WT and activated $CPC^{\Delta CEN}$ metaphase extracts treated with STLC and after ten-fold dilution of STLC. All values are normalized to the nocodazole treated control samples shown in Figure 4A. Error bars represent the SEM of 3 independent experiments; n = 96 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$); NS indicates no significant difference.

(**D**) Western blot for the CPC components, Histone H3 phosphorylation (H3S10ph) and tubulin for samples in (A-C) and Figures 2D and 3D-G.

Figure S5

related to Figures 3 and 4

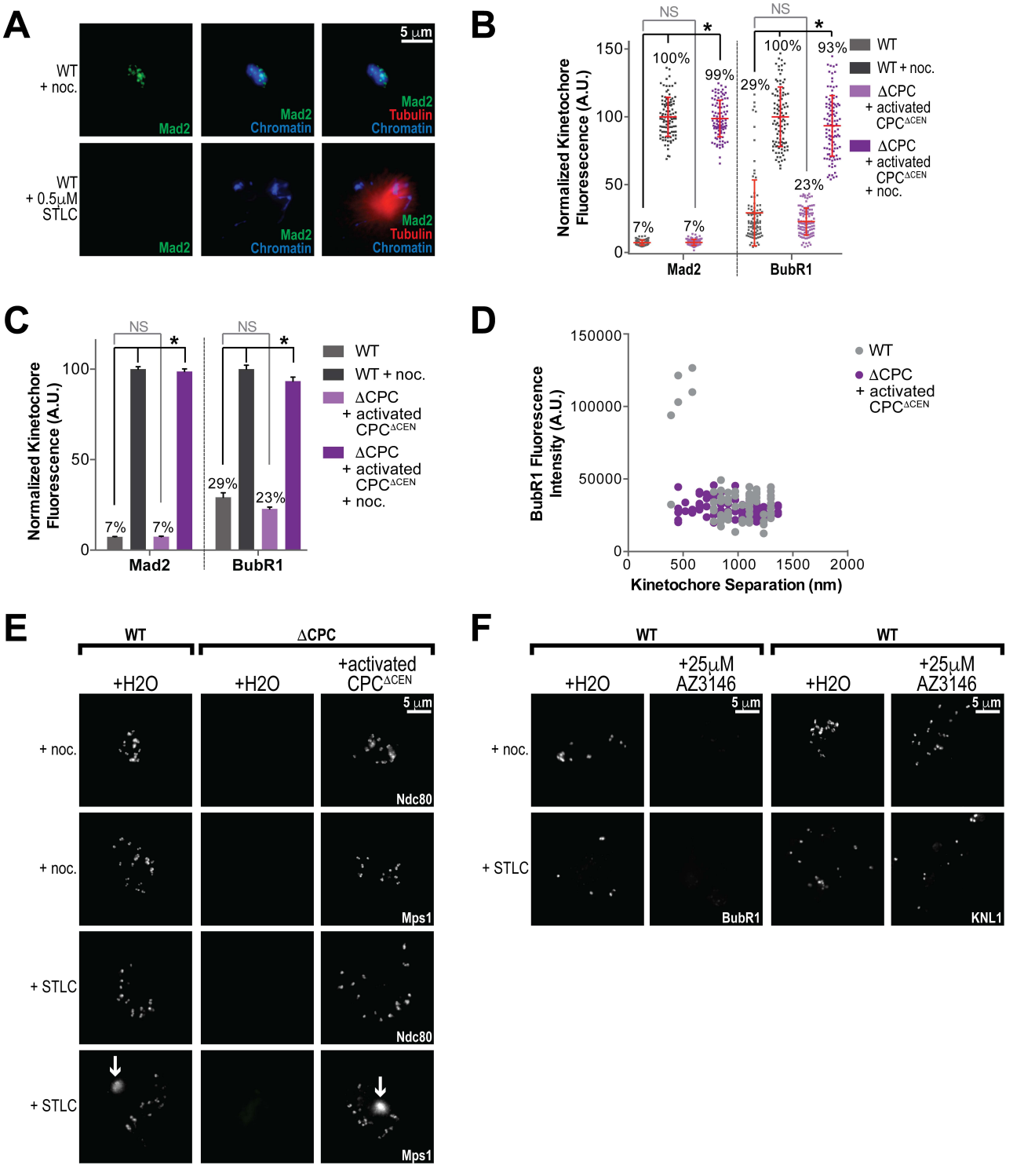


Figure S5. Centromeric CPC is Required for Mps1-dependent BubR1 Enrichment at Tensionless Attachments but not at Unattached Kinetochores; Related to Figures 3 and 4

(**A**) Representative IF images of metaphase spindles formed in WT extract in the presence of nocodazole or 0.5 μM STLC as indicated. Samples were stained for Mad2 (green).

(**B**) Scatter plots of integrated fluorescence intensities of Mad2 and BubR1 from WT and activated $CPC^{\Delta CEN}$ metaphase extracts +/- nocodazole. This is the raw data used to generate the graph in (C). The normalized intensity relative to the WT nocodazole treated condition is displayed above each data set. Error bars represent the SD of 3 independent experiments; n = 96 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$); NS indicates no significant difference.

(**C**) Mean integrated fluorescence intensities of checkpoint components from WT and activated $CPC^{\Delta CEN}$ metaphase extracts +/- nocodazole normalized to the WT condition treated with nocodazole. Error bars represent the SEM of 3 independent experiments; n = 96 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$); NS indicates no significant difference.

(**D**) Scatter plot of integrated BubR1 fluorescence intensity relative to sister kinetochore separation during metaphase in WT and activated $CPC^{\Delta CEN}$ extracts. Kinetochore separation is a proxy for tension across sister kinetochores. n = 100 kinetochores.

(**E**) Representative IF images of metaphase spindles formed in the presence of nocodazole or 0.5 μM STLC in WT and activated $CPC^{\Delta CEN}$ extracts. Arrows indicate staining of poles of monopolar spindles by Mps1 antibodies. Samples were stained for Ndc80 or Mps1 (white).

(**F**) Representative IF images of metaphase spindles formed in the presence of nocodazole or 0.5 μM STLC in WT extracts +/- 25 μM AZ3146 (Mps1 inhibitor). Samples were stained for BubR1 or KNL1 (white).

Figure S6

related to Figure 5

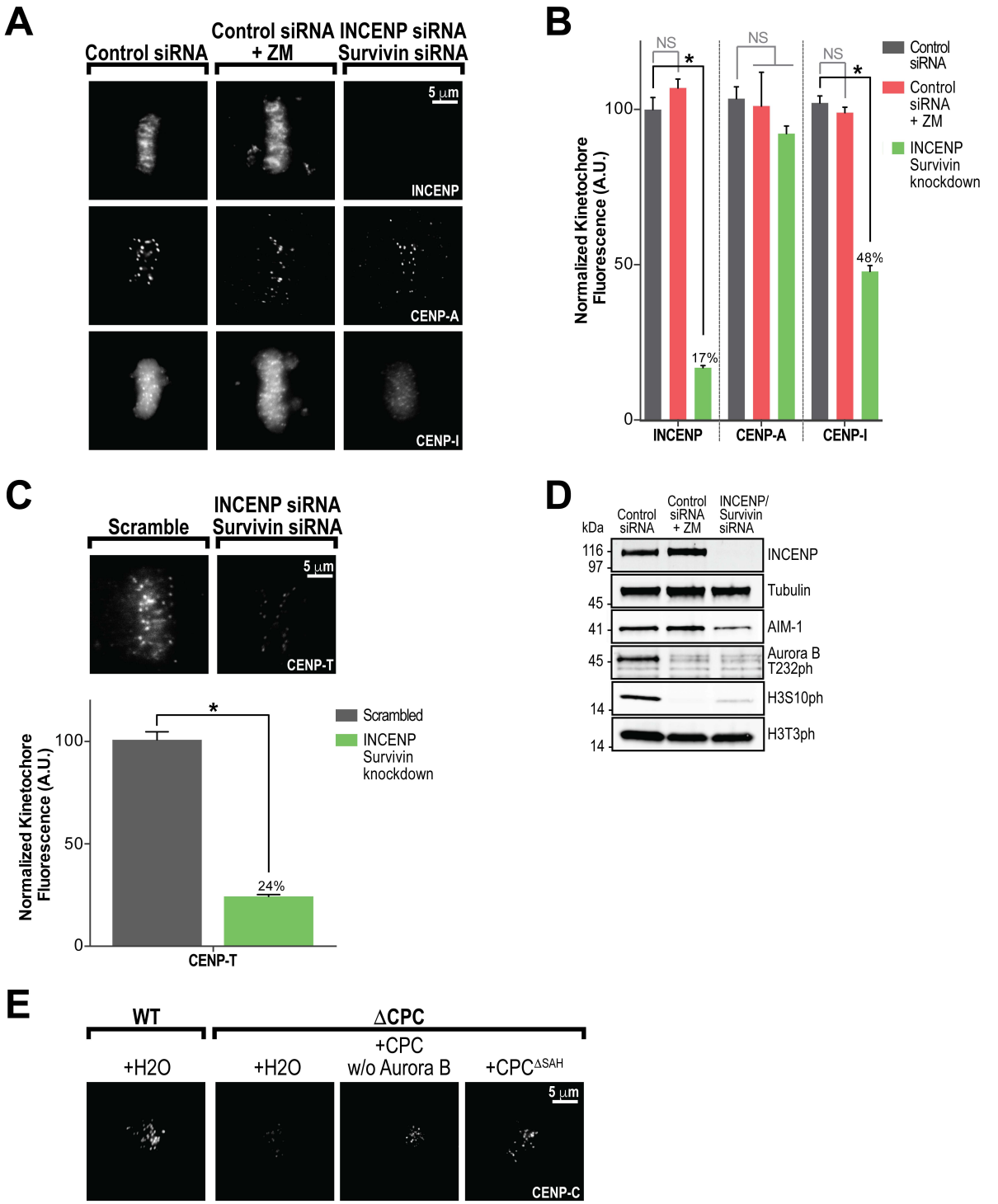


Figure S6. Proper Inner Kinetochore Composition Requires Centromeric CPC; Related to Figure 5

(**A**) Representative IF images of metaphase kinetochores in Hela cells treated with control siRNA, control siRNA + ZM447439 (Aurora B kinase inhibitor), or INCENP + Survivin siRNAs. Samples were stained for the indicated kinetochore components (white).

(**B**) Mean integrated fluorescence intensities of kinetochore components shown in (A) were normalized to the intensity of the control sample. Error bars represent the SEM of 3 independent experiments; n = 96 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$); NS indicates no significant difference.

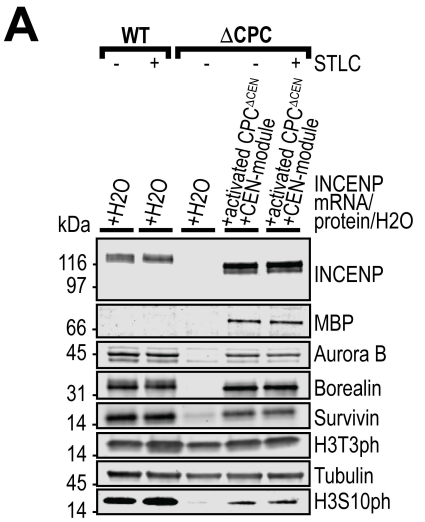
(**C**) Upper panel: Representative IF images of metaphase kinetochores in Hela cells treated with control siRNA or INCENP + Survivin siRNAs. Samples were stained for CENP-T. Lower panel: Mean integrated fluorescence intensities of CENP-T shown in upper panel were normalized to the intensity of the control sample. Error bars represent the SEM of 3 independent experiments; n = 96 kinetochores. Asterisk indicates a statistically significant difference ($P<0.0001$).

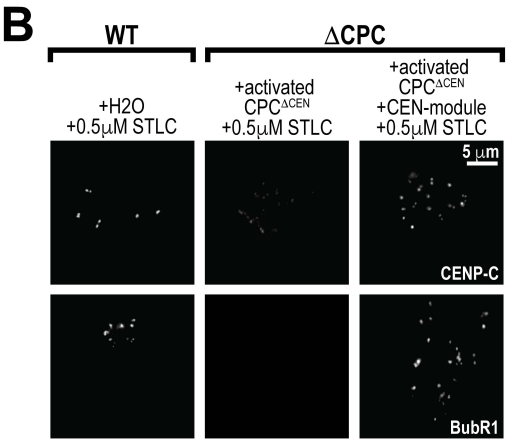
(**D**) Western blot for the CPC components, metaphase marker Histone H3 (H3T3ph), Histone H3 phosphorylation (H3S10ph), Aurora B T232 phosphorylation, and tubulin for samples shown in (A-D) above and those in Figure 5C-D.

(**E**) Representative IF images of replicated chromosomes in metaphase in Xenopus laevis WT, ΔCPC, CPC lacking Aurora B, and $CPC^{\Delta SAH}$ extracts. Spindles were stained for CENP-C (white).

Figure S7

related to Figure 7





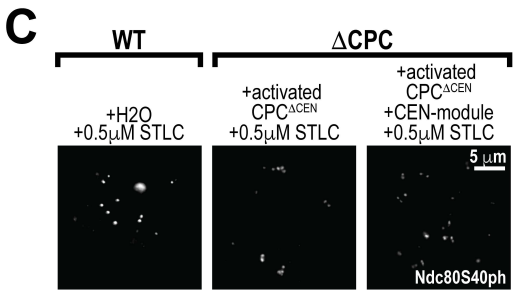


Figure S7. Inner Kinetochore Assembly and Aurora B Localization to Centromeres are Required for the Proper Response to Erroneous Attachments; Related to Figure 7

(**A**) Western blot for the CPC components, metaphase marker Histone H3 (H3T3ph), Histone H3 phosphorylation (H3S10ph) and tubulin for samples in (B-C) and Figure 7.

(**B**) Representative IF images of metaphase spindles formed in the presence of 0.5 μM STLC in WT, activated $CPC^{\Delta CEN}$, and activated $CPC^{\Delta CEN}$ + CEN-module extracts. Samples were stained for CENP-C or BubR1 (white).

(**C**) Representative IF images of metaphase spindles formed in the presence of 0.5 μM STLC in WT, activated $CPC^{\Delta CEN}$, and activated $CPC^{\Delta CEN}$ + CEN-module extracts. Samples were stained for Ndc80S40ph (white).