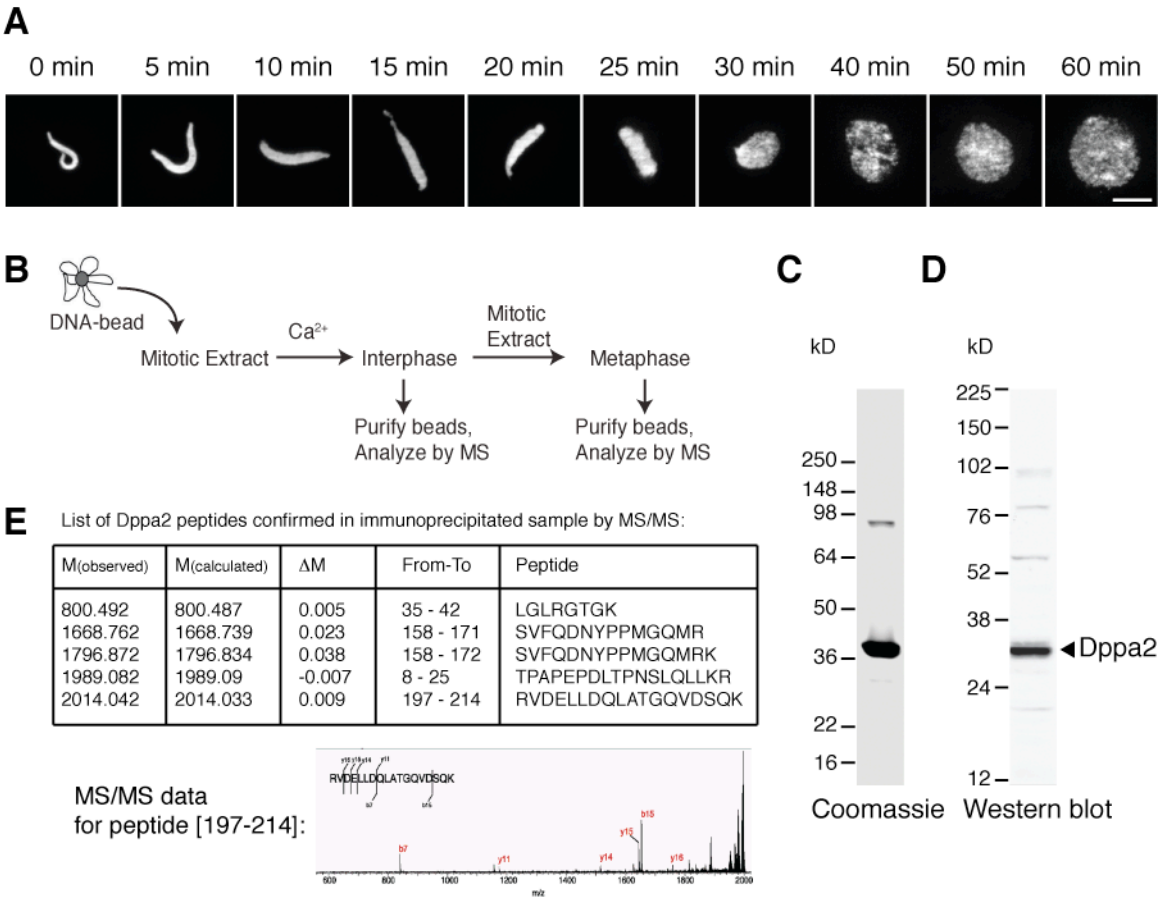

Figure S1 related to Figure 1
Identification of the novel chromatin-binding protein Dppa2 by mass spectrometry
(A) Pronuclear assembly in Xenopus egg extracts. Sperm nuclei were added to meiotic metaphase II-arrested extracts together with calcium to induce release into interphase and initiate pronuclear assembly. DNA was stained with Hoechst 33342. Scale bar, 10 µm.
(B) Identification of chromatin-binding proteins. DNA beads were purified from interphase and metaphase *Xenopus* egg extracts and analyzed by mass spectrometry (MS) to identify associated proteins.
(C) Purity of recombinant Dppa2 protein. GST-tagged Dppa2 was purified from *E. coli* and GST removed by Prescission protease cleavage, then subjected to SDS-PAGE and Coomassie staining.
(D) Anti-Dppa2 antibodies detect a single major species in *Xenopus* egg extracts. 0.5 µl CSF extract was subjected to Western blotting with anti-Dppa2 antibodies.
(E) Validation of anti-Dppa2 antibodies. Anti-Dppa2 antibodies were used to immunoprecipitate specifically recognized proteins from CSF extract, and eluted proteins analyzed by MS/MS. The table lists Dppa2 peptides confirmed by MS/MS, with MS/MS spectrum shown for one example.

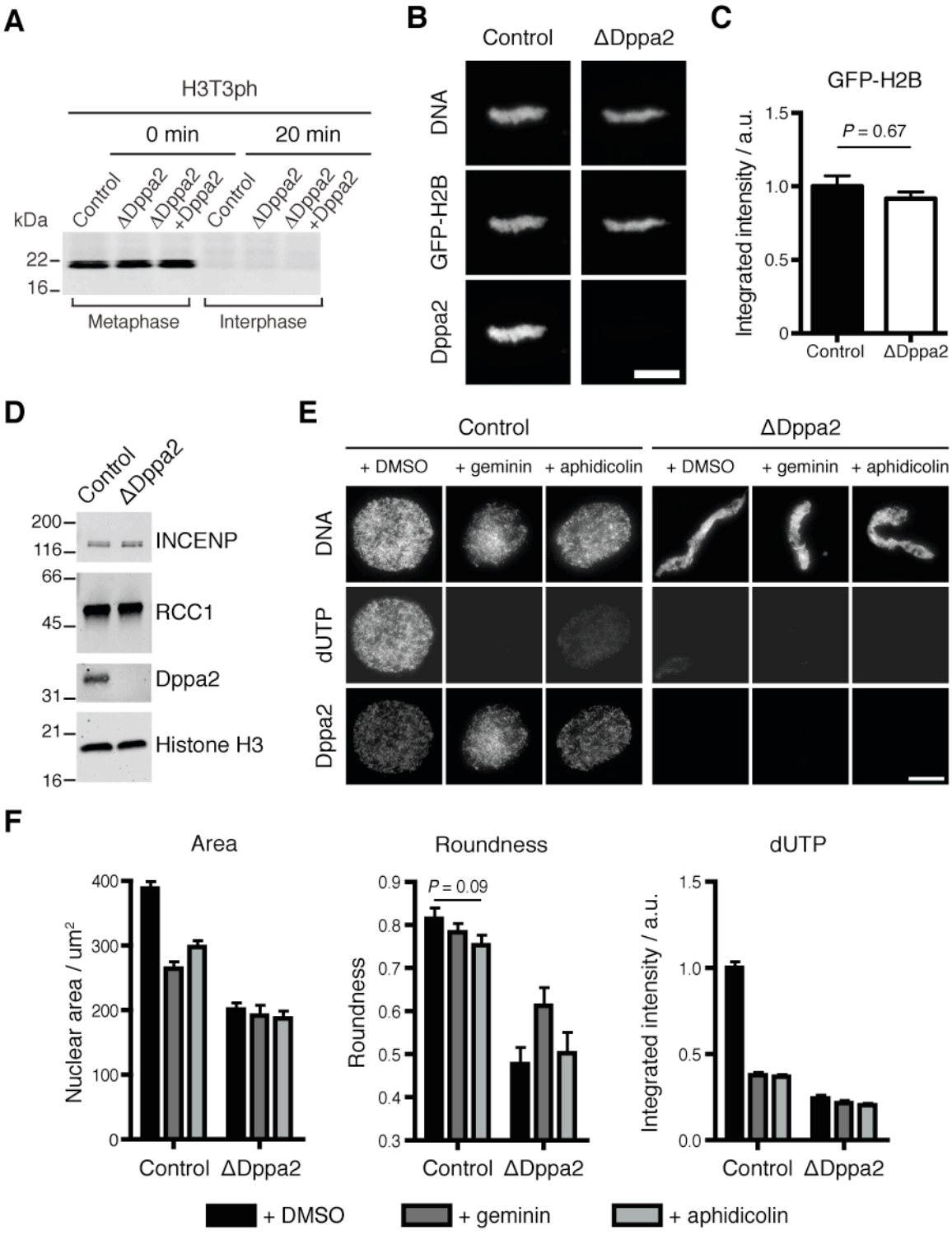

Figure S2 related to Figure 2
Cell cycle progression and sperm remodeling are not affected in ΔDppa2 extracts
Legend on next page

Figure S2 related to Figure 2

Cell cycle progression and sperm remodeling are not affected in ΔDppa2 extracts

(A) M phase-specific histone H3 threonine 3 phosphorylation (H3T3ph) is down-regulated with normal timing in ΔDppa2 extracts. Extract samples taken at 0 and 20 min after calcium addition were probed for H3T3ph by Western blotting.

(B) Exchange of sperm protamines for histone H2B is normal in ΔDppa2 extracts. Demembranated sperm were added to metaphase extracts containing GFP-H2B, and GFP-H2B incorporation visualized by immunofluorescence with anti-GFP antibodies.

(C) Quantification of GFP fluorescence intensity from (B). Bars indicate mean and standard error from > 60 nuclei; two-tailed unpaired *t*-test.

(D) Dppa2 depletion did not perturb chromosome binding of RCC1 or the CPC. Biotinylated sperm chromosomes were purified from control and ΔDppa2 extracts and probed by Western blotting for RCC1 and INCENP (a subunit of the CPC).

(E) Inhibition of DNA replication does not lead to nuclear morphology defects. Nuclei were assembled in control and ΔDppa2 extracts supplemented with 10 μM Cy3-dUTP and 8 μM recombinant non-degradable geminin or 40 μM aphidicolin. Nuclei were spun down at 60 min after calcium addition and stained with anti-Dppa2 antibodies and Hoechst 33342.

(F) Quantification of nuclei from (E). Each bar represents mean and standard error of > 20 nuclei. Inhibition of replication led to minor reduction of nuclear size but no significant reduction in nuclear roundness (comparing control extracts: DMSO vs. geminin, $P = 0.37$; DMSO vs. aphidicolin, $P = 0.09$ by two-tailed unpaired t-test).

Scale bars, 10 μm.

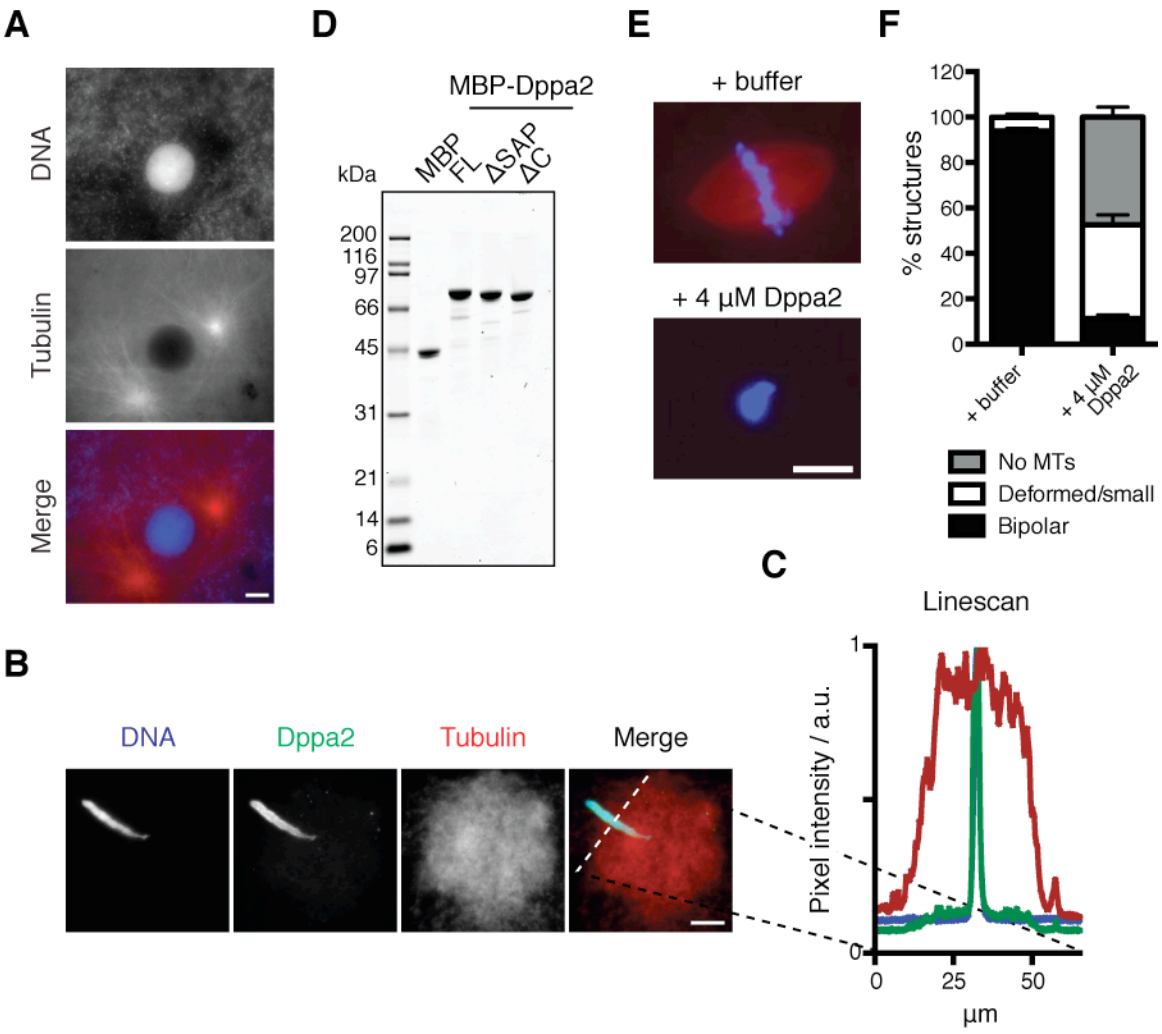

Figure S3 related to Figure 3
Dppa2 inhibits microtubule assembly at the metaphase-interphase transition
(A) Interphase microtubules are longer but less dense. Sperm were added together with calcium to metaphase extracts supplemented with rhodamine-labeled tubulin (red). Unlike in Figure 3A, these samples were not fixed with formaldehyde. Instead, 4 µl extracts containing sperm were squashed under a glass coverslip and incubated live at 20 °C. The above image was taken after 56 min, showing arrays of interphase microtubules which are less dense than those in Figure 3A and therefore difficult to visualize under the same fixation conditions.
(B) Dppa2 is localized to chromatin and not astral microtubules during early nuclear assembly. Sperm were added together with calcium to metaphase extracts supplemented with rhodamine-labeled tubulin (red) as in Figure 3A. Sperm nuclei and centrosomal asters were spun down after 12 min and stained with anti-Dppa2 antibodies (green) and Hoechst 33342 (blue).
(C) Linescan of fluorescence intensity across the indicated region in (B).
(D) Purity of recombinant MBP-Dppa2 proteins. Equimolar amounts of MBP and MBP-Dppa2 fusion proteins were analyzed by Coomassie brilliant blue staining.
(E) Untagged recombinant Dppa2 inhibits metaphase spindle assembly. Metaphase spindles were assembled in extracts supplemented with buffer or 4 µM recombinant Dppa2 protein and rhodamine-tubulin (red). DNA was stained with Hoechst 33342 (blue). Scale bar, 10 µm.
(F) Quantification of spindles in (C). Values shown are mean and standard error from 3 independent experiments, > 150 structures counted per sample per experiment.
Scale bars, 10 µm.

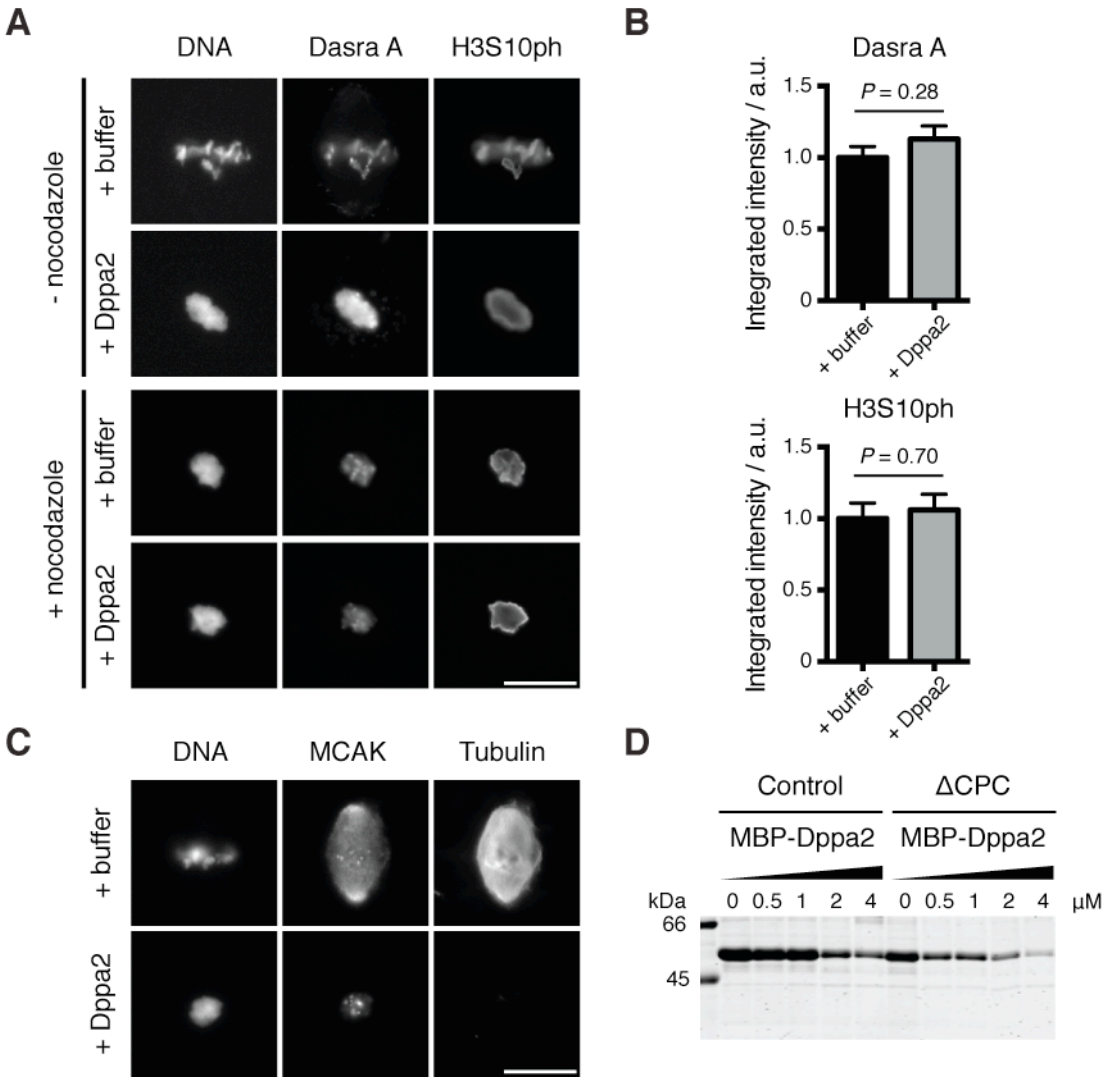

Figure S4 related to Figure 4

Dppa2 does not act through inhibiting the chromosomal passenger complex (CPC)

(A – C) Excess Dppa2 does not inhibit the CPC

(A) Metaphase spindles were assembled in extracts supplemented with buffer or 4 μM Dppa2 protein. Chromosomal structures were processed for immunofluorescence using antibodies against Dasra A (a subunit of the CPC) and phosphorylated histone H3 serine 10 (H3S10ph) to detect CPC localization and substrate phosphorylation. Excess Dppa2 abolished spindle formation, causing metaphase chromosomes to cluster. For fair comparison of immunofluorescence signals, samples were also treated with 16 μM nocodazole to cluster chromosomes independently of Dppa2. Scale bar, 20 μm.

(B) Quantification of Dasra A and H3S10ph on chromatin in nocodazole-treated samples from (A). Bars indicate mean and standard error from > 30 structures per sample and are representative of 3 independent experiments; two-tailed unpaired *t*-test.

(C) Experiment performed as in (A), but stained using antibodies against MCAK and tubulin. The punctate, centromeric localization of MCAK in control extracts is dependent on CPC activity and is retained in the presence of excess Dppa2. Scale bar, 20 μm.

(D) The CPC is dispensable for inhibition of microtubule assembly by Dppa2. Control or ΔCPC extracts were supplemented with MBP-Dppa2 and treated with 0.5 % DMSO to stimulate microtubule assembly. Microtubule polymer was recovered by pelleting and visualized by Coomassie stain.

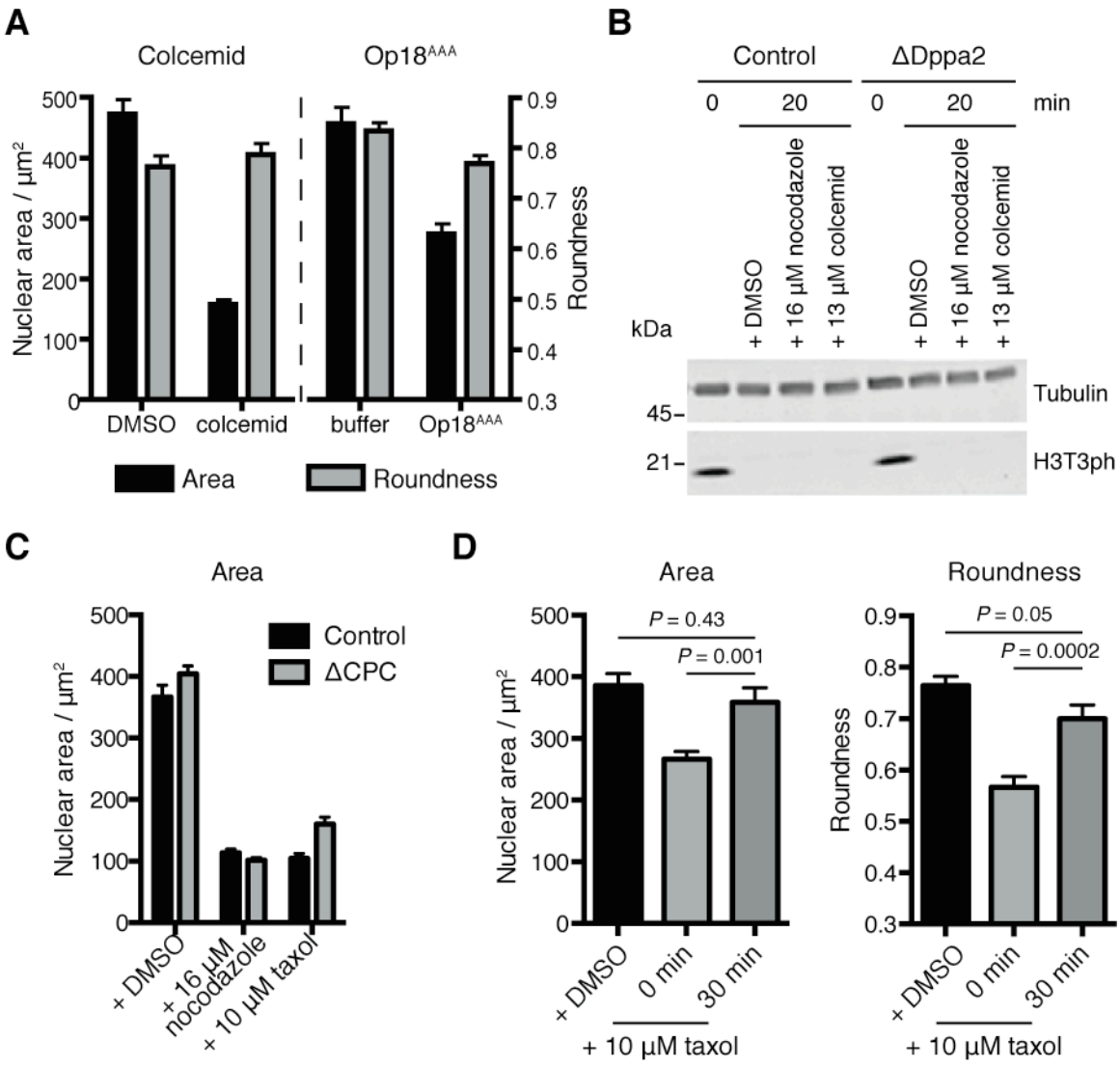

Figure S5 related to Figure 5

Microtubule poisons perturb nuclear formation during an early time window but do not activate the spindle assembly checkpoint (SAC)

(A) Depolymerizing microtubules with colcemid or Op18^{AAA} (Budde et al., 2001) reduces nuclear size but does not affect roundness. Nuclei were assembled in extracts containing 13 µM colcemid or 4 µM Op18^{AAA} and imaged at 60 min after calcium addition. Each bar represents mean and standard error of > 20 nuclei, nuclear area is shown on the left axis, roundness on the right.

(B) Microtubule depolymerization does not trigger the SAC during pronuclear assembly. Sperm were added to control and ΔDppa2 metaphase extracts together with the indicated drugs and calcium to release into interphase. Samples were taken at 0 and 20 min after calcium addition and probed for M phase-specific H3T3ph by Western blotting.

(C) Nocodazole and taxol treatment impair nuclear expansion in a SAC-independent manner. Sperm and calcium were added to control and ΔCPC metaphase extracts together with the indicated drugs. Nuclei were fixed at 60 min and stained with Hoechst 33342. Bars indicate mean and standard error from > 30 nuclei per sample.

(D) Inducing ectopic microtubules after 30 min does not perturb subsequent pronuclear assembly. Sperm and calcium were added to control metaphase extracts, and 10 µM taxol added at 0 min or after 30 min. Nuclei were fixed at 60 min and stained with Hoechst 33342. Bars represent mean and standard error from > 30 nuclei per sample; two-tailed unpaired t-test.

Supplemental Experimental Procedures

DNA beads

150 µg pBluescript SK+ plasmid DNA was digested with BamHI and NotI (New England Biolabs; NEB) and recovered by ethanol precipitation. Overhangs were filled in with the Klenow fragment of DNA polymerase I (NEB M0210) and 50 µM each of biotin-dATP, biotin-dUTP, thio-dCTP and dGTP in 70 µl volume for 2 h. DNA was cleaned up using NICK columns (GE Healthcare 17-0855-01). 3 µl magnetic streptavidin-coupled beads (Invitrogen 11206D) were used per µg DNA recovered. Beads were first washed 3 times with 400 µl bead buffer (10 mM Tris, 2 M NaCl, 1 mM EDTA, pH 7.6), then resuspended with biotinylated DNA in 1.5 ml total volume (50 mM Tris, 1 M NaCl, 2 mM EDTA, 2.5 % polyvinyl alcohol, pH 8). This mixture was incubated with rotation at room temperature for > 3 h, then washed 5 times with 400 µl bead buffer.

Chromatin bead purification

Metaphase chromatin was assembled as previously described (Heald et al., 1996) by incubating 1 µg DNA on beads, prepared as above, with 50 µl CSF egg extracts for 45 min at 22 °C. The metaphase chromatin beads were then collected on a magnet in the presence of 32 µM nocodazole, washed five times with 30SDB2+150 mM NaCl (10 mM Hepes, 150 mM NaCl, 10 mM β-glycerophosphate, 50 mM NaF, 20 mM EGTA, 2 mM EDTA, 0.5 mM spermin, 30% sucrose, 0.05 % Triton X-100, pH 8), and proteins were eluted with SDS-PAGE sample buffer. Interphase chromatin beads were produced by incubation of DNA beads in CSF extracts plus 0.3 mM $CaCl_2$ and 100 µg/ml cycloheximide for 80 min at 22 °C. To purify these beads, nuclear membranes were first disrupted by addition of 2.5 volumes of ice-cold DB2+Triton (10 mM Hepes, 10 mM β-glycerophosphate, 50 mM NaF, 20 mM EGTA, 2 mM EDTA, 0.5 mM spermin, 0.1% Triton X-100) to the extracts containing beads, and incubation for 10 min on ice. This mixture was layered on top of 400 µl of 60SDB2+Triton (10 mM Hepes, 10 mM β-glycerophosphate, 50 mM NaF, 20 mM EGTA, 2 mM EDTA, 0.5 mM spermin, 60% sucrose, 0.1% Triton X-100) and spun at 4000 g for 10 min at 4 °C in a centrifuge with a swinging bucket rotor. The sucrose interface was washed twice with DB2+Triton. The supernatant was removed, and the beads were collected on a magnet. The beads were then washed five times with DB2+Triton, and proteins were eluted with SDS-PAGE sample buffer.

Mass spectrometric identification of proteins

To identify proteins associating with interphase and metaphase chromatin beads, proteins were separated by SDS-PAGE and the entire gel lane was cut into 1 mm slices using a gel slicer (Brinkman Instruments). A comprehensive protocol for matrix-assisted laser desorption ionization (MALDI) sample preparation is available online at www.rockefeller.edu/labheads/chait/. Briefly, proteins were alkylated in-gel with 25 mM iodoacetamide, then digested with trypsin (Roche). Peptides were extracted from the gel slices with a slurry of 25 µg/µl POROS R2 20 reversed-phase resin (Applied Biosystems) in 5 % formic acid/0.2 % trifluoroacetic acid (TFA) at 4 °C for 6 h. The slurry was transferred to C18 Ziptips (Millipore) and washed with 0.1 % TFA. Peptides were eluted on a metal plate with saturated α-cyano-4-hydroxycinnamic acid in two parts water, one

part acetonitrile. MALDI-MS was performed with a prOTOF 2000 MALDI-time-of-flight (MALDI-TOF) mass spectrometer (Perkin-Elmer). Tandem mass spectrometry (MS/MS) was performed using a vMALDI-LTQ instrument (Thermo Scientific). The XProteo program (http://www.xproteo.com) was used for data analysis.

Recombinant MBP-Dppa2 fusion proteins

Protein expression constructs were transformed into Rosetta 2(DE3)pLysS *E. coli* (EMD Millipore 71403), and single colonies inoculated into 25 ml of protein expression medium (1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, 0.1 % NH_4Cl, 0.6 % Na_2HPO_4, 0.3 % KH_2PO_4, 1 mM $MgCl_2$, 0.4 % glycerol, 0.5 % glucose). These starter cultures were grown at 37 °C for 16 h, then transferred into 1 l fresh medium and grown to OD_{600} of 0.7 before induction with 0.3 mM IPTG. Cultures were grown for a further 5 h at 30 °C, then harvested and stored at -20 °C. Frozen pellets were resuspended in 15 ml wash buffer (20 mM HEPES, 200 mM NaCl, 1 mM DTT, pH 8) supplemented with Complete protease inhibitor cocktail (Roche 04693132001), 0.2 mg/ml lysozyme and 20 µg/ml DNase I and incubated at 4 °C for 30 min. The lysate was sonicated for 2 min at 35 % amplitude in 10 s pulses, and clarified by centrifugation at 46,000 rpm (*k*-factor 100) in a type 70 Ti rotor (Beckman 337922 rotor, 355618 tubes). Clarified lysates were filtered through a 0.45 µm syringe filter and applied to 2 ml amylose resin (NEB E8021). Binding was performed with rotation for 1 h at 4 °C. The resin was then collected in a 20 ml chromatography column (Bio-rad 732-1010), washed with 100 ml wash buffer and eluted in 10 × 1 ml fractions with wash buffer supplemented with 10 mM maltose.

Antibody-coated beads for immunodepletion

Magnetic protein A Dynabeads (Invitrogen 10008D) were washed 5 times with equal volumes of wash buffer (100 mM HEPES, 150 mM NaCl, pH 8), then resuspended in wash buffer and supplemented with 10 µg rabbit IgG or anti-Dppa2 antibodies. Beads were incubated with rotation at room temperature for 1 h, then moved to a fresh tube and washed 3 times. Prior to immunodepletion, beads were resuspended in sperm dilution buffer (5 mM HEPES, 100 mM KCl, 1 mM $MgCl_2$, 150 mM sucrose, pH 8).

Immunoprecipitation

Beads coated with anti-Dppa2 antibodies prepared as described above were cross-linked with dimethyl pimelimidate (DMP; Pierce 21667). 50 µl beads were resuspended in 0.5 ml wash buffer containing 30 mM DMP and incubated in darkness with rotation for 45 min at room temperature. The reaction was quenched with 50 µl 1 M Tris, pH 8, followed by 15 min further rotation. The beads were then recovered and resuspended in 0.5 ml wash buffer, plus 50 µl 1 M Tris, pH 8. After 10 min rotation, beads were washed 3 times with wash buffer and 2 times with sperm dilution buffer. These beads were incubated with 100 µl CSF extract for 30 min at 4 °C and then washed 5 times with 30SDB2+150 mM NaCl (10 mM Hepes, 150 mM NaCl, 10 mM β-glycerophosphate, 50 mM NaF, 20 mM EGTA, 2 mM EDTA, 0.5 mM spermin, 30% sucrose, 0.05 % Triton X-100, pH 8). Eluted proteins were digested with trypsin (7 h, 37 °C), and tryptic peptides collected using C18 resin (Millipore) and analyzed by MS/MS.

Sperm remodeling
Capped mRNA encoding GFP-H2B (gift of Christopher Jenness) was synthesized using the mMessage mMachine kit (Invitrogen AM1340). CSF-arrested extracts were supplemented with 150 μg/ml mRNA and cycled through interphase to metaphase for translation. Sperm nuclei were incubated in these extracts for 30 min and visualized by immunofluorescence using 2 μg/ml anti-GFP antibodies (Roche 11814460001). Biotinylated chromosomes were purified as described (Funabiki and Murray, 2000) and analyzed by Western blotting.

Microscopy equipment and settings
Images in Figures 1A and 2 were captured on an Olympus IX70 microscope with PlanApoN 60 ×/1.42 NA oil objective, Sedat quad colour filter (Chroma 89000) and Coolsnap HQ camera (Photometrics). Z stacks were acquired at 0.2 μm intervals and subjected to 10-pass iterative deconvolution using SoftWorx (Applied Precision). All other images were captured as single optical sections on a Zeiss Axioplan 2 microscope with Plan Neofluar 40 ×/0.75 NA air objective, 3 colour filters (Chroma 31000, 41001, 41004) and Coolsnaq HQ camera.

Statistical analysis
Quantitative microscopy data was obtained by measuring at least 30 individual structures per sample. Sample means were compared by two-tailed, unpaired heteroscedastic *t*-test with $\alpha = 0.05$.