# Supporting Information

## Chan et al. 10.1073/pnas.1108705109

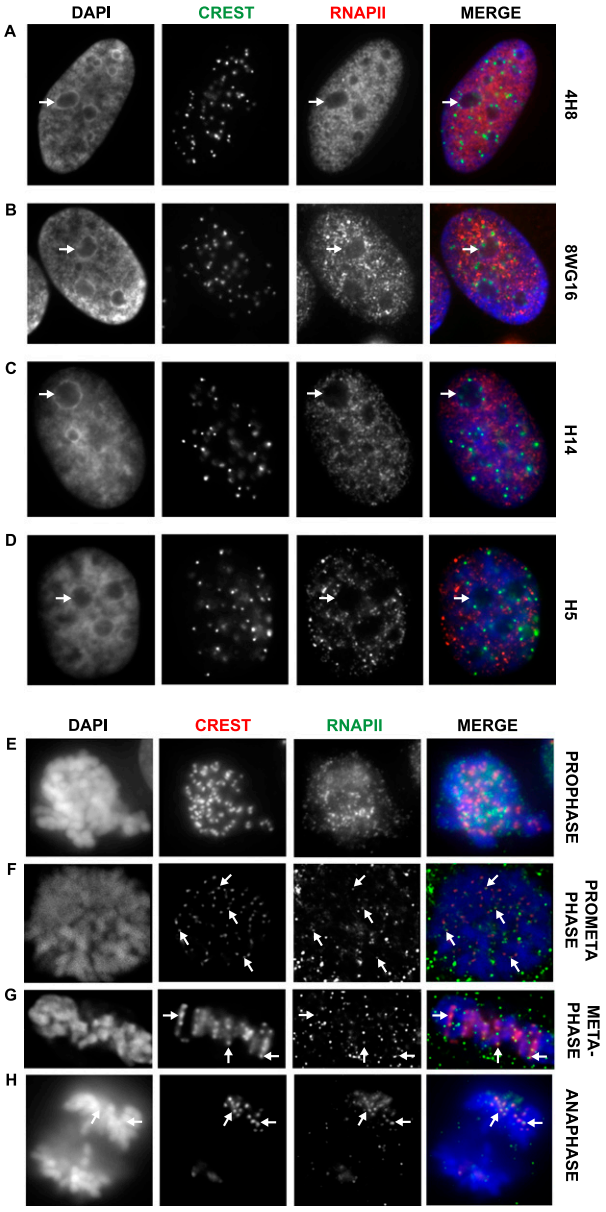


**Fig. S1.** RNA polymerase II (RNAPII) was not enriched specifically at interphase cells but was recruited to mitotic kinetochores as early as prometaphase. Anti-RNAPII antibodies (Table S1) were used to detect the presence of RNAPII at the centromeres of NIH 3T3 interphase cells. All four anti-RNAPII antibodies—$4H8_{\text{total RNAPII}}$ (*A*), $8WG16_{\text{unphosphorylated}}$ (*B*), $H14_{\text{phosphoSer5}}$ (*C*), and $H5_{\text{phosphoSer2}}$ (*D*)—showed strong nuclear staining that was excluded from the nucleolar regions (arrows in *A*–*D*). However, because of the overall intense nuclear staining, no significant enrichment or colocalization of anti-RNAPII with Calcinosis, Raynaud phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia (CREST) antisera immunostaining was observed. (*E*) A maximal image projection of a prophase 14ZBHT cell showing no specific enrichment of RNAPII ($H5_{\text{phosphoSer2}}$) at the kinetochores. (*F*) A single Z-stack image of a prometaphase 14ZBHT cell showing RNAPII ($H5_{\text{phosphoSer2}}$) localization at a subset of kinetochores (arrows). (*G*) A single Z-stack image of an aligned metaphase 14ZBHT cell showing RNAPII ($H5_{\text{phosphoSer2}}$) localization at the kinetochores (arrows). (*H*) A single Z-stack image of an anaphase 14ZBHT cell showing RNAPII ($H5_{\text{phosphoSer2}}$) localization at the kinetochores (arrows).

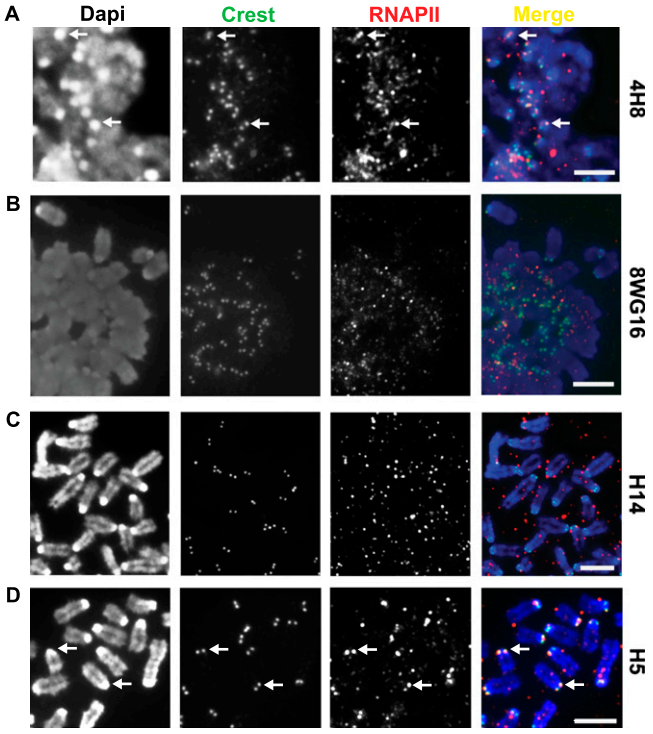


**Fig. S2.** RNAPII localizes to the mitotic kinetochore of mouse NIH 3T3 cells. (*A*) The colocalization of $4H8_{\text{total RNAPII}}$ and CREST signals (arrows) indicates the presence of RNAPII at the mitotic kinetochore in mouse NIH 3T3 cells. (*B*) $8WG16_{\text{unphosphorylated}}$ and (*C*) $H14_{\text{phosphoSer5}}$ do not show colocalization with CREST signals. (*D*) Colocalization of $H5_{\text{phosphoSer2}}$ with CREST signals on the mitotic kinetochore (arrows). This result, together with the immunofluorescence data for human cells (Fig. 1), suggests that the presence of RNAPII at the mitotic kinetochore is conserved in mammalian systems. (Scale bars: 5 μm.)

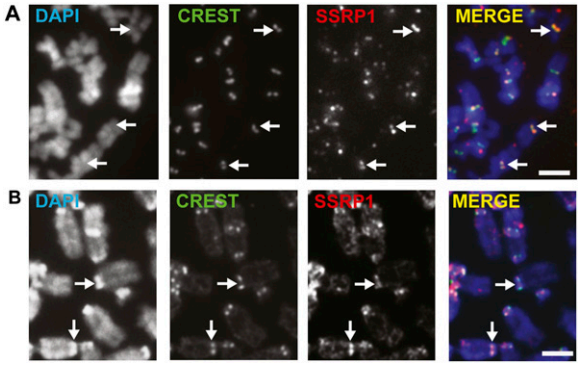


**Fig. S3.** RNAPII-associated transcription factor structure-specific recognition protein 1 (SSRP1) localizes to the mammalian mitotic kinetochore. (*A* and *B*) SSRP1 localized to the kinetochores of human HeLa and mouse NIH 3T3 mitotic cells, respectively, as shown by the costaining of SSRP1 and CREST (arrows). (Scale bars: 5 μm.)

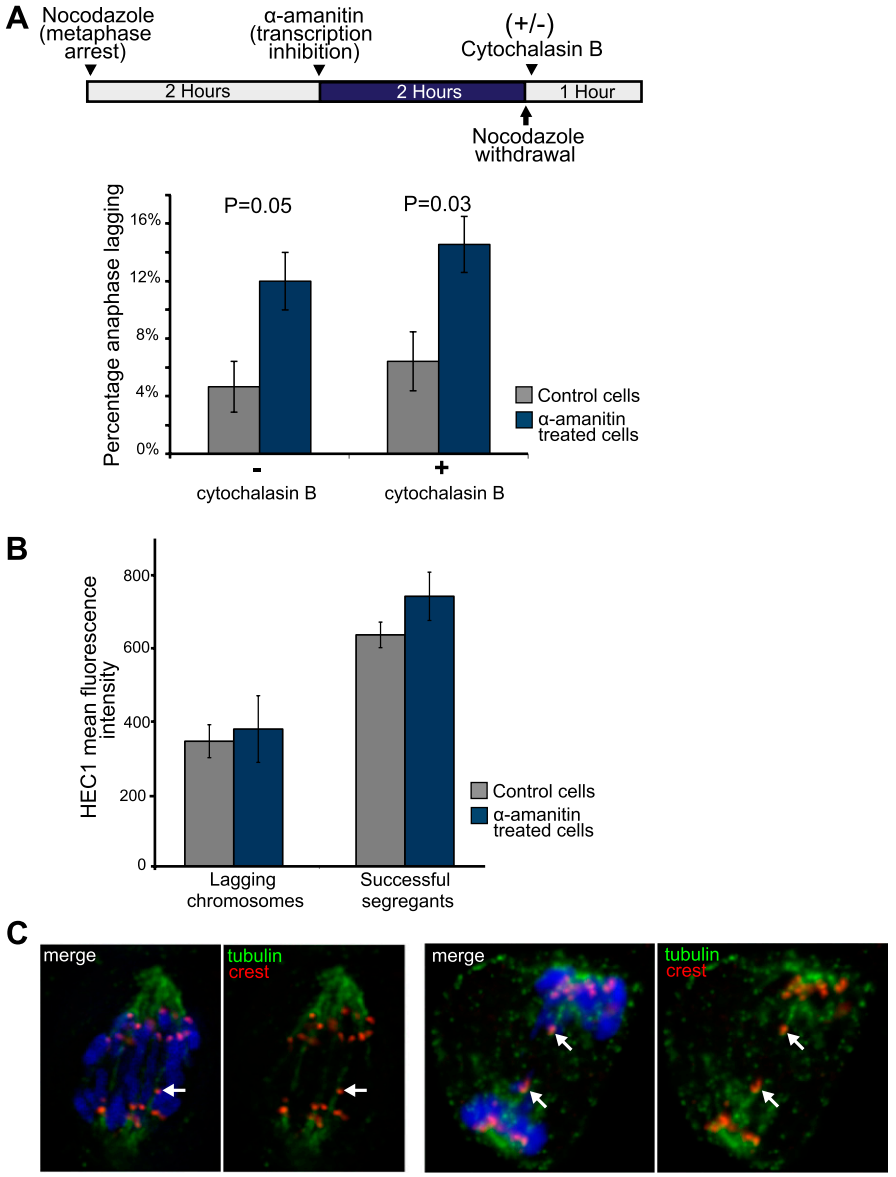


**Fig. S4.** RNAPII inhibition in mitotic cells causes an increase in anaphase-lagging cells, which do not show loss of the outer kinetochore protein HEC1. (*A*) An anaphase-lagging assay was used to detect kinetochore dysfunction in α-amanitin–treated ATCC CCL-171 cells (a minimally transformed normal lung fibroblast cell line with a normal, stable karyotype). ATCC CCL-171 cells were treated with nocodazole for 2 h to enrich for mitotic cells; then cells were treated with α-amanitin (50 μg/mL) or PBS for a further 2 h. Cells then were released from mitotic arrest and placed in fresh medium. In a separate experiment, cells were released from mitotic arrest into medium containing cytochalasin B to inhibit cytokinesis. In each case, 50 anaphase events were scored as normal or anaphase-lagging. α-Amanitin treatment caused a significant increase in anaphase-lagging cells, from 4.7% in control cells to 12% in α-amanitin–treated cells ($P = 0.05$; $n = 4$) without the use of cytochalasin B and from 6.4% in control cells to14.6% in α-amanitin–treated cells ($P = 0.03$; $n = 3$) when released into cytochalasin B medium. (*B*) The fluorescence intensities of the outer kinetochore protein NDC80 kinetochore complex component homolog (S. cerevisiae) (HEC1) at the kinetochores of anaphase-lagging cells and successfully segregated chromosomes of anaphase-lagging cells were measured. After α-amanitin inhibition, no change was detected in HEC1 mean fluorescence intensity at the kinetochores of the successfully segregated chromosomes and lagging chromosomes. (Data are based on measurements from three biological replicates). (*C*) Two examples of α-amanitin–treated anaphase-lagging ATCC CCL-171 cells are shown, with tubulin and CREST immunofluorescence. The images are of single Z-stack deconvolution images, showing clear microtubule–kinetochore attachment of the lagging chromosomes (arrows).

**Table S1. Monoclonal anti-RNAPII antibodies used in this study recognizing differentially phosphorylated populations of RNAPII**

| RNAPII antibody | Specific recognition |
|---|---|
| $4H8_{\text{total RNAPII}}$ | Total RNAPII regardless of phosphorylation status |
| $8WG16_{\text{unphosphorylated}}$ | Unphosphorylated RNAPII (promoter-bound RNAPII) |
| $H14_{\text{phosphoSer5}}$ | RNAPII-ser5 (transcription-initiation RNAPII) |
| $H5_{\text{phosphoSer2}}$ | RNAPII-ser2 (transcription-élongation RNAPII) |

**Table S2. Primer sequences used in quantitative PCR**

| | |
|---|---|
| α-satellite | Forward: CTCACAGAGTTGAACCTTCC |
| | Reverse: GAAGTTTCTGAGAATGCTTCTG |
| G protein-coupled receptor kinase 5 | Forward: AAGTTCCCTCACATTAGCCA |
| | Reverse: CTTCATATTCTGCCACGGAG |
| β-Actin | Forward: GTGGCCTTGGAGTGTGTA |
| | Reverse: GTCAGGATCTTCATGAGGTAGTC |
| GAPDH | Forward: CGAGATCCCTCCAAAATCAA |
| | Reverse: TTCACACCCATGACGAACAT |
| Hypoxanthine phosphoribosyltransferase | Forward: CTGAAGAGCTATTGTAATGACCAG |
| | Reverse: CATCTTTGGATTATACTGCCTGAC |