Moree et al., http://www.jcb.org/cgi/content/full/jcb.201106079/DC1

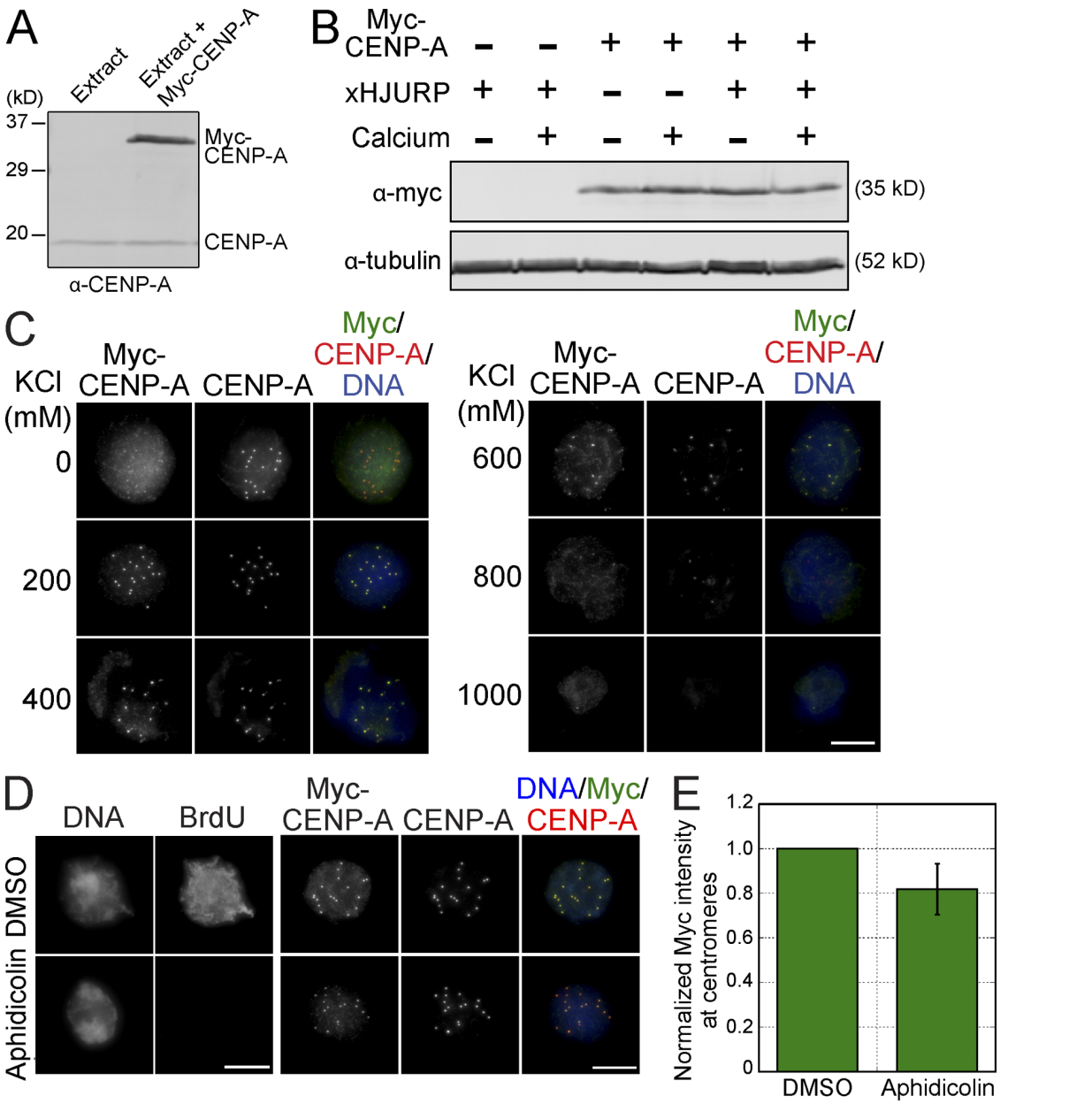

Figure S1. **Characterization of xHJURP-mediated CENP-A assembly assay in *Xenopus* egg extract.** (A) Representative Western blot from a CENP-A assembly assay showing relative levels of myc–CENP-A and endogenous CENP-A in bulk extract. The ratio of myc–CENP-A to CENP-A in this experiment was 4.4; the mean ratio across all experiments was 5. (B) Representative Western blot from a CENP-A assembly assay. Myc–CENP-A, xHJURP, or calcium addition is shown above each lane. (top) Anti-myc blot, which shows that myc–CENP-A protein levels in bulk extract were the same in all samples supplemented with myc–CENP-A RNA. Tubulin is shown as a loading control. (C) Myc–CENP-A is stably incorporated into sperm centromeric chromatin. Representative images from CENP-A assembly reactions were subjected to salt extraction before fixation. The concentration of KCl used for salt extraction is shown to the left of the images, and immunolocalized proteins are indicated above. $n = 3$. (D) Representative images from a CENP-A assembly assay performed in the presence of aphidicolin, a small molecular inhibitor of DNA polymerase. DMSO or aphidicolin addition is listed to the left of the images, and immunolocalized antigens are indicated above. Aphidicolin treatment prevented BrdU incorporated into chromatin, indicating that DNA replication has been blocked. (E) Quantification of myc–CENP-A fluorescence intensity at centromeres in the CENP-A assembly reactions described in D, which were normalized to levels in the DMSO-treated sample. Error bars show SEM; $n = 4$. Bars, 10 µm.

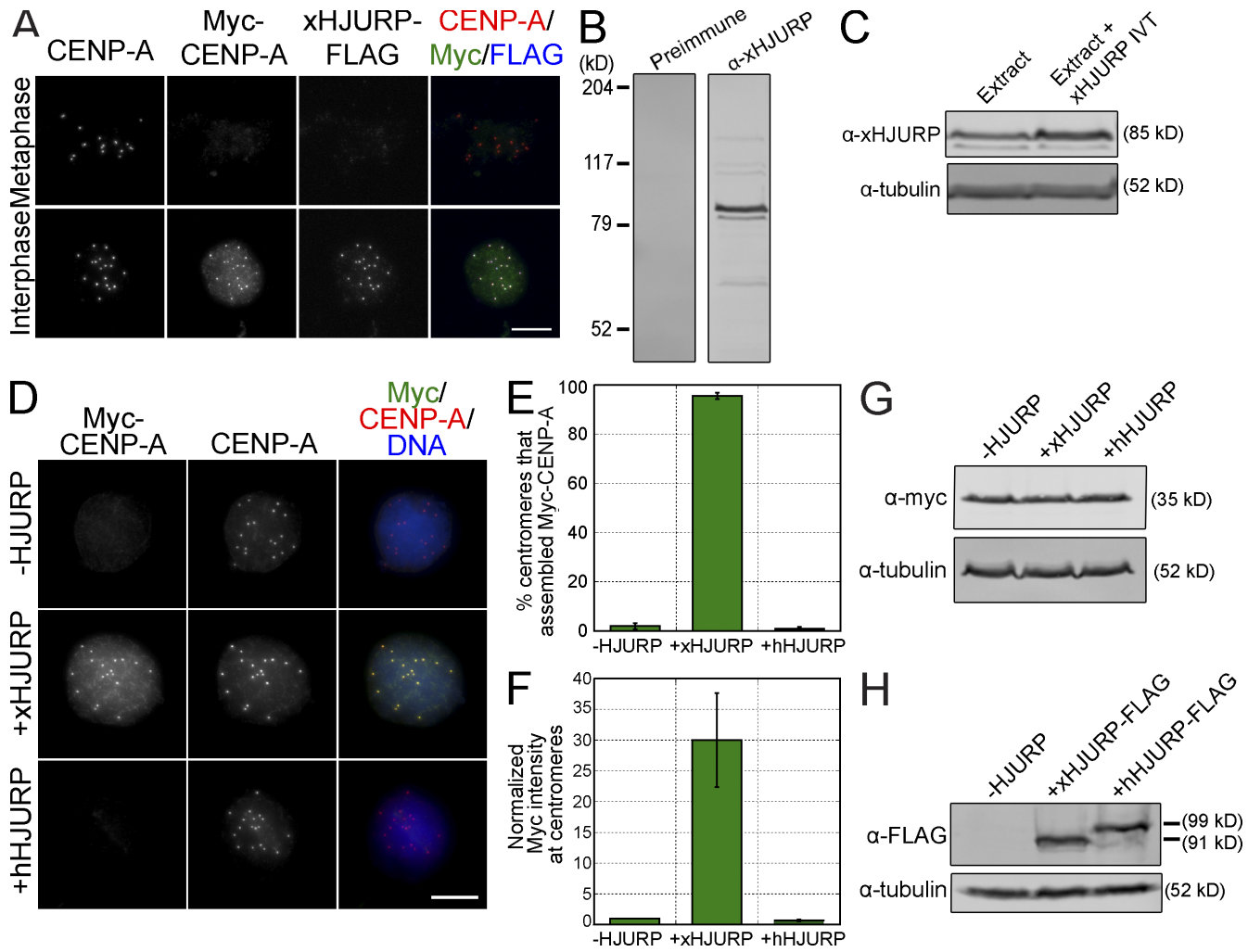

Figure S2. **xHJURP localizes to interphase centromeres and promotes CENP-A assembly.** (A) Exogenous xHJURP localizes to centromeres in interphase. Representative images from a CENP-A assay using FLAG epitope–tagged HJURP IVT protein. The cell cycle state of the extract is listed to the left of the images, and immunolocalized antigens are listed above. (B) Western blot of *Xenopus* egg extract with preimmune sera (Preimmune) or affinity-purified rabbit antibody raised against xHJURP (α-xHJURP). The calculated molecular mass for xHJURP is 85 kD. (C) Representative Western blot from a CENP-A assembly reaction showing relative amounts of xHJURP in extract with or without added xHJURP IVT. Addition of xHJURP led to a 1.6-fold increase in the total amount of xHJURP in extract. Tubulin is shown as a loading control. (D) Representative images from CENP-A assembly reactions without added HJURP, supplemented with xHJURP, or supplemented with hHJURP. HJURP addition is indicated to the left of the images, and immunolocalized proteins are indicated above. (E) Quantification of the frequency of myc–CENP-A assembly at centromeres for CENP-A assembly reactions described in D. (F) Quantification of myc–CENP-A fluorescence intensity at centromeres for CENP-A assembly described in D, which was normalized to the no-HJURP control sample. Quantification was performed as in C. (G) Representative Western blot from CENP-A assembly assay described in D. (top) Anti-myc blot, which shows that myc–CENP-A protein levels in bulk extract are the same across all samples. Tubulin is shown as a loading control. (H) Representative Western blot of extract without added HJURP, supplemented with xHJURP-FLAG, or supplemented with hHJURP-FLAG. (top) Anti-FLAG blot shows that equal amounts of xHJURP and hHJURP proteins are present in bulk extract when extracts are supplemented with the HJURP IVT protein. Tubulin is shown as a loading control. Error bars show SEM; $n = 3$. Bars, 10 µm.

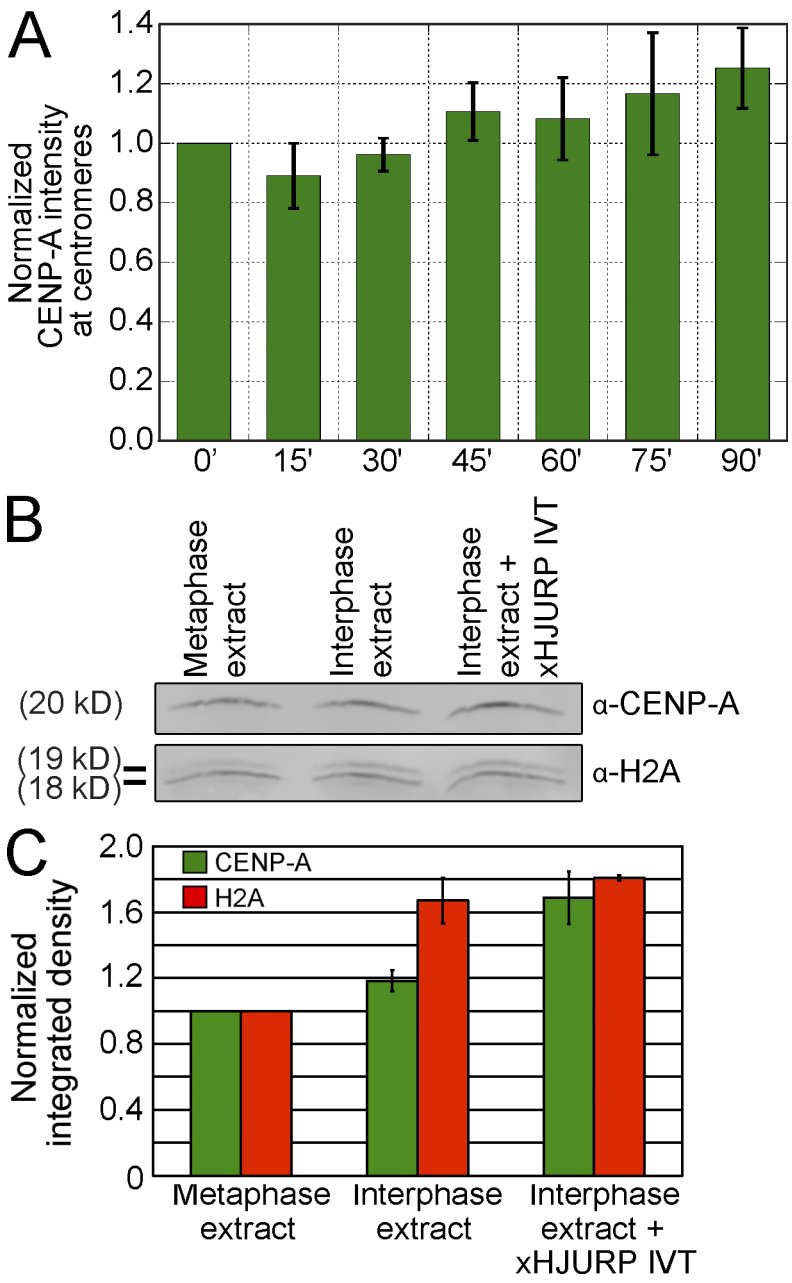

Figure S3. **xHJURP promotes the assembly of the endogenous CENP-A in *Xenopus* egg extracts.** (A) Quantification of total CENP-A levels at centromeres at various time points after release from metaphase arrest. CENP-A levels are normalized to levels in metaphase-arrested *Xenopus* egg extract (t = 0 min). Mean per pixel intensity at centromeres was quantified, and >100 centromeres were quantified per condition per experiment. Time is shown in minutes. (B) Representative Western blot of chromatin fractions from metaphase extract, interphase extract, and interphase extract supplemented with xHJURP IVT protein. Histone H2A is used as a loading control. The histone H2A antibody recognizes both histone H2A (bottom band; 18 kD) and an embryonic H2A variant, H2A.X-F (top band; 19 kD). In egg extract, H2A.X-F is incorporated into chromatin with approximately equal stoichiometry to H2A. (Shechter et al., 2009). (C) Quantification of CENP-A– and H2A-integrated densities under each condition, which were normalized to the levels in metaphase chromatin. For H2A quantification, the integrated densities of the H2A and H2A.X-F bands were summed. Error bars show SEM; $n = 3$

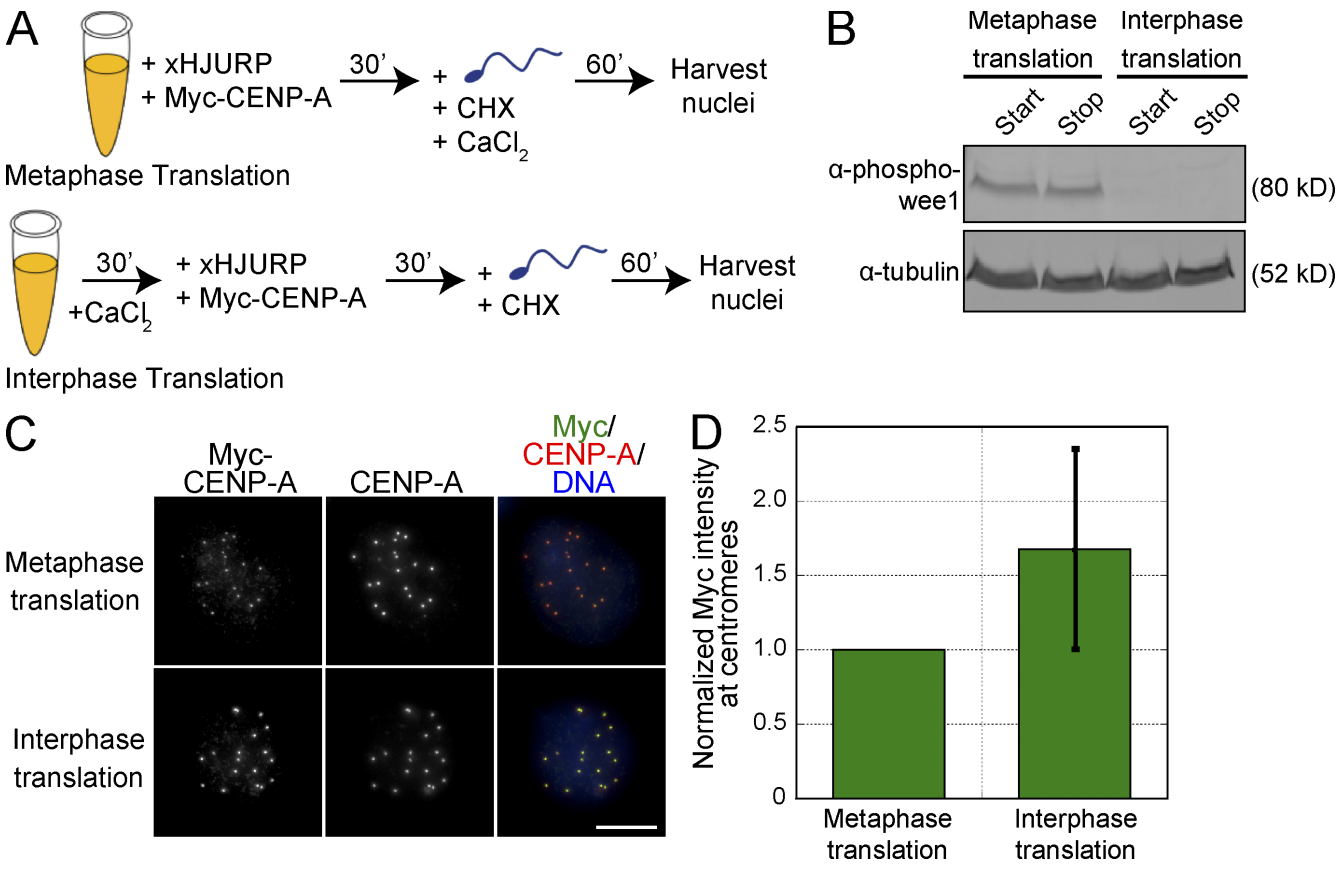

Figure S4. **xHJURP, myc–CENP-A, and the sperm chromatin template do not need to pass through mitosis for efficient myc–CENP-A assembly.** (A) Schematic of CENP-A assembly assay comparing the efficiency of assembly after myc–CENP-A translation in metaphase versus interphase extracts. (B) Representative Western blot from CENP-A assembly assays described in A. Samples were taken at the start and end of the 30-min myc–CENP-A translation period. Samples were blotted for phospho-wee1, a mitotic marker, to ensure that extracts maintained metaphase arrest throughout translation period (metaphase translation) or that extracts had entered interphase before initiation of translation (interphase translation). Tubulin is shown as a loading control. (C) Representative images from the CENP-A assembly assay described in A. Translation conditions are indicated to the left of the images, and the immunolocalized proteins are listed above. Bar, 10 µm. (D) Quantification of myc–CENP-A fluorescence intensity at centromeres in CENP-A assembly assays described in A, which were normalized to the metaphase translation sample. Error bars show SEM; *n* = 3.

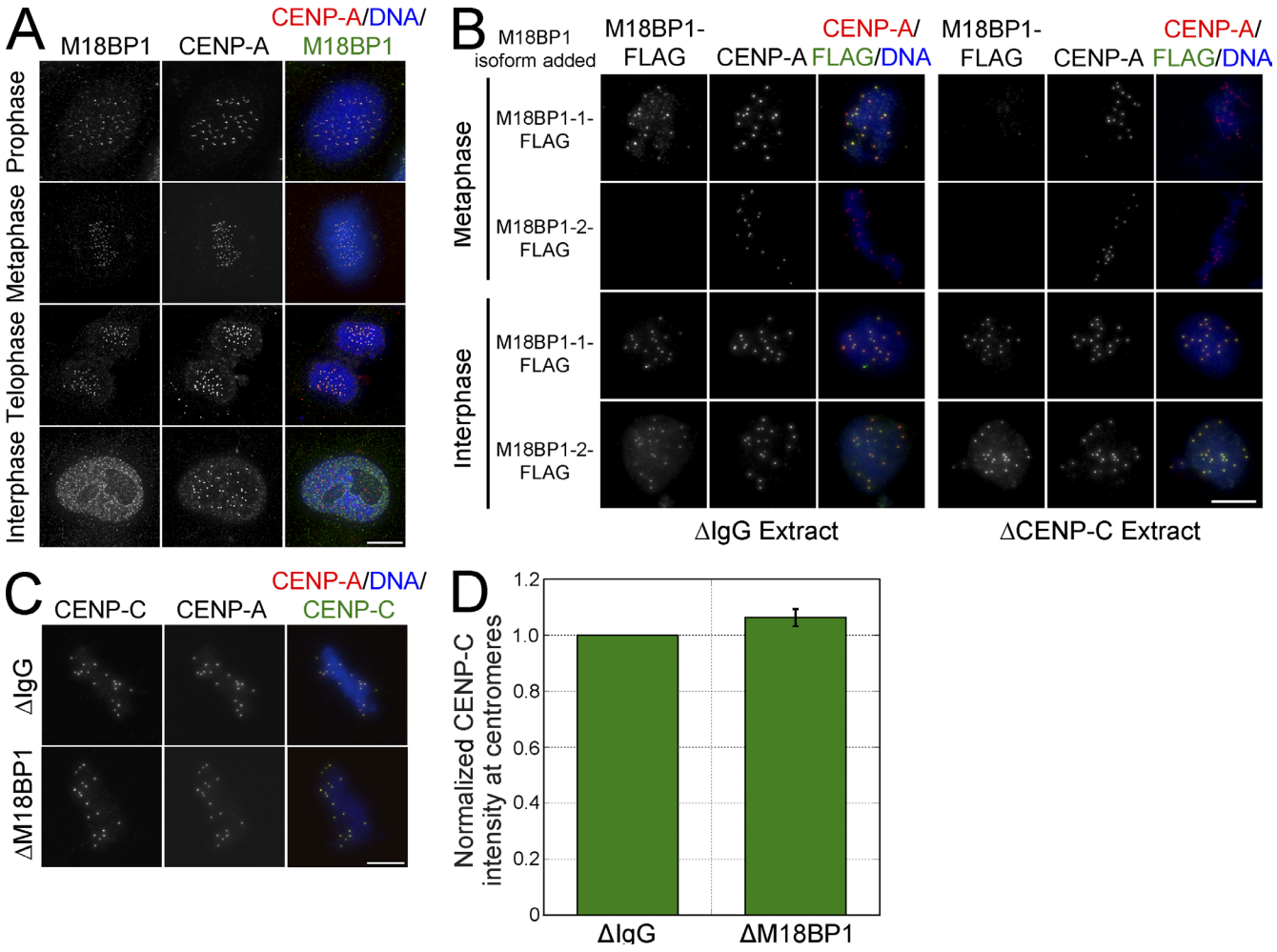

Figure S5. **Additional characterization of xM18BP1.** (A) xM18BP1 localizes to centromeres throughout mitosis in *Xenopus* S3 cells. The cell cycle phase is indicated to the left of the images, and immunolocalized antigens are indicated above. Images were deconvolved as described in Materials and methods. (B) CENP-C depletion inhibits targeting of M18BP1-1 to metaphase centromeres but does not affect targeting of either M18BP1 isoform to interphase centromeres. The cell cycle state of the extract and addition of FLAG epitope–tagged M18BP1 isoforms are indicated to the left of the images, depletion conditions are indicated below the images, and immunolocalized proteins are listed above the images. (C) M18BP1 depletion does not affect CENP-C assembly at centromeres. Depletion conditions are listed to the left of the images, and the immunolocalized proteins are listed above. (D) Quantification of CENP-C fluorescence intensity at centromeres after M18BP1 depletion, which were normalized to the levels in mock-depleted extracts. Quantification was performed as described in Fig. 1. Error bars show SEM; $n = 3$. Bars, 10 µm.

Reference

Shechter, D., R.K. Chitta, A. Xiao, J. Shabanowitz, D.F. Hunt, and C.D. Allis. 2009. A distinct H2A.X isoform is enriched in *Xenopus laevis* eggs and early embryos and is phosphorylated in the absence of a checkpoint. *Proc. Natl. Acad. Sci. USA*. 106:749–754. http://dx.doi.org/10.1073/pnas.0812207106