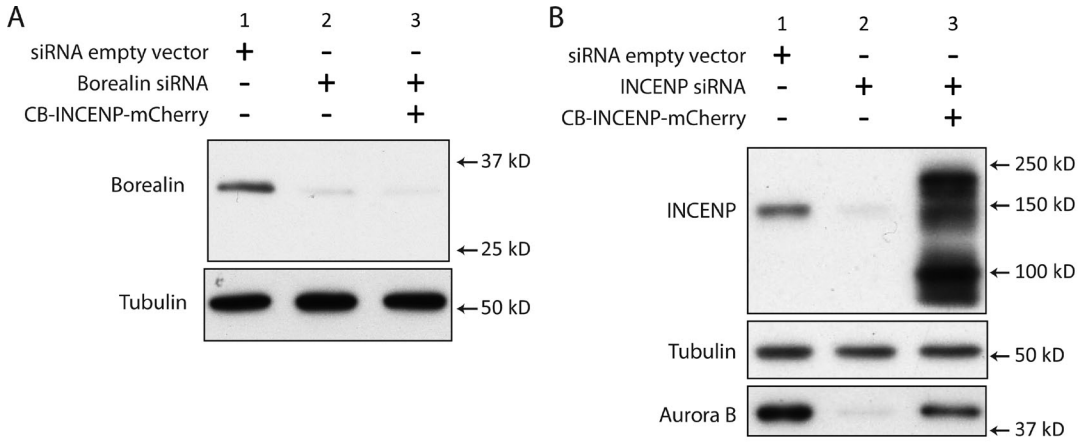
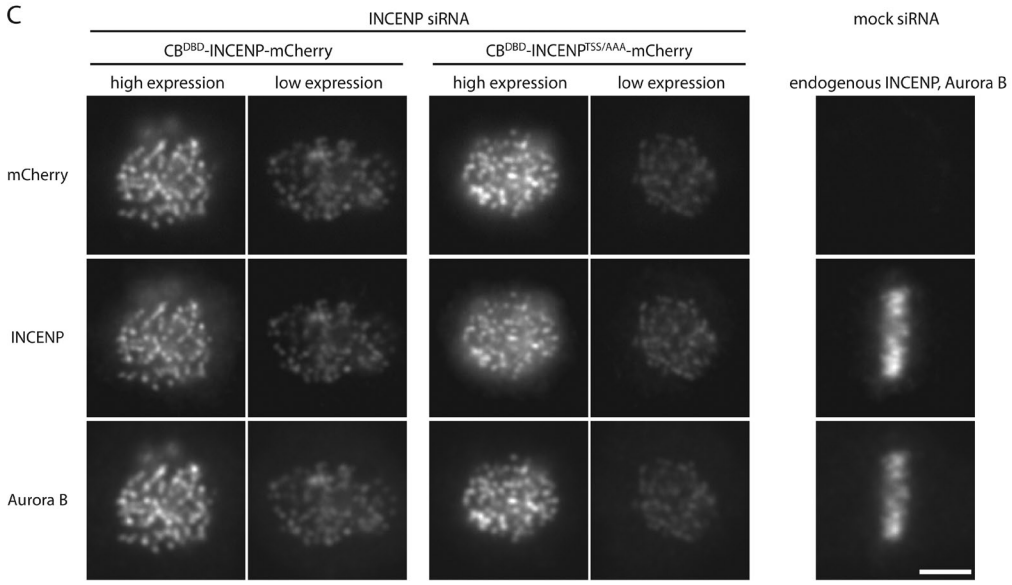
Wang et al., http://www.jcb.org/cgi/content/full/jcb.201103044/DC1





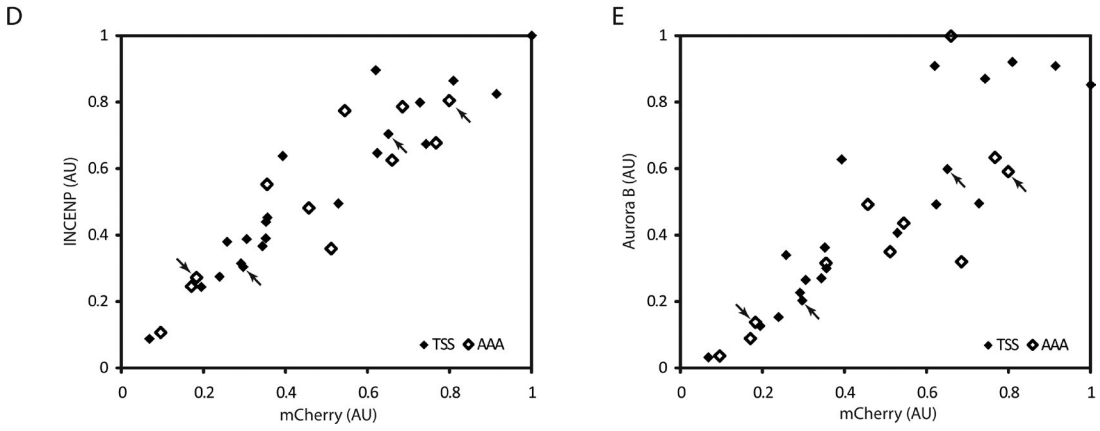


Figure S1. **Western blot analysis of siRNA depletions and analysis of Aurora B recruitment by INCENP.** (A and B) HeLa cells were transfected with either empty siRNA vector or with Borealin siRNA (A) or INCENP siRNA (B) with or without CB^{DBD}-INCENP–mCherry as indicated. Cells were arrested for 16 h with nocodazole, and mitotic cell lysates were prepared and analyzed by Western blotting for either Borealin (A) or INCENP and Aurora B (B) with tubulin as a loading control. Note that Aurora B levels are greatly reduced in INCENP-depleted cells but are approximately normal in cells expressing CB^{DBD}-INCENP–mCherry (B, lane 3), suggesting normal INCENP levels in most cells. The top band in CB^{DBD}-INCENP–mCherry lysates runs at the expected size, but a significant portion of the overexpressed INCENP migrates faster, suggesting partial degradation. By fluorescence, we observed a subpopulation of cells with very high levels of CB^{DBD}-INCENP–mCherry, which appear to be dead or dying (not depicted). These cells are not included in our analyses, but they likely contribute disproportionally to the total INCENP seen by Western blotting. (C–E) HeLa cells were transfected with INCENP siRNA vector, together with siRNA-resistant CB^{DBD}-INCENP–mCherry or CB^{DBD}-$INCENP^{TSS/AAA}$–mCherry. Cells were fixed and stained for INCENP and Aurora B. A range of expression levels is observed for the CB^{DBD}-INCENP constructs. (C) Images show examples of high and low expression for each. Endogenous INCENP and Aurora B (mock siRNA conditions) are shown for comparison in an untransfected cell. Bar, 5 µm. (D and E) Mean intensities of INCENP and Aurora B immunofluorescence at centromeres were quantified and plotted against mCherry intensity over a range of expression levels. Each data point represents a single cell. Arrows indicate data points corresponding to the cells shown in C. Note that INCENP and Aurora B levels correlate with mCherry intensity, independent of the TSS/AAA mutation, indicating that the mutation does not affect Aurora B recruitment. AU, arbitrary unit.

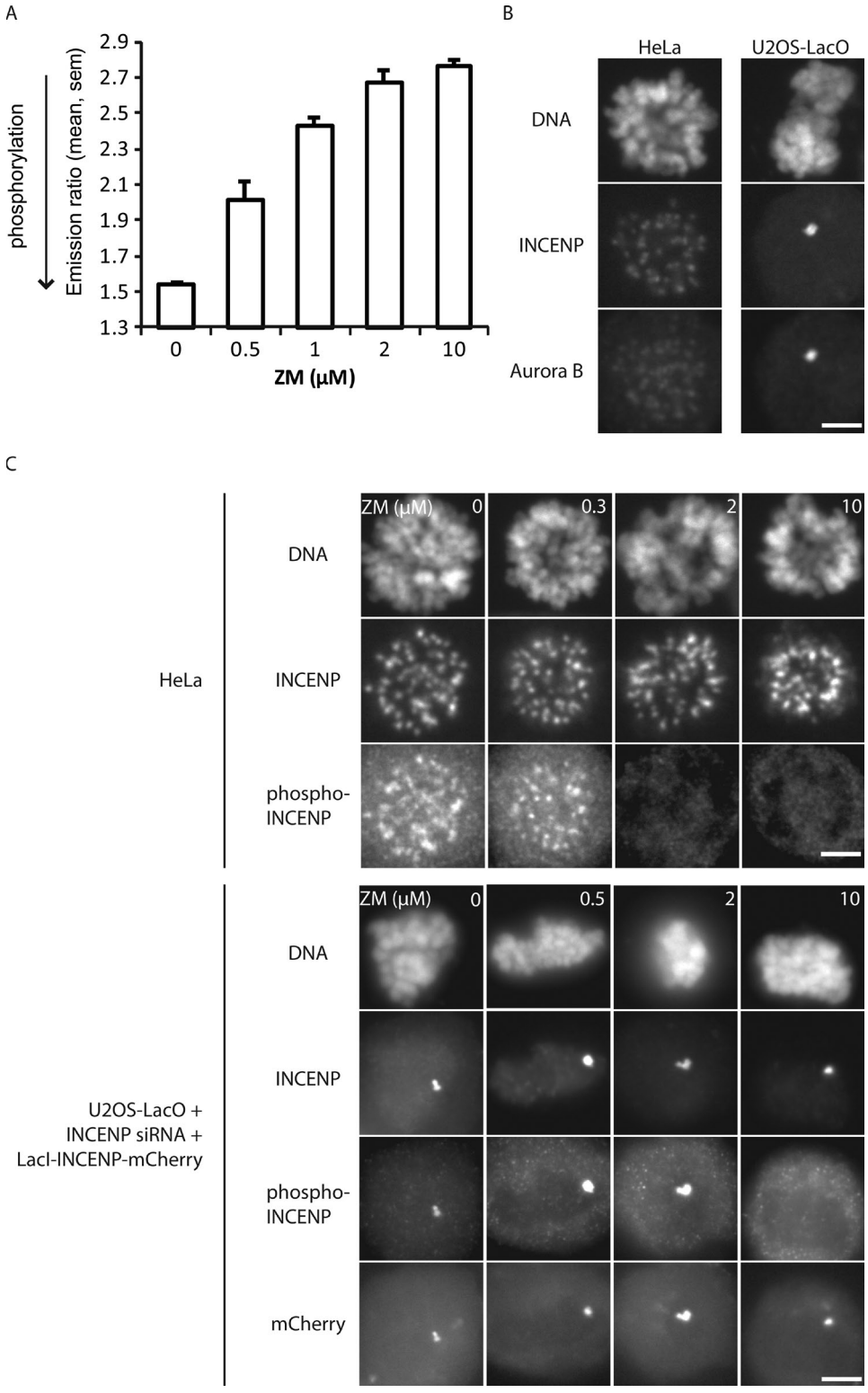


Figure S2. **LacI-INCENP is highly concentrated and phosphorylated in U2OS-LacO cells.** (A) U2OS-LacO cells were treated and imaged as in Fig. 4 (D and E). The YFP/CFP emission ratio was averaged over multiple cells ($n \geq 8$ for each concentration). (B and C) U2OS-LacO cells were transfected with INCENP siRNA vector and siRNA-resistant LacI-INCENP–mCherry and treated for 1 h with nocodazole (B) or nocodazole and ZM at the indicated concentrations (C). Untransfected HeLa cells were treated for 1 h with monastrol, MG132 (to prevent mitotic exit), and ZM. Cells were fixed and stained for DNA, INCENP, and either Aurora B (B) or phospho-INCENP (C), using a phospho-specific antibody against the C-terminal TSS motif. Note that LacI-INCENP levels at the LacO site are higher than endogenous INCENP levels at centromeres (B). Endogenous INCENP is dephosphorylated at 2 μM ZM, whereas LacI-INCENP is still phosphorylated (C), suggesting that the kinase is active, consistent with the phosphorylation shown in Fig. 4 (D and E). Note that in C, the images of HeLa cells are processed differently from the images of U2OS cells, so that centromeres do not appear dim relative to the LacO spots. Bars, 5 μm.

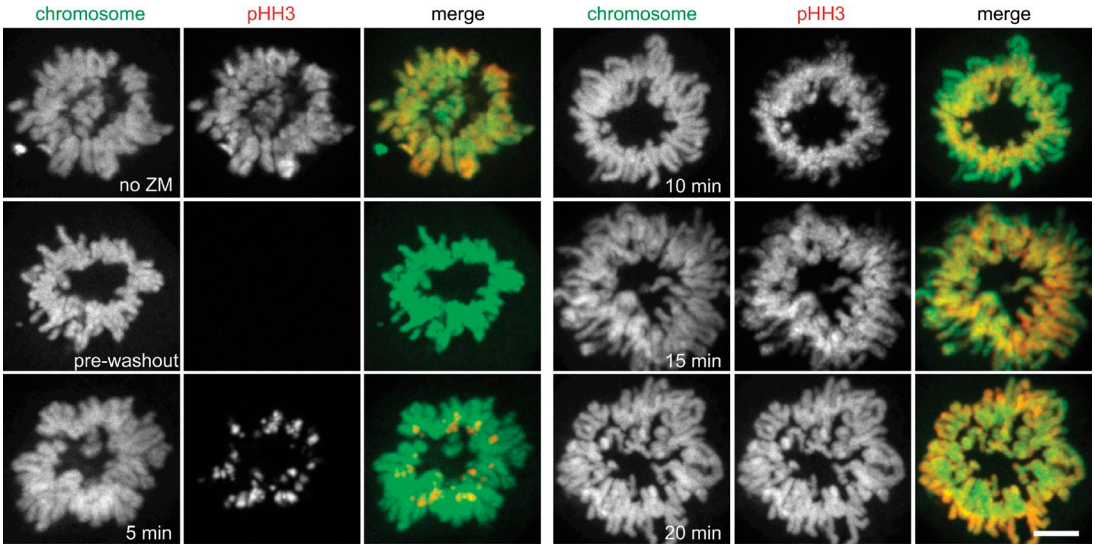
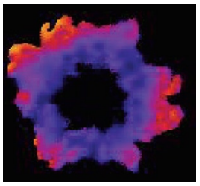


Figure S3. **Phosphorylation of endogenous substrates spreads from centromeres during Aurora B activation.** Cells were treated with monastrol, MG132, and ZM and then were fixed at the indicated times after activation of Aurora B by washing out ZM. Fixed cells were stained for DNA and phospho-H3 (pHH3) Ser10. Representative images are shown at each time point. Bar, 5 µm.



Video 1. **Phosphorylation spreading from centromeres after Aurora B activation.** Cells were transfected with the chromatin-targeted Aurora B phosphorylation sensor together with CB-mCherry to label centromeres and then were treated with monastrol, MG132, and ZM. Cells were imaged live during activation of Aurora B by ZM washout. The color-coded YFP/CFP emission ratio is shown for each time point. The timestamp (minutes) is relative to ZM washout at t = 0. This video corresponds to the data in Fig. 5 B.