Banerjee et al., http://www.jcb.org/cgi/content/full/jcb.201307119/DC1

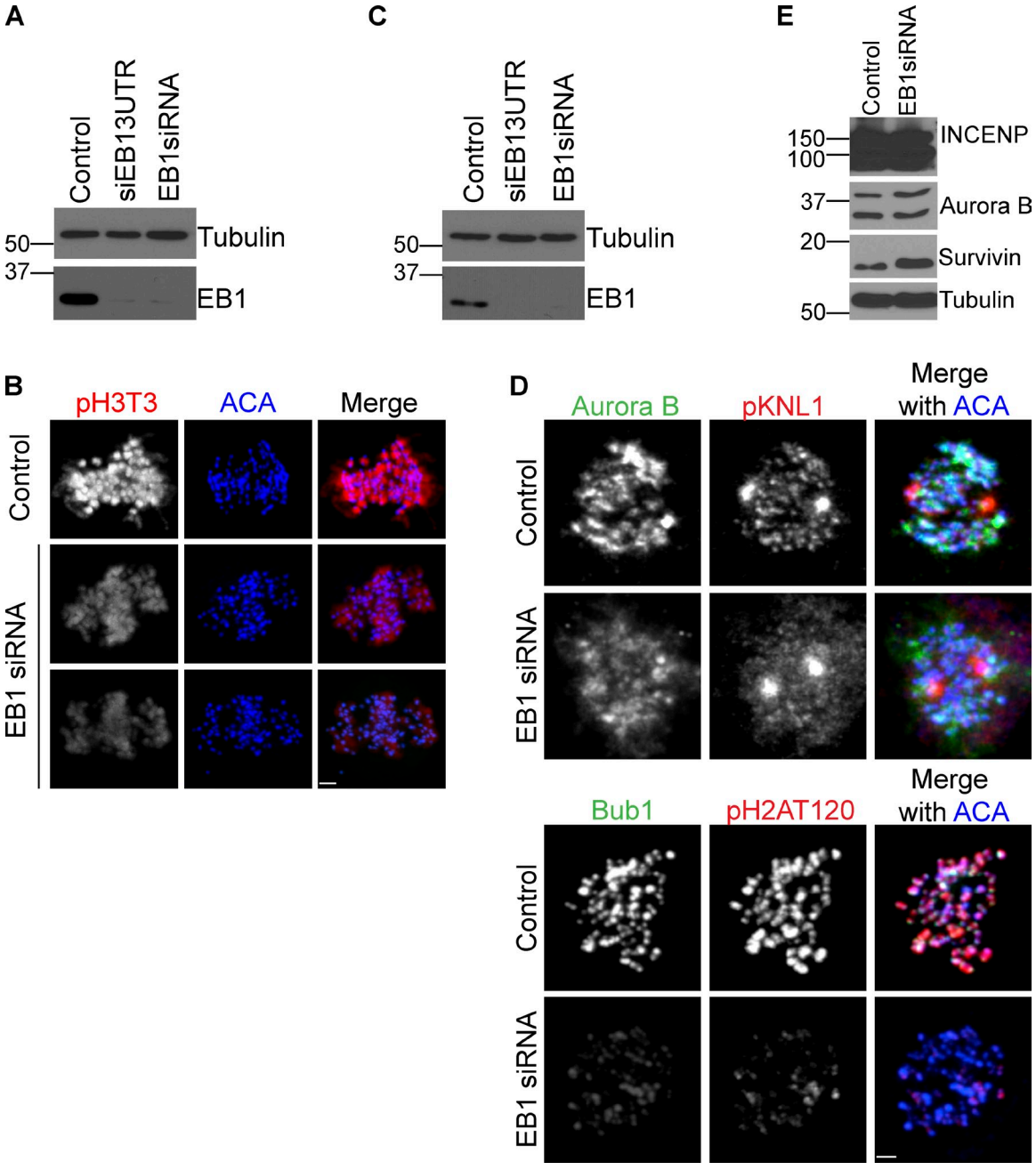


Figure S1. **Phenotypes of EB1 depletion.** (A) Protein levels as measured by immunoblotting of the experiment in Fig. 1. HeLa cells treated with either an siRNA targeting the EB1-coding sequence (10 µM EB1 siRNA) or a combination of two EB1 3′UTR-targeted siRNAs (siEB13UTR; 10 nM each) for 48 h. (B) Immunostaining of EB1-depleted HeLa cells with antiphosho–histone H3Thr3 (pH3T3) antibodies. Bar, 1.9 µm. (C and D) EB1 depletion in HEK293T cells gives the same phenotype as HeLa cells. (C) HEK293T cells were depleted as in A, and protein levels were measured. (D) EB1 depletion in HEK293T cells shows reduced levels of Aurora B at the inner centromere, reduced Bub1 at kinetochores, and reduced phospho-KNL1 levels. Bar, 1.4 µm. (E) Immunoblot to compare CPC proteins—INCENP, Aurora B, and survivin—in control-depleted and EB1-depleted cells. Molecular mass markers are given in kilodaltons.

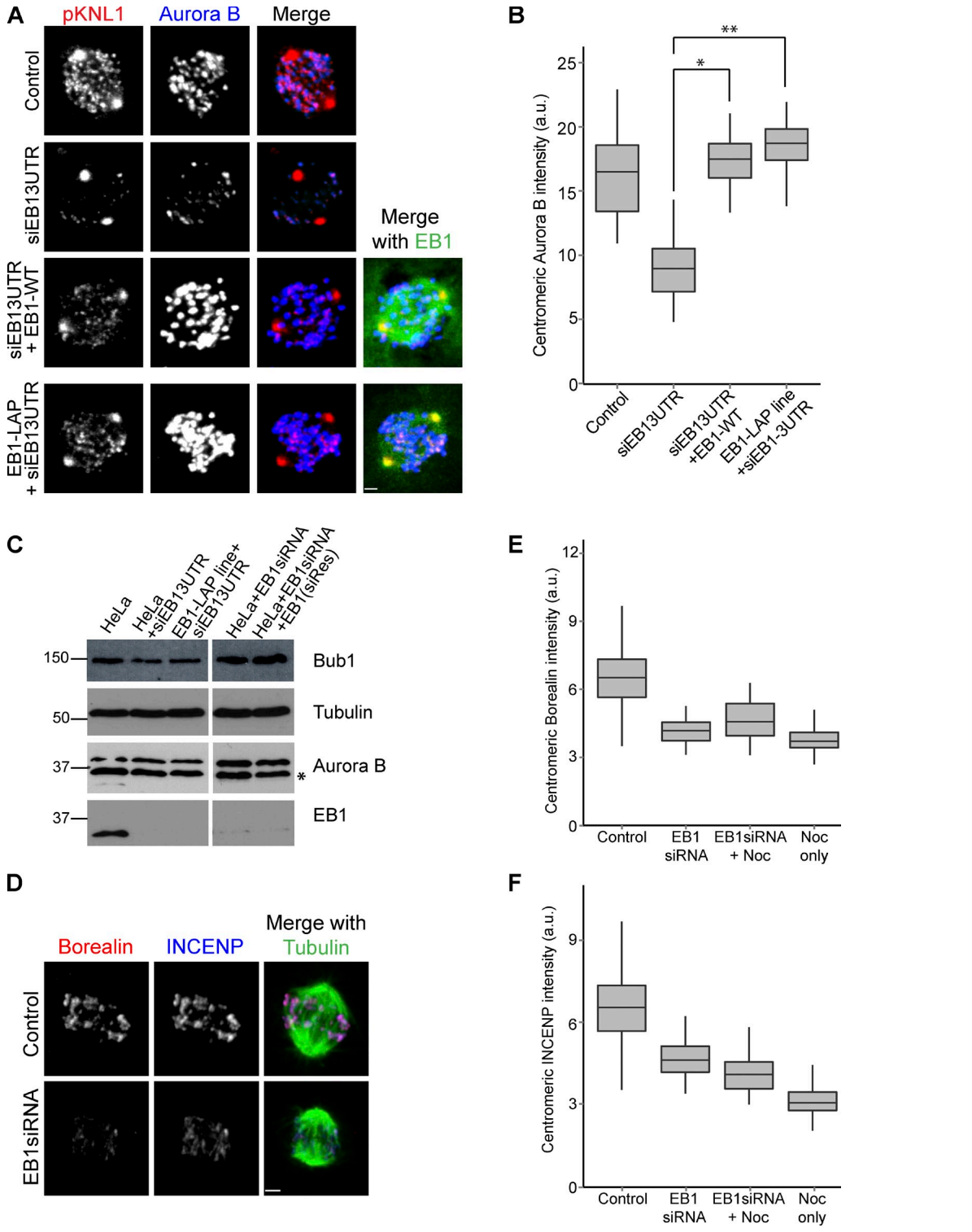


Figure S2. **Additional approaches to rescue EB1 depletion effects on centromeric Aurora B and compare it with microtubule perturbation.** (A) HeLa cells were treated with siEB13UTR for 48 h. Centromeric Aurora B levels were rescued by transfecting pDLAP-EB1 24 h after siRNA transfection (siEB13UTR + EB1-wild type [WT]). siRNA-resistant EB1-GFP expression is shown along with Aurora B and phospho-KNL1 staining. An EB1-LAP–expressing stable line also showed no reduction in inner centromeric Aurora B after transfecting siEB13UTR siRNAs for 48 h (EB1-LAP + siEB13UTR). Bar, 1.6 µm. (B) Quantification of immunostaining intensities in A. *, $P = 3.16 \times 10^{-102}$; **, $P = 2.55 \times 10^{-116}$. (C) Immunoblots to measure protein levels for A. Tubulin staining is shown as a loading control. The asterisk indicates a nonspecific band recognized by the anti–Aurora B antibody (Bethyl Laboratories, Inc.). Molecular mass markers are given in kilodaltons. (D) Immunostaining of INCENP and Borealin showing effects of EB1 depletion. Bar, 2.1 µm. (E) HeLa cells treated with EB1 siRNA or 3.3 µM nocodazole separately or in combination show similar loss in inner centromeric Borealin. (F) HeLa cells treated with EB1 siRNA or 3.3 µM nocodazole (Noc) separately or in combination show similar loss in inner centromeric INCENP. The height of the boxes represents the IQR. The central horizontal lines depict the median. The top whiskers represent the 75th percentile + 1.5× IQR, and the bottom whiskers represent the 25th percentile − 1.5× IQR. a.u., arbitrary units.

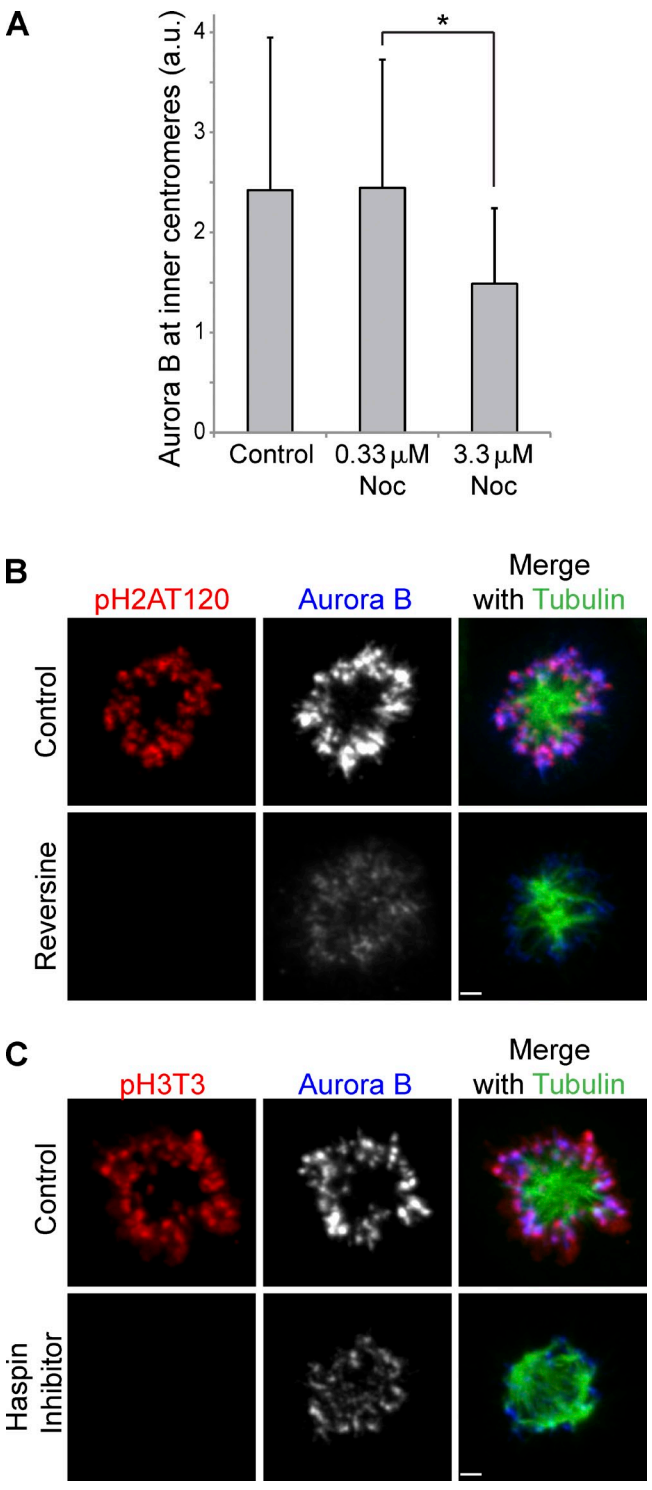


Figure S3. **Centromeric Aurora B is reduced by reversine or 5-iodotubericidine (HI) treatment in HeLa cells.** (A) Mean inner centromeric Aurora B levels measured at the indicated treatment conditions. Error bars show standard deviations. a.u., arbitrary units; Noc, nocodazole. *, P = 0.0118. (B and C) HeLa cells were treated with 10 μM reversine (B) or 1 μM 5-iodotubericidine (HI; C) along with MG132 for 30 min. Cells were then fixed and stained with phospho–histone H2AThr120, anti-tubulin, and anti–Aurora B antibodies. Bars, 1.7 μm. The control cells in C also are shown in Fig. 3 C.

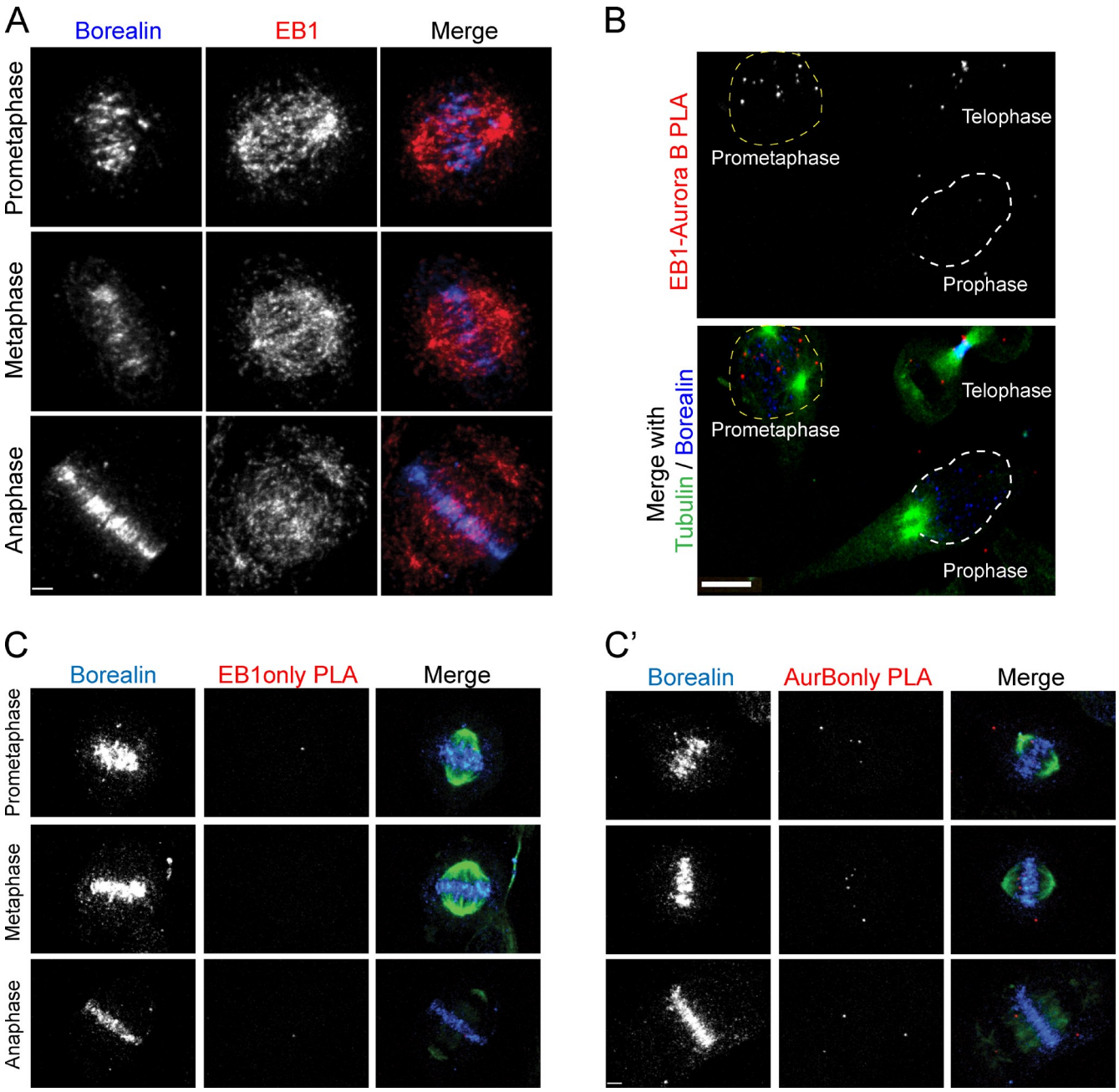


Figure S4. **The bulk of EB1 and CPC localize to distinct subcellular domains but interact near the kinetochores in prometaphase.** (A) Single-channel split of images shown in Fig. 4 D. Bar, 2.6 µm. (B) EB1–Aurora B PLA signals are absent in prophase cells. A representative prophase nucleus is outlined in white dashed lines. A prometaphase cell with numerous PLA dots is highlighted by yellow dashed lines in the same images. Bar, 8 µm. (C and C′) PLA controls for Fig. 4 E. Bar, 2.8 µm, same for both C and C′.

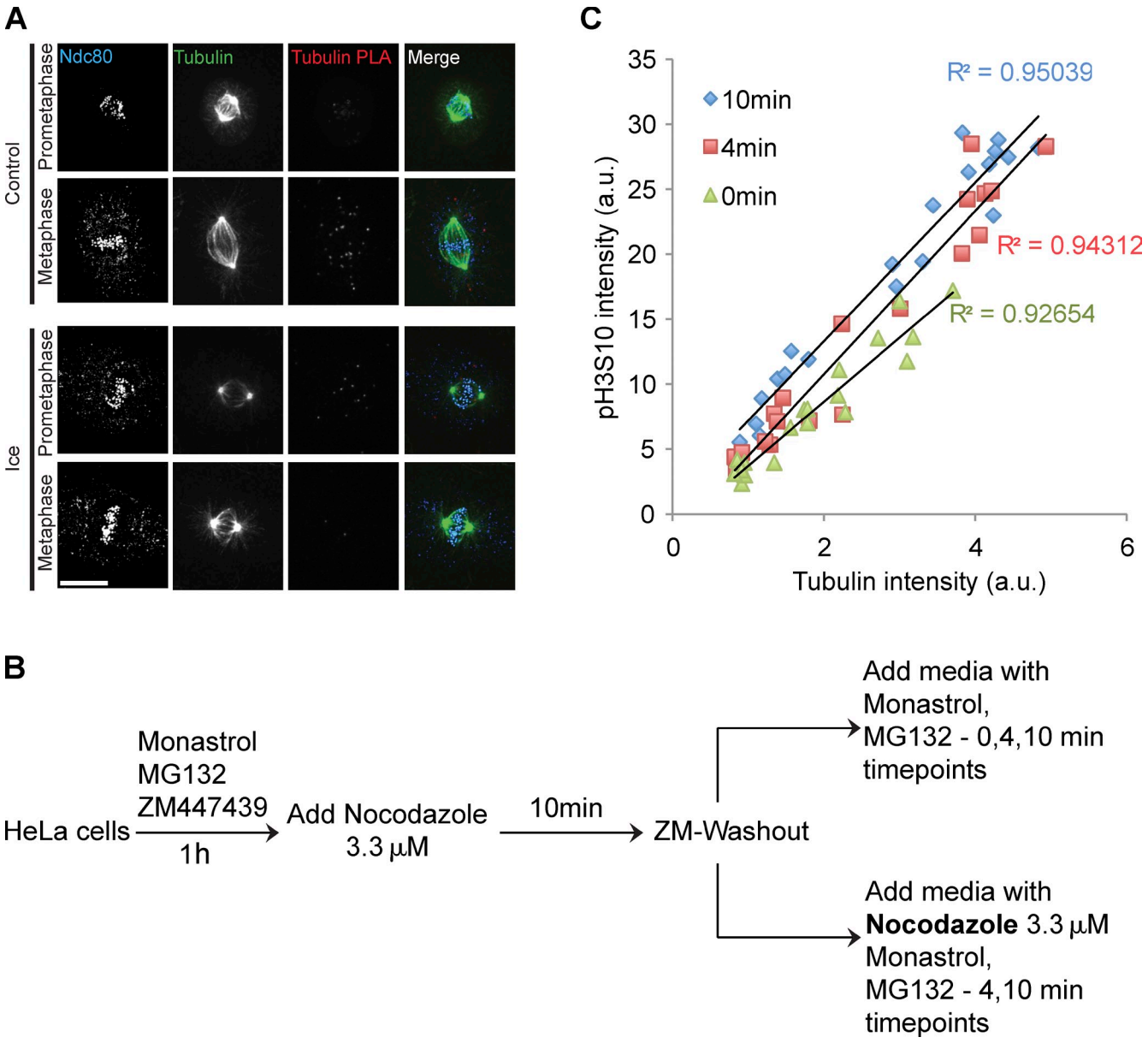
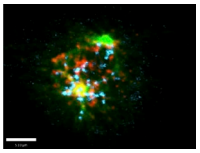
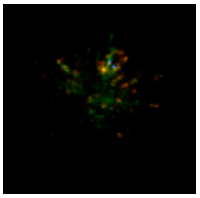


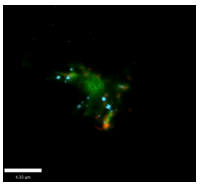
Figure S5. **Microtubules regulate spreading Aurora B activity from centromeres to chromosome arms.** (A) PLA controls for Fig. 5. Bar, 13 µm. (B) Scheme representing the timeline of ZM washout assay to measure spreading of activity that is used in Fig. 6 G and Fig. 7 (A and B). (C) Scatter plot showing correlation of total tubulin intensities and total phospho-H3S10 in individual HeLa cells at the indicated time points in the experiment shown in Fig. 7 D and outlined in B. R^2 values are mentioned in respective colors. The data shown are from a single representative experiment out of three repeats (n = 58). a.u., arbitrary units.



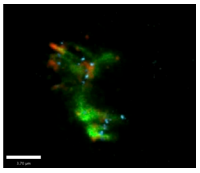
Video 1. **Sequential z sections (0.4-µm step size) of a prometaphase cell presented as a video generated in Volocity 5.5.** A fixed *Xenopus* S3 cell stained for INCENP (blue), tubulin (green), and Aurora B–tubulin PLA (red) was imaged using a spinning-disk confocal microscope. Z sections were stitched sequentially using Volocity 5.5 software to make a MOV file. Fig. 5 A represents a whole-cell projection of this video.



Video 2. **Sequential z sections (0.4-µm step size) of a monastrol-treated cell presented as a video generated in Volocity 5.5.** A fixed *Xenopus* S3 cell stained for Hec1 (blue), tubulin (green), and Aurora B–tubulin PLA (red) was imaged using a spinning-disk confocal microscope. Z sections were stitched sequentially using Volocity 5.5 software to make a MOV file. Fig. 5 B includes four z sections of this video.



Video 3. **Sequential z sections (0.4-µm step size) of a prometaphase cell fixed and stained 5 min after nocodazole washout, presented as a video generated in Volocity 5.5.** A fixed *Xenopus* S3 cell stained for Hec1 (blue), tubulin (green), and Aurora B–tubulin PLA (red) was imaged using a spinning-disk confocal microscope. Z sections were stitched sequentially using Volocity 5.5 software to make a MOV file. Fig. 5 C includes seven z sections of this video.



Video 4. **Sequential z sections (0.4-µm step size) of a prometaphase cell fixed and stained 15 min after nocodazole washout, presented as a video generated in Volocity 5.5.** A fixed *Xenopus* S3 cell stained for Hec1 (blue), tubulin (green), and Aurora B–tubulin PLA (red) was imaged using a spinning-disk confocal microscope. Z sections were stitched sequentially using Volocity 5.5 software to make a MOV file. Fig. 5 C includes nine z sections of this video.

The R code for the box and whisker plot graphs is also provided online as a Word file.