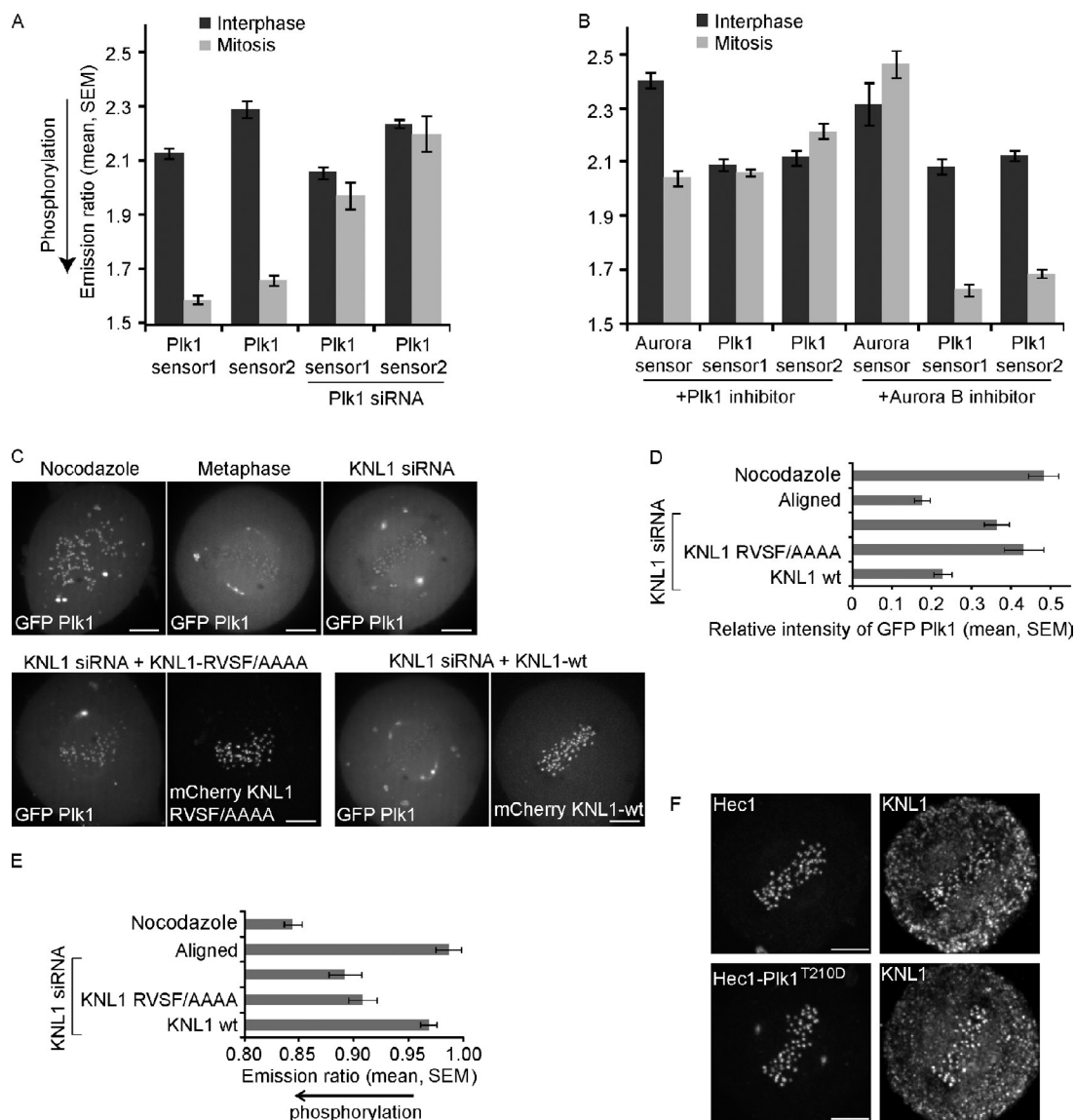
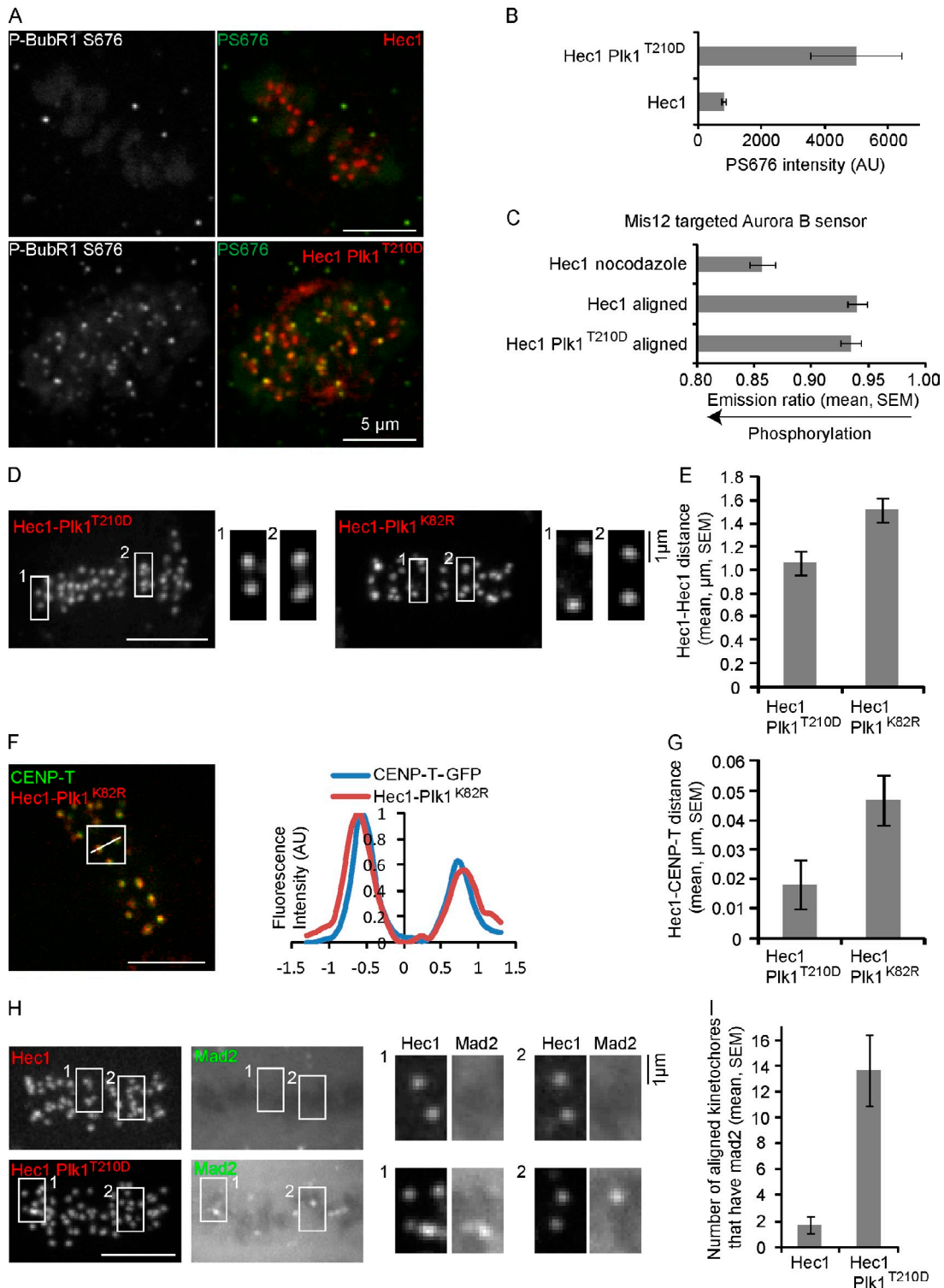


Liu et al., <http://www.jcb.org/cgi/content/full/jcb.201205090/DC1>

**Figure S1. Characterization of Plk1 phosphorylation sensors and Hec1-Plk1<sup>T210D</sup>.** (A) Cells expressing untargeted phosphorylation sensors for Plk1 were imaged live either in interphase or in mitosis. Sensors with two different substrate sequences are shown: PPSLSSTVLIVRN from BRCA2 (sensor 1) and DDALNAIFLPSEG from c-Jun (sensor 2), with the Plk1 target residues italicized. Plk1 was depleted by RNAi, as indicated. The YFP/CFP emission ratio was calculated and averaged over multiple cells. Each bar represents  $\geq 10$  cells, and the experiment was repeated with similar results. Note that both sensors are phosphorylated in mitosis relative to interphase, indicated by decreased emission ratio, and phosphorylation depends on Plk1. (B) Cells expressing untargeted phosphorylation sensors for Plk1 or Aurora B were treated with either the Aurora B inhibitor ZM447439 (2  $\mu$ M) or the Plk1 inhibitor BI2536 (100 nM) for 1 h and analyzed as in A. Each bar represents  $\geq 10$  cells, and the experiment was repeated with similar results. Note that phosphorylation of the Aurora B sensor depends on Aurora B activity, whereas phosphorylation of the Plk1 sensors depends on Plk1 activity. (C) Cells expressing GFP-Plk1 were treated with nocodazole (100 ng/ml), analyzed at metaphase, or treated with KNL1 siRNA to disrupt PP1 targeting to kinetochores (top row). Cells treated with KNL1 siRNA were also transfected with rescue plasmids: either KNL1-wt or KNL1 mutant in which the PP1-binding motif RVSF was mutated to AAAA (bottom row). Cells were imaged live to assay for Plk1 localization to kinetochores. Images are maximal intensity projects of confocal z series. (D) To measure GFP-Plk1 levels at kinetochores for cells treated as in C, an intensity threshold was selected manually to cover all kinetochores. After background subtraction, the mean kinetochore intensity was normalized by dividing by the GFP intensity in the cytoplasm to control for variation in expression levels between cells. Only kinetochores aligned at metaphase were analyzed in cells treated with KNL1 siRNA. Each bar represents a mean over multiple cells, and the experiment was repeated three times with similar results. Note that removal of Plk1 from kinetochores depends on PP1 recruitment because cells depleted of KNL1 and expressing the RVSF/AAAA mutant maintain Plk1 at metaphase kinetochores. (E) Cells expressing the kinetochore-targeted Plk1 sensor were treated as in C and imaged live. The YFP/CFP emission ratio was calculated and averaged over multiple cells (each bar represents  $n \geq 10$  cells,  $n \geq 30$  kinetochores per cell). The experiment was repeated twice with similar results. Only kinetochores aligned at metaphase were analyzed in cells treated with KNL1 siRNA. Note that dephosphorylation of the Plk1 sensor depends on PP1 recruitment. (F) Cells expressing either Hec1 or Hec1-Plk1<sup>T210D</sup> were fixed and stained for endogenous KNL1. KNL1 levels are not affected by Hec1-Plk1<sup>T210D</sup>, indicating that the kinetochore is intact. (C and F) Bars, 5  $\mu$ m.



**Figure S2. Effects of Hec1-Plk1<sup>T210D</sup> expression.** (A and B) Cells expressing either Hec1 or Hec1-Plk1<sup>T210D</sup> were fixed and stained with a phosphospecific antibody against the Plk1 substrate BubR1-S676-P. Representative images are shown (A), and the mean intensity of BubR1-S676-P staining was calculated over multiple cells in metaphase (each bar represents  $n \geq 7$  cells; B). Expression of Hec1-Plk1<sup>T210D</sup> increases BubR1-S676 phosphorylation. Error bars are SEM. AU, arbitrary unit. (C) Phosphorylation of a Mis2-targeted Aurora B phosphorylation sensor was calculated in cells expressing either Hec1 or Hec1-Plk1<sup>T210D</sup>. Metaphase-aligned kinetochores were analyzed, or cells were treated with nocodazole, as indicated. Each bar represents  $n \geq 10$  cells, with  $n \geq 30$  kinetochores per cell. Phosphorylation is not affected by expression of Hec1-Plk1<sup>T210D</sup>. (D and E) Interkinetochore distance was measured in cells expressing Hec1-Plk1<sup>T210D</sup> or Hec1-Plk1<sup>K82R</sup> (kinase-inactive mutant). (F and G) Intrakinetochore stretch was analyzed in cells expressing CENP-T-GFP together with either Hec1-Plk1<sup>T210D</sup> or Hec1-Plk1<sup>K82R</sup>, as in Fig. 2 (F-I). Expression of Hec1-Plk1<sup>K82R</sup> does not collapse interkinetochore tension and intrakinetochore stretch in contrast to Hec1-Plk1<sup>T210D</sup>. The boxed area shows the individual kinetochore pair used for the line scans. (E and G)  $n \geq 80$  kinetochore pairs from multiple cells. (H and I) Cells expressing Mad2-GFP, together with either Hec1 or Hec1-Plk1<sup>T210D</sup>, were examined at metaphase. Insets show individual kinetochore pairs. The number of kinetochores with detectable Mad2-GFP was counted and averaged over multiple cells ( $n = 7$ ). Mad2 is detectable on multiple kinetochores in cells expressing Hec1-Plk1<sup>T210D</sup> but not in control cells expressing Hec1. (A, D, F, and H) Bars, 5 μm.

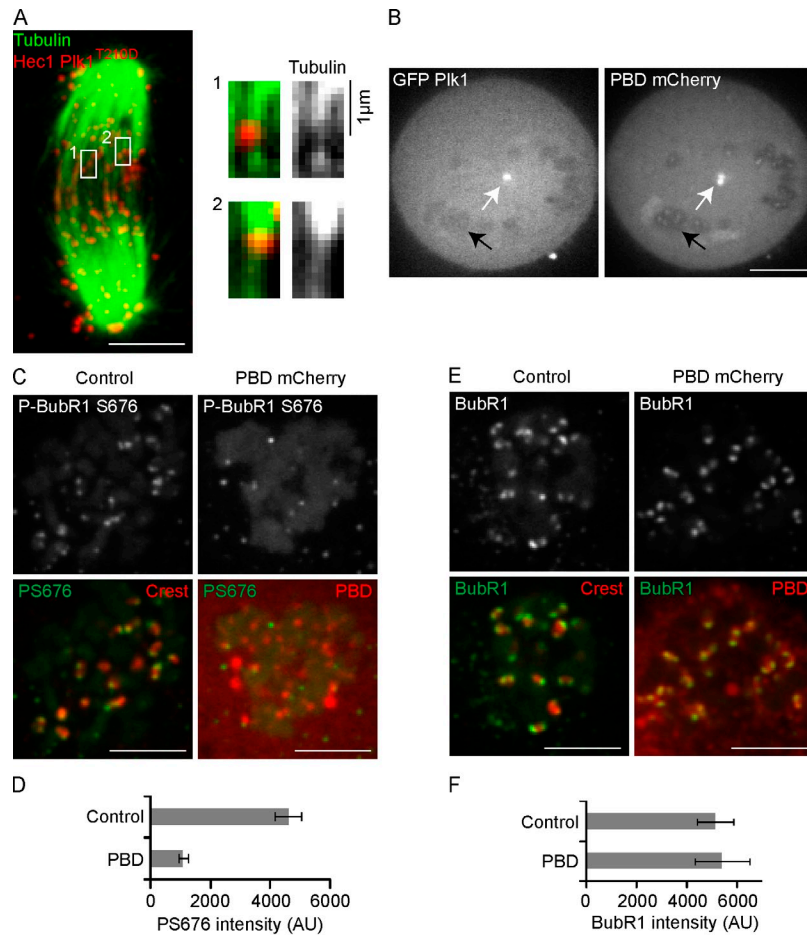
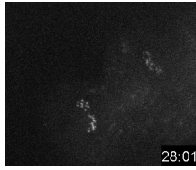
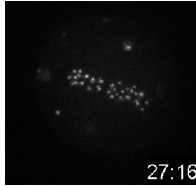


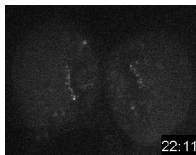
Figure S3. **Effects of Hec1-Plk1<sup>T210D</sup> or PBD-mCherry expression.** (A) A cell expressing Hec1-Plk1<sup>T210D</sup> and treated with reversin, as in Fig. 4, was fixed in anaphase and stained for microtubules. Insets show examples of merotelic kinetochores. (B) An example of a cell expressing GFP-Plk1 and PBD-mCherry. The PBD localizes to both kinetochores (black arrows) and centrosomes (white arrows), whereas Plk1 localizes only to centrosomes. (C–F) Cells expressing PBD-mCherry or untransfected control cells were treated with nocodazole (100 ng/ml) for 30 min and then fixed and stained with a phosphospecific antibody against BubR1-S676-P (C and D) or stained for total BubR1 (E and F). Control cells were stained with CREST to label centromeres. Top images show BubR1 staining, and bottom images show a merge either with CREST or with PBD-mCherry. Staining intensities were quantified and averaged over multiple cells (each bar represents  $n \geq 8$  cells). The experiment was repeated twice with similar results. Error bars are SEM. AU, arbitrary unit. (A–C and E) Bars, 5  $\mu$ m.



Video 1. **Control cell expressing Hec1-mCherry, imaged from metaphase to anaphase.** Images were acquired every 2 min (five z planes per time point with 0.5- $\mu\text{m}$  spacing) using a spinning-disk confocal with a 100 $\times$  1.4 NA objective. Maximal intensity projections were used to make the video.



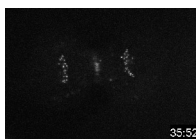
Video 2. **Cell expressing Hec1-mCherry-Plk1<sup>T210D</sup>.** The cell was imaged as in Video 1 from metaphase for 60 min with no anaphase onset.



Video 3. **Control cell expressing Hec1-mCherry.** The cell was imaged as in Video 1 from metaphase to anaphase with reversine added at  $t = 0$ . Lagging chromosomes were rare compared with cells expressing Hec1-Plk1<sup>T210D</sup>, as quantified in Fig. 4 (D and E).



Video 4. **Cell expressing Hec1-mCherry-Plk1<sup>T210D</sup>.** The cell was imaged as in Video 1 from metaphase to anaphase with reversine added at  $t = 0$ . This cell corresponds to the images shown in Fig. 4 F. Note that kinetochores occasionally come on and off the metaphase plate.



Video 5. **Another example of a cell expressing Hec1-mCherry-Plk1<sup>T210D</sup>.** The cell was imaged as in Video 1 from metaphase to anaphase with reversine added at  $t = 0$ .