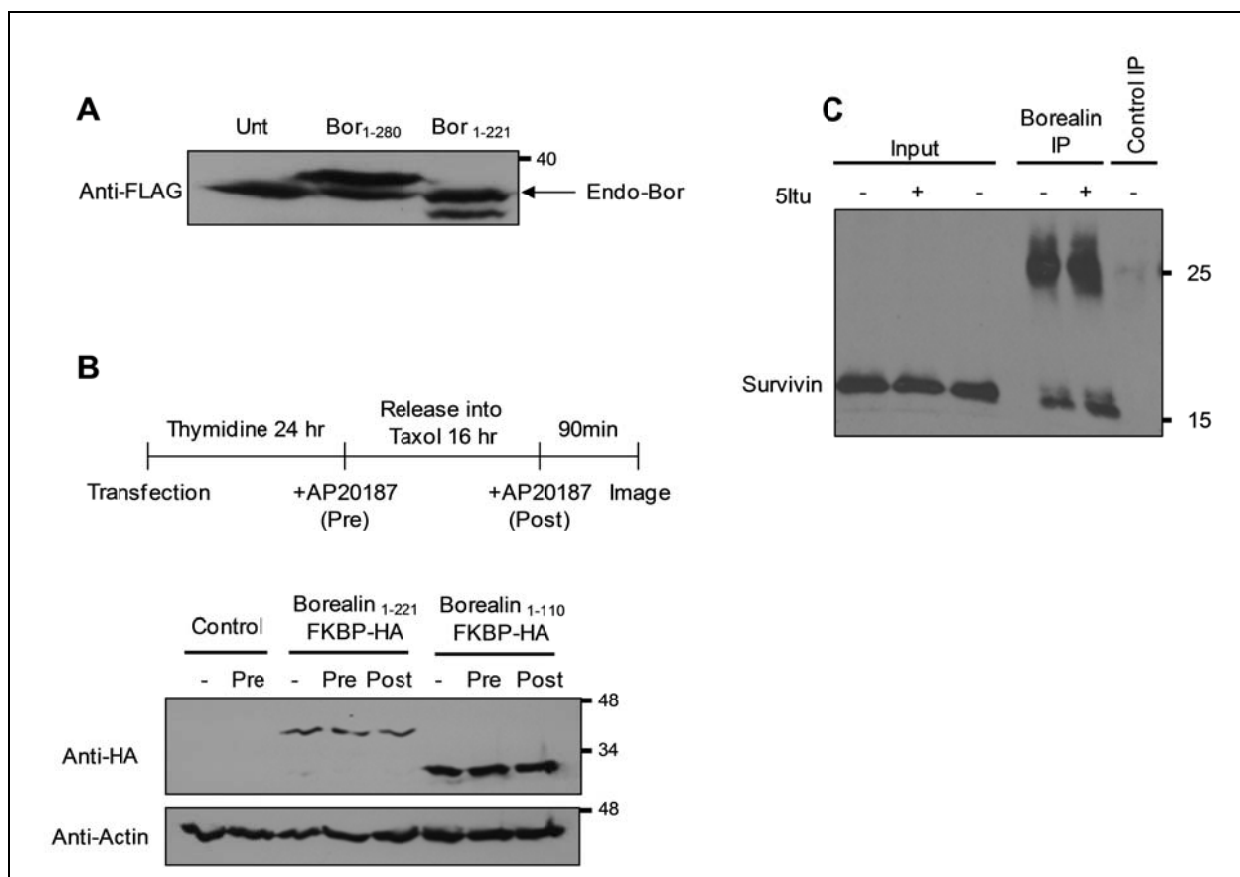
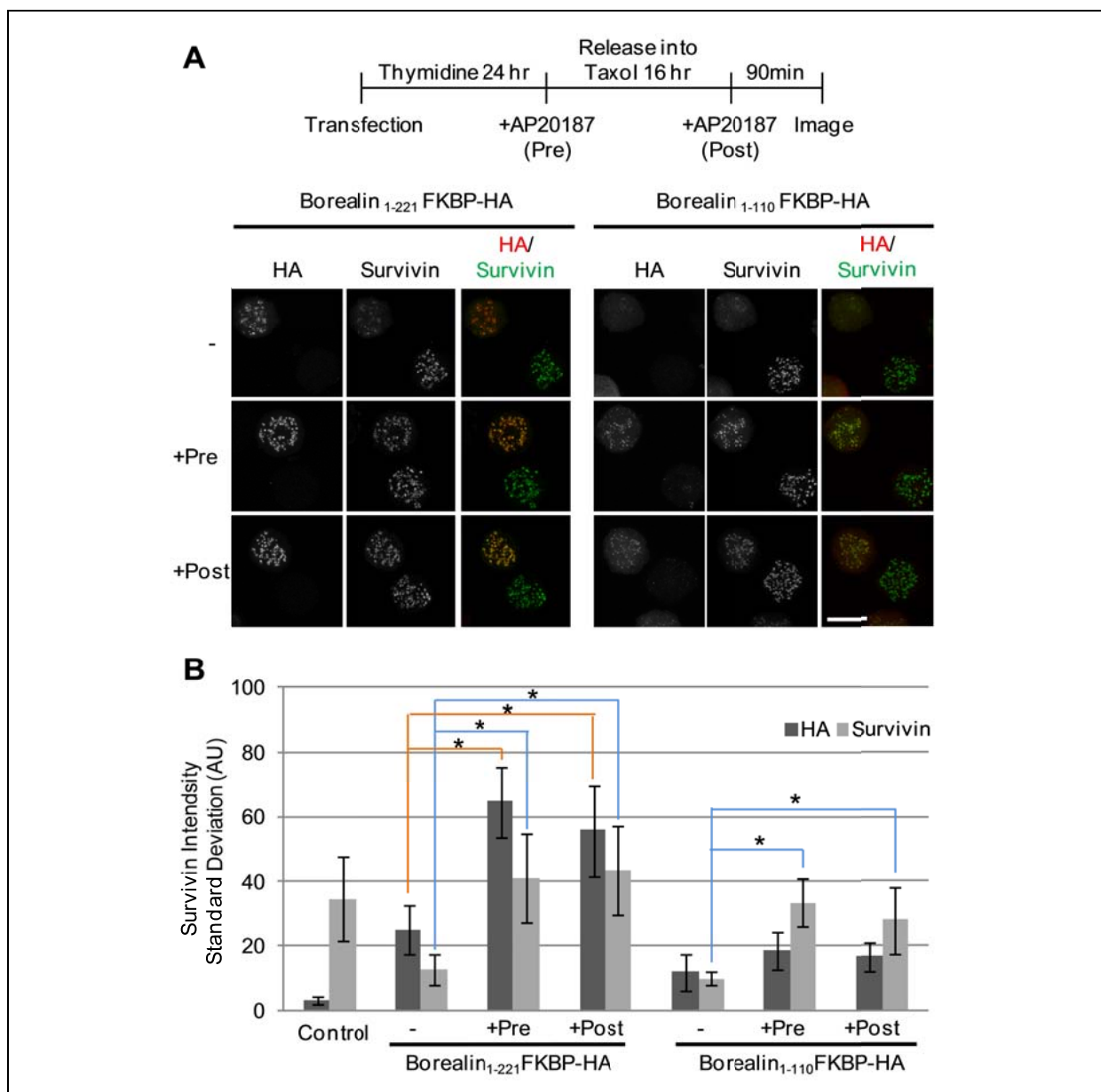


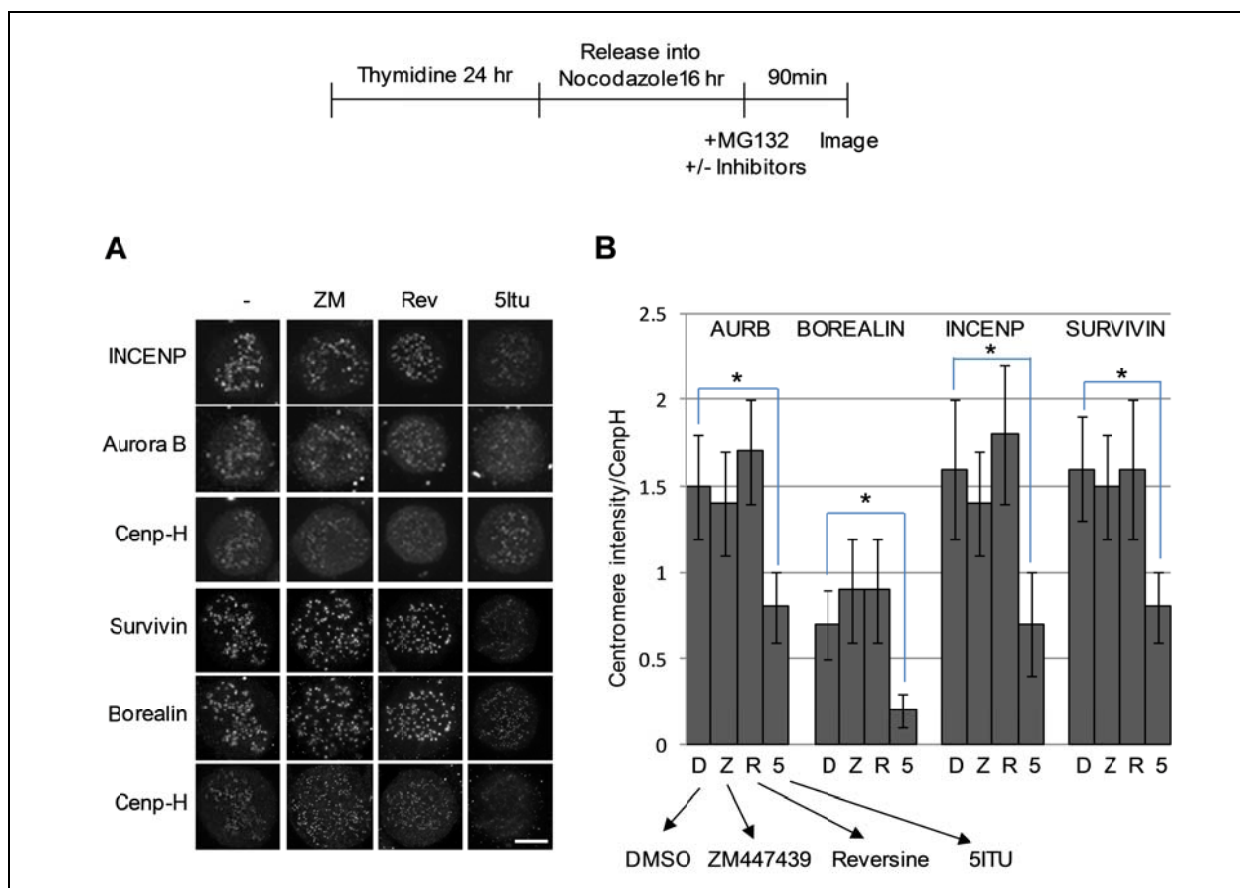
Supplementary Figure 1. Localization of truncated Borealin co-transfected with Borealin shRNA. HeLa M cells were transfected with empty pSUPER or a vector producing a shRNA targeting the 3'UTR (missing in the Borealin-FLAG constructs). After transfection, cells were released from a thymidine block into nocodazole. Immunofluorescence was carried out to detect either endogenous Borealin using a polyclonal serum or the transfected versions using anti-FLAG antibodies. Examples of confocal images are shown. Both scale bars represent 10 μ m.



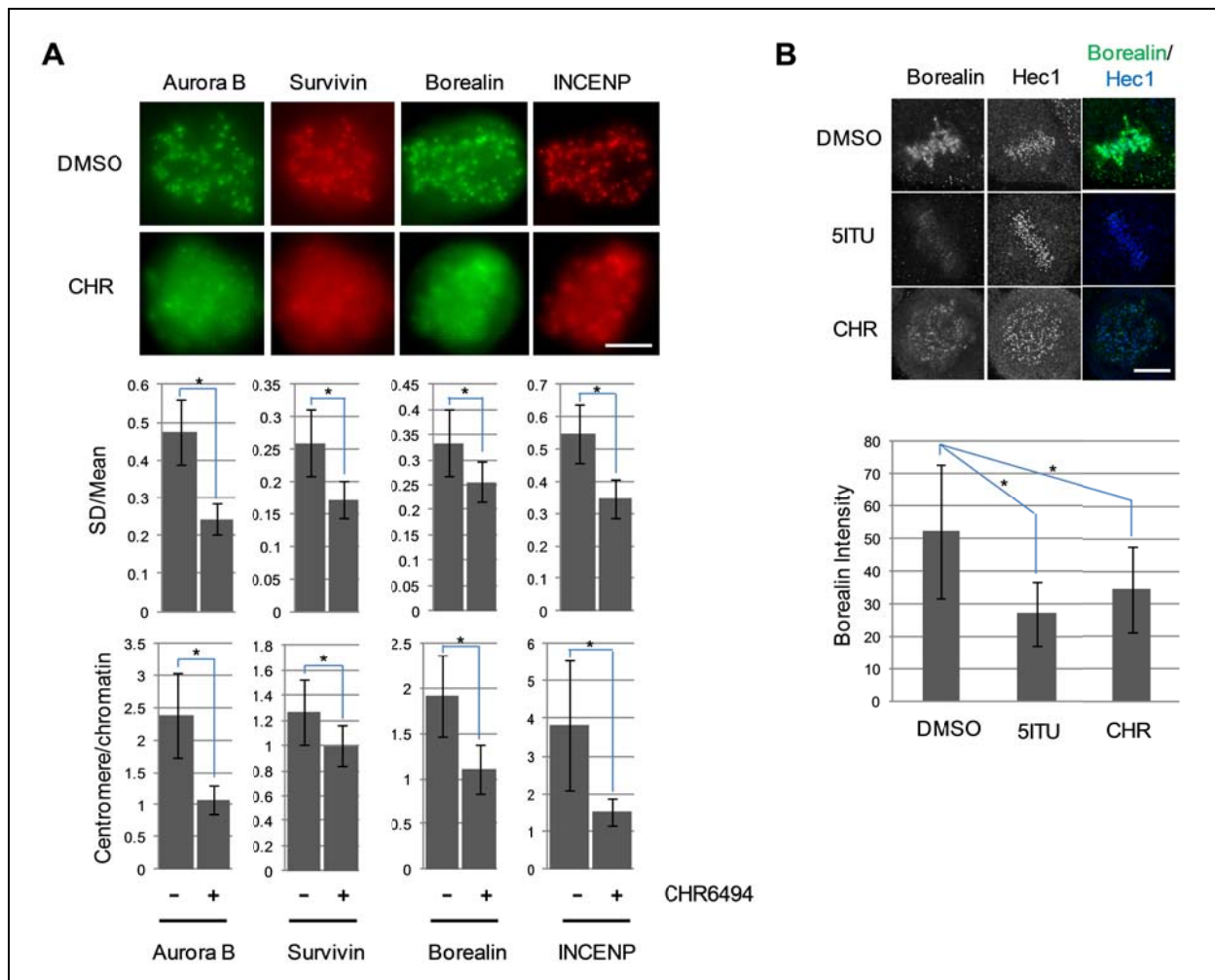
Supplementary Figure 2. Western analysis of Borealin and Survivin. A.) HeLa M cells were transfected with either Borealin₁₋₂₈₀-FLAG or Borealin₁₋₂₂₁-FLAG. Cell lysates were analyzed by western blotting with a polyclonal Borealin antiserum to detect endogenous and transfected proteins in the same lane. Borealin₁₋₂₈₀-FLAG is expressed at ~130% of the endogenous protein, while Borealin₁₋₂₂₁-FLAG is expressed ~60 of the endogenous protein. Both Borealin₁₋₂₈₀-FLAG and Borealin₁₋₂₂₁-FLAG were detected at similar levels using antibodies to the FLAG tag (Figure 1H). Our polyclonal serum may recognize epitopes in the deleted region resulting in lower detection levels compared to FLAG westerns (Fig. 1H). Also, smaller truncations were difficult to detect with the polyclonal serum possibly for the same reason. B.) Dimerization does not affect steady state levels of Borealin. The indicated Borealin-FKBP fusions were expressed in HeLa M cells which were synchronized and treated as shown in the schematic. After AP20187 treatment, cells were harvested and lysates were separated by SDS-PAGE. Proteins were transferred to PVDF membranes and HA-tagged FKBP fusions were detected using an antibody to HA. Actin serves as a loading control. C.) 5Itu does not disrupt the Borealin-Survivin interaction. HeLa M cells were released from a thymidine block in the presence of nocodazole. 5Itu was added 16 hours after thymidine release and cells harvested 90 minutes later. Cell lysates were immunoprecipitated with a polyclonal antibody to Borealin or beads alone (Control IP). The western blot was detected with an antibody to Survivin.



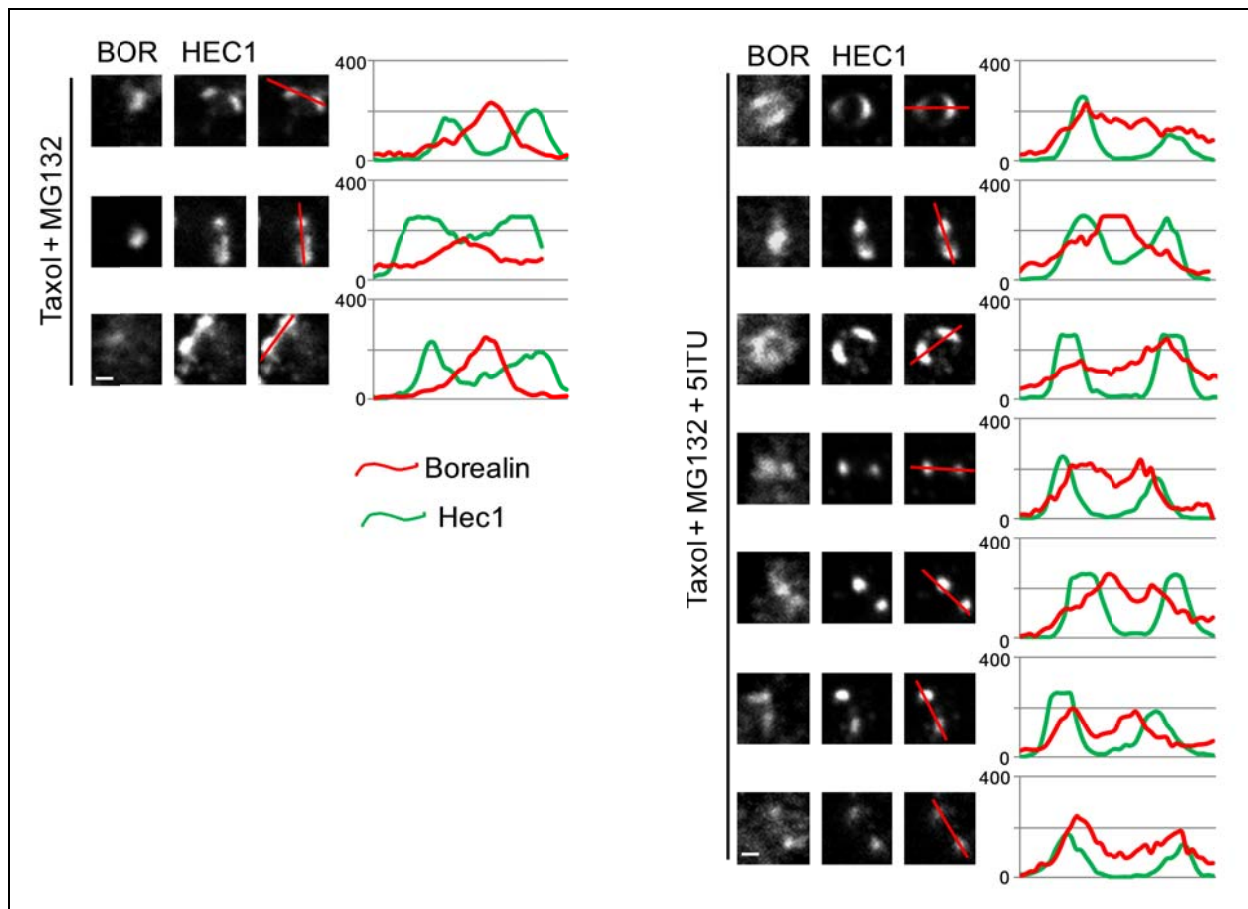
Supplementary Figure 3. Effect of inducing Borealin dimerization before or after mitotic entry on CPC abundance at centromeres A.) Schematic of experimental design and maximum intensity projections of taxol-arrested HeLa M cells expressing HA-tagged Borealin-FKBP fusion proteins in the absence (-) or presence (+PRE, +POST) of the homo-dimerizing agent AP20187. +PRE indicates dimerization prior to mitotic entry, while +POST indicates dimerization following mitotic entry. Immunofluorescence was performed using antibodies against HA and Survivin (scale bar = 20 μ m). B.) Whole cell standard deviation of HA and Survivin signal intensity as a measure of signal heterogeneity and centromere localization from A. More than 30 cells were measured for each condition. Bars represent standard deviation. * $p < 0.05$ using a student's t-test.



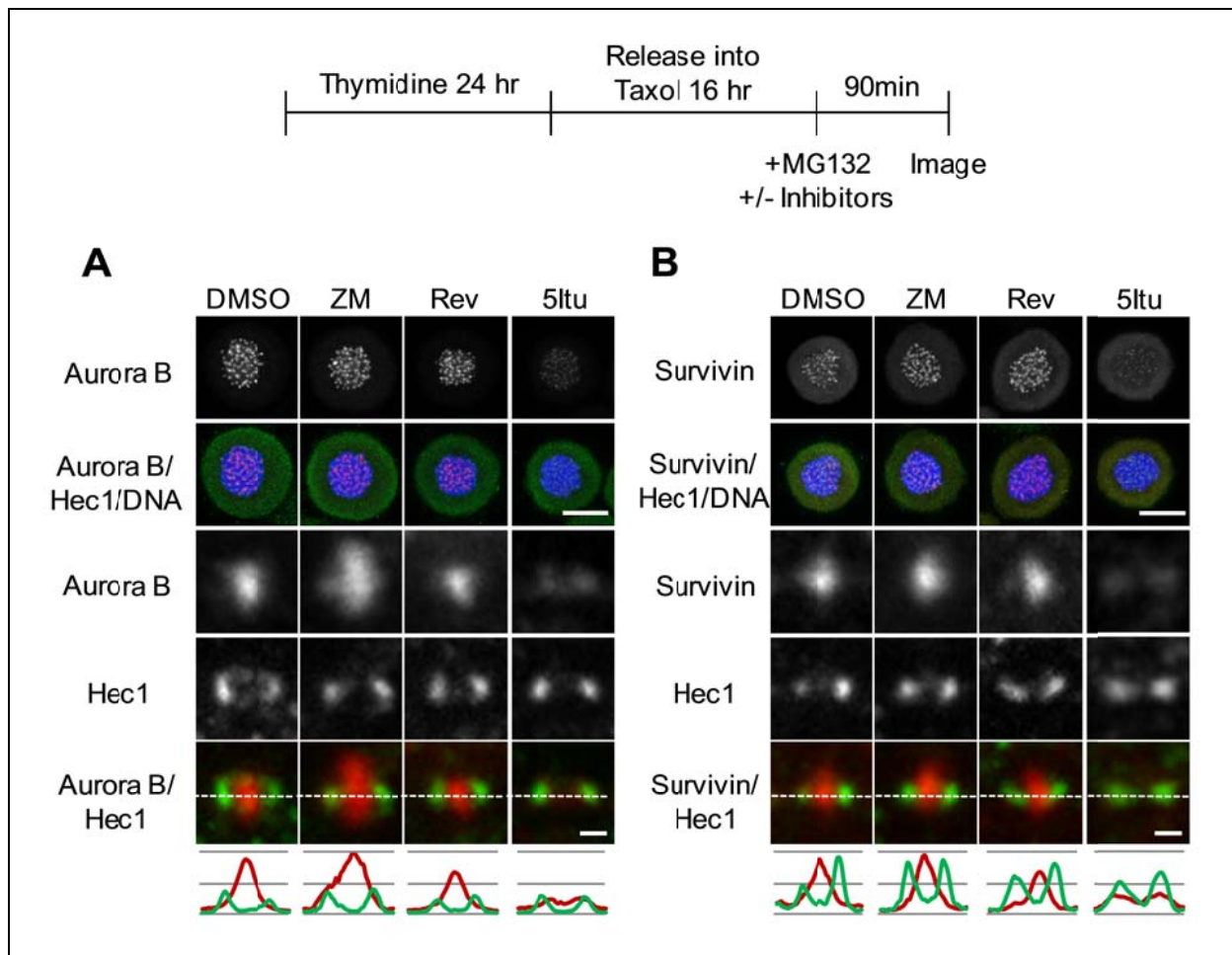
Supplementary Figure 4. Effects of Aurora B, Mps1, and Haspin inhibitors on CPC localization to the centromere. HeLa M cells were exposed to specific kinase inhibitors and analyzed by immunofluorescence to detect CPC family members. A.) HeLa M cells were arrested in mitosis with nocodazole and then exposed to MG132 (to prevent mitotic block) and either DMSO (-), ZM447439 (ZM), reversine (Rev), or 5Itu using the scheme shown. Cells were stained with antibodies to either INCENP, Aurora B and CenpH or Survivin, Borealin, and CenpH. Examples of maximum projection images are shown (scale bar = 10 μ m). B.) HeLa M cells stained as in “A” were quantified to determine the amount of CPC subunits relative to the CenpH kinetochore marker. At least 200 centromeres from 20 different cells were quantified for each condition. Bars represent standard deviation. * $p < 0.05$ using a student’s t-test.



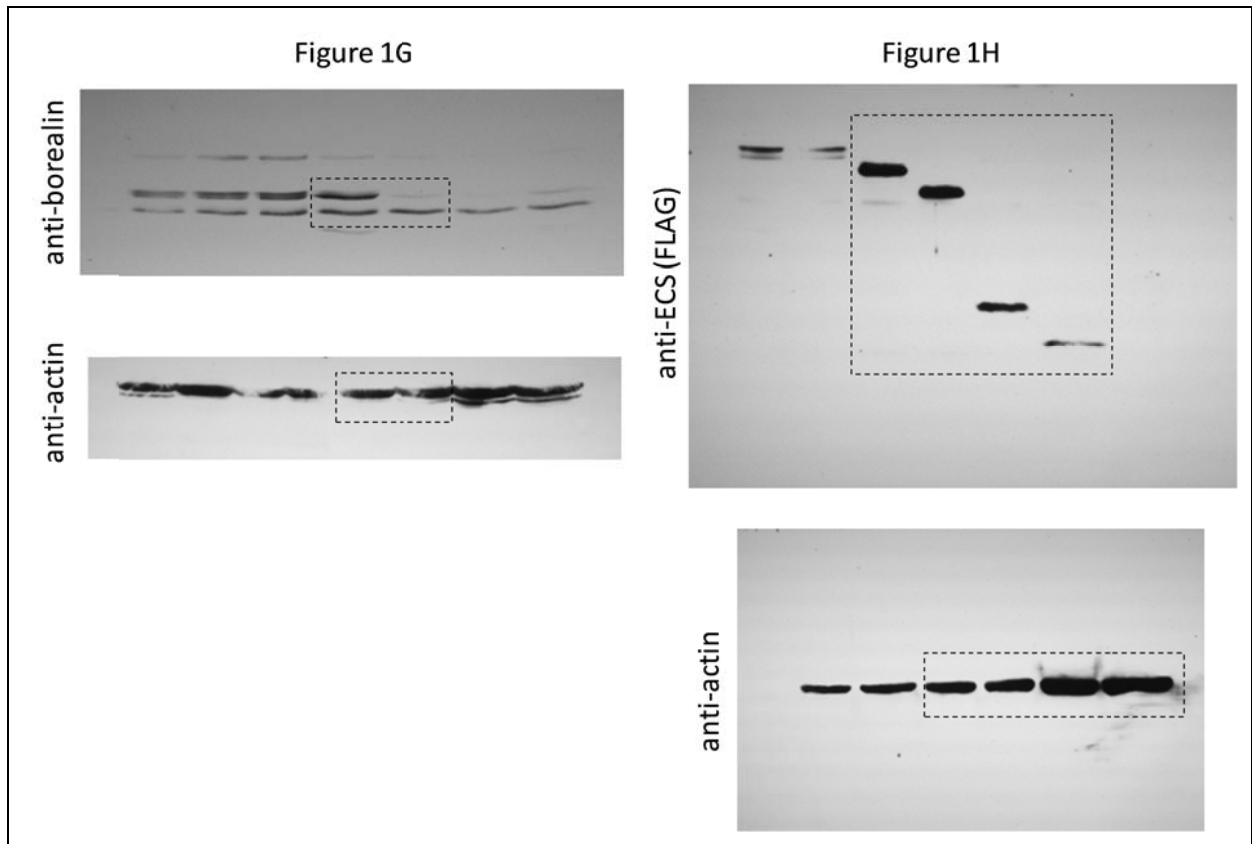
Supplementary Figure 5. 5Itu and CHR6494 have similar effects on CPC localization. A.) HeLa M cells were exposed to taxol for 6 hours, and then with MG132, and either DMSO or CHR6494 for 90 minutes. Immunofluorescence was used to detect Aurora B, Borealin, INCENP and Survivin (scale bar = 10 μ m). Deconvoluted wide-field images were used to quantify CPC centromere localization using two methods. First, standard deviations of pixel intensities of 20 whole cell images relative to mean intensity was compared in cells with or without CHR6494 treatment. All CPC subunits showed a significant reduction in pixel variation. Secondly, average staining intensities of >40 kinetochores relative to staining intensity on chromatin was compared for the same treatments. Examples of maximum projection images are shown along with quantitation. B.) Asynchronously growing HeLa M cells were exposed to either 5Itu or CHR6494 (CHR) and analyzed by immunofluorescence and confocal microscopy to detect Borealin and Hec1. Examples of maximum projection images are shown (scale bar = 10 μ m). 50 centromeres were analyzed for each condition. In both A and B, error bars represent standard deviations and asterisks indicate $p < 0.05$ using a student's t-test.



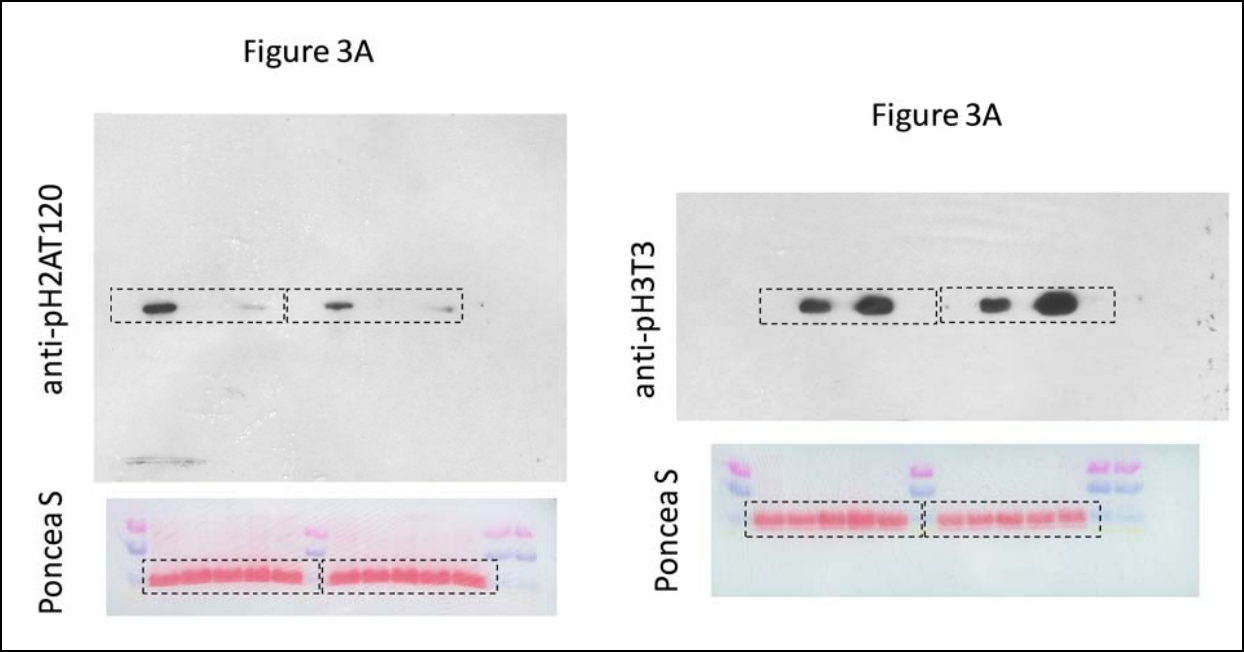
Supplementary Figure 6. Survey of multiple kinetochores after 5Itu-treatment. HeLa M cells were released from a thymidine block into taxol. Sixteen hours later, cells were exposed to Taxol +MG132 with or without 5Itu for 90 minutes. Immunofluorescence was carried out with antibodies to Borealin (BOR) and Hec1. Close-up images and line scans of individual sister kinetochores/centromeres are shown. Images are maximum projections of between 2 to 3 Z planes, selected so as to include both Hec1-stained kinetochores. Both scale bars represent 0.5 μ m.



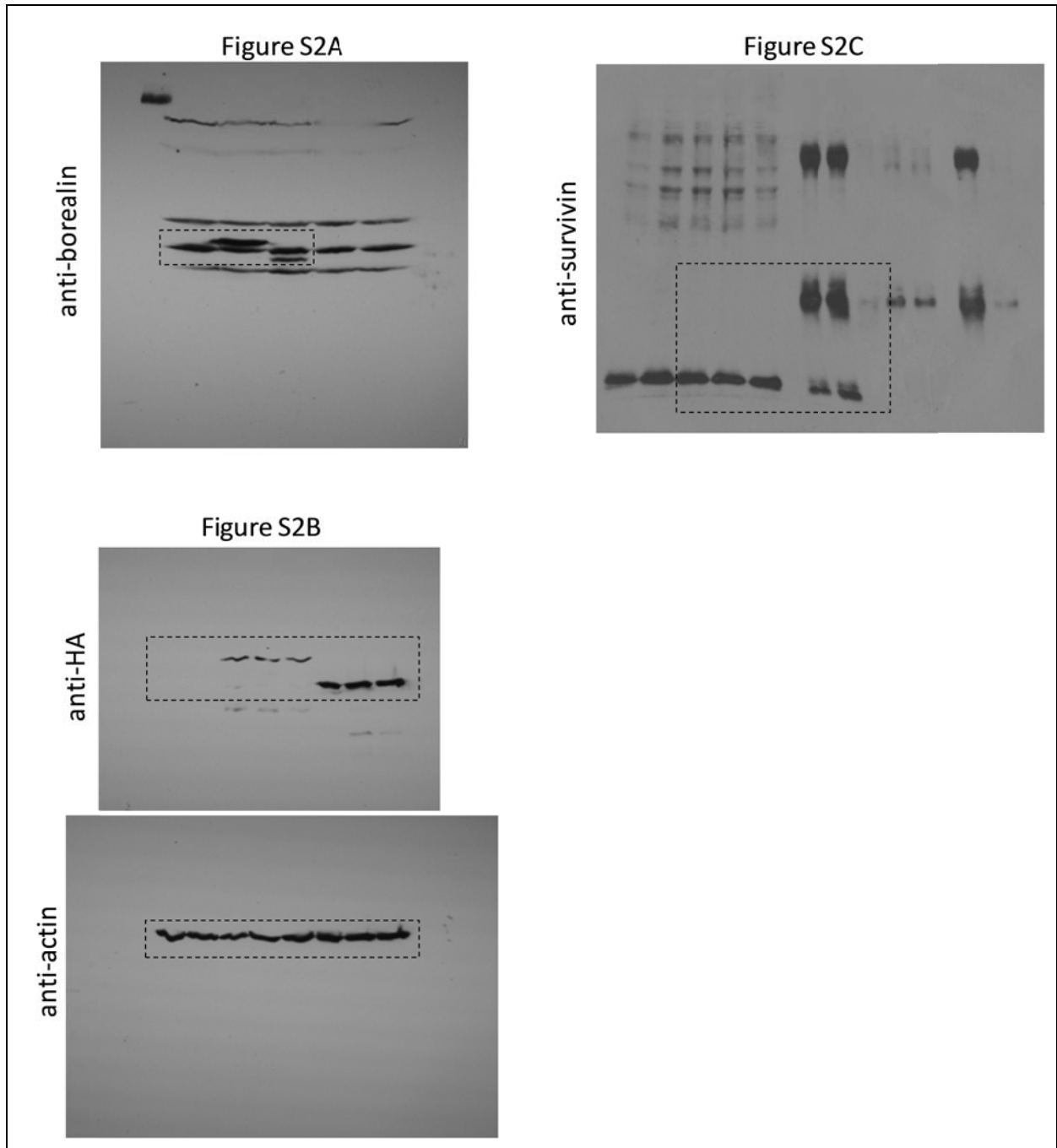
Supplementary Figure 7. Localization of Aurora B and Survivin in taxol-blocked, 5Itu-treated cells. HeLa M cells were synchronized and treated as indicated in the schematic. ZM: ZM447439; REV: reversine; 5Itu: 5-iodotubercidin. A.) Immunofluorescence analysis of Aurora B and Hec1. B.) Immunofluorescence analysis of Survivin and Hec1. For both staining experiments, examples of maximum projection confocal images are shown in the top two rows (scale bar = 10 μ m). Close-up images are shown in the bottom three rows (scale bar = 0.5 μ m) with lines scans also included. Hoechst 33342 was used to visualize DNA.



Supplementary Figure 8. Un-cropped scanned films shown in figure 1.



Supplementary Figure 9. Un-cropped scanned films shown in figure 3.



Supplementary Figure 10. Un-cropped scanned films shown in figure S2A, B and C.