JCB

Supplemental material

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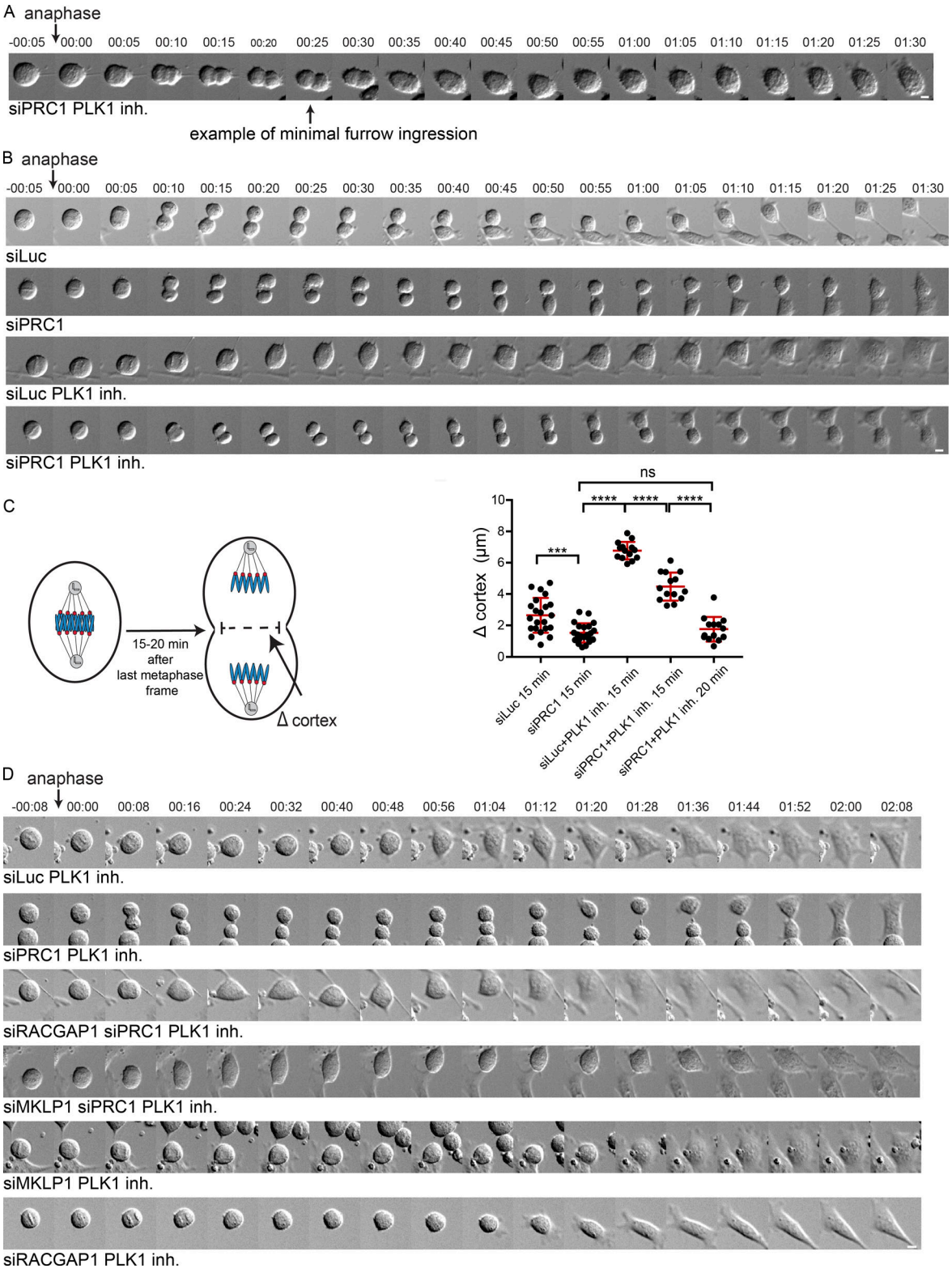


Figure S1. **PLK1-independent and centralspindlin-dependent furrow ingression. (A)** Representative DIC stills of a cell showing minimal furrow ingression. **(B)** More DIC stills of the live cell imaging experiment shown in Fig. 1 C of HeLa cells transfected with either siLuc or siPRC1 with (PLK1 inh.) or without addition of BI2536 (100 nM) before anaphase onset. Time point 00:00 (hours:minutes) refers to the first frame where we observed separating sisters. **(C)** Left: Scheme explaining how furrow ingression was measured 15–20 min after the last frame in metaphase. Right: Measurements of furrow ingression 15 min after the last frame in metaphase. For the siPRC1 + PLK1 inhibitor (PLK1 inh.) condition, a 20 min time point was also measured. Each dot represents an individual cell. Error bars depict SDs of the mean. ****, $P < 0.0001$; ***, $P < 0.001$; Student's *t* test for comparison of the indicated conditions; ns, not significant. **(D)** DIC stills of a live cell imaging experiment of HeLa cells treated with indicated siRNAs with or without addition of BI2536 (100 nM) before anaphase onset. Note that the top four rows are more DIC stills of the live imaging experiment shown in Fig. 2 E. Bars: 10 µm (A, B, and D).

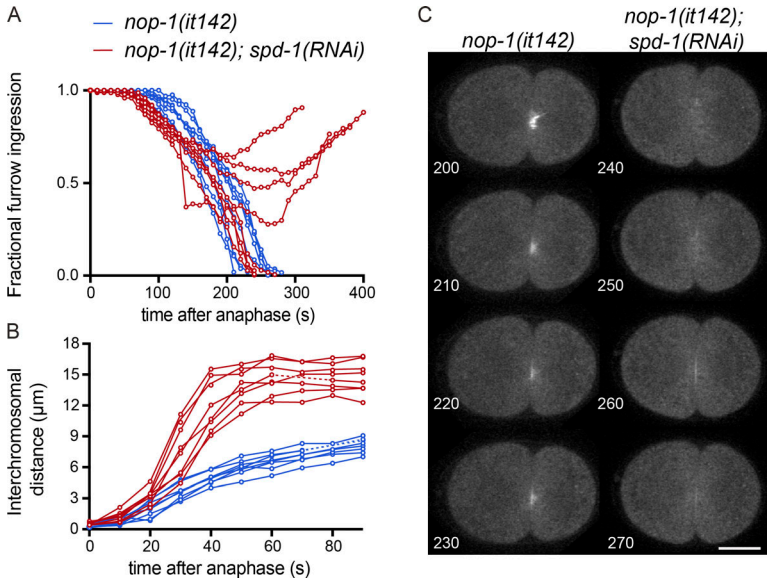


Figure S2. **Centralspindlin-independent furrow ingression in *C. elegans* embryos. (A and B)** Quantification of the rate of furrow ingression (A) and chromosome separation (B) in *nop-1(it142)* embryos treated with *spd-1* (*n* = 8 embryos) or *vector* (*RNAi*; *n* = 8 embryos). Dotted lines reflect missing time points on account of a filter wheel that was out of adjustment and failed to reposition itself for some time points. Embryos expressed mCherry::Histone and mCherry::PH, as well as CYK-4::GFP. **(C)** Stills of embryos imaged 200, 210, 220, and 230 s for *vector* (*RNAi*) or 240, 250, 260, and 270 s for *spd-1* (*RNAi*) after anaphase onset. CYK-4::GFP is shown. Bar: 10 µm.

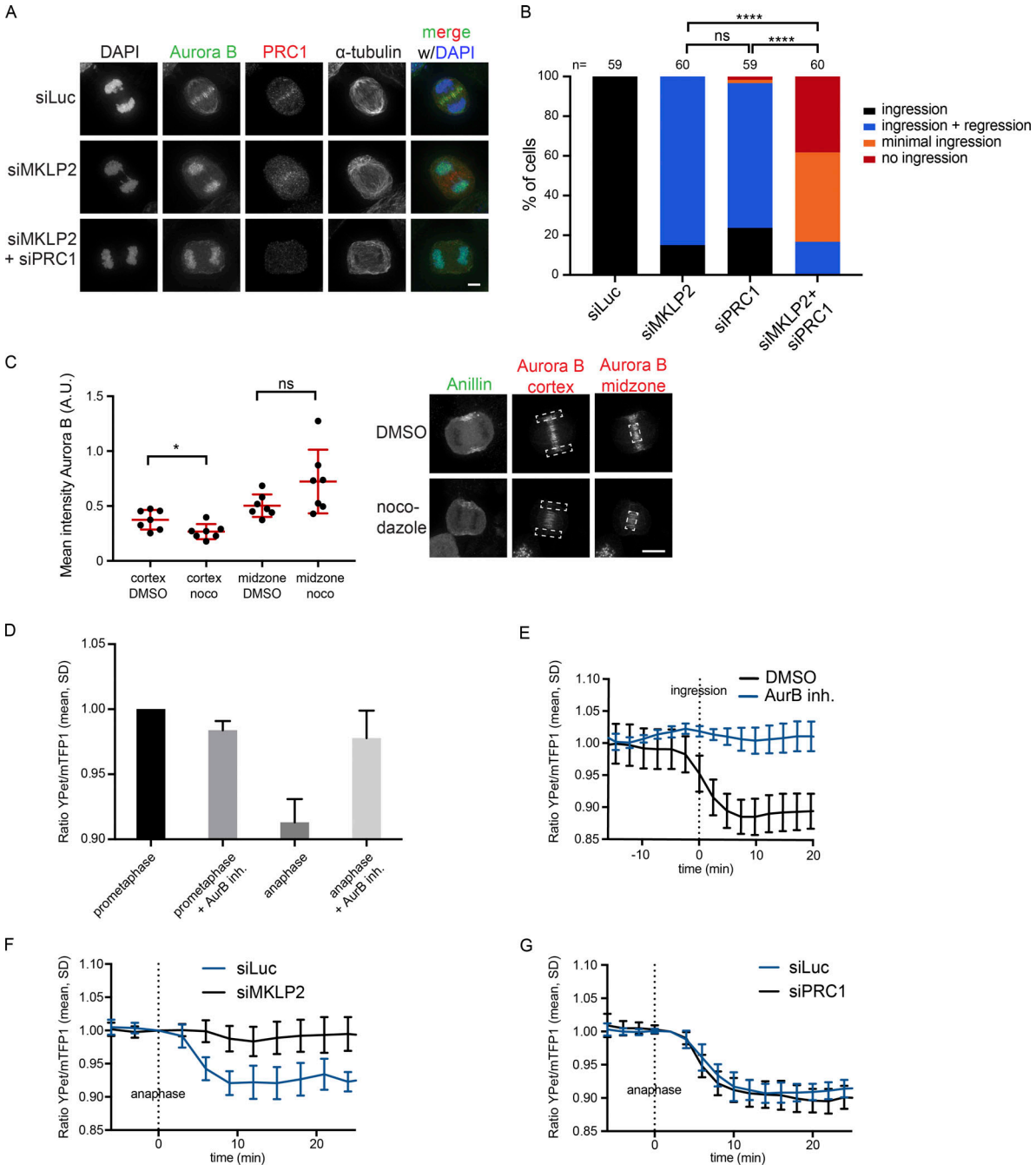


Figure S3. **Aurora B localization and activity at the equatorial cortex. (A)** IF for Aurora B, PRC1, and α-tubulin of HeLa cells in anaphase transfected with the indicated siRNAs. **(B)** HeLa cell lines were transfected with the indicated siRNAs and imaged live. The number of cells showing complete furrow regression, full-furrow regression followed by regression, visible but minimal furrow ingression, or no furrow ingression was scored. The number of cells analyzed is indicated (n). One representative experiment out of two is shown. ****, $P < 0.0001$; χ^2 test for comparison of the indicated conditions; ns, not significant. **(C)** Left: Quantification of Aurora B fluorescent intensity at the equatorial cortex or the midzone, with or without a low dose of nocodazole (83 nM). Each dot represents an individual cell. Error bars depict SDs of the mean. *, $P < 0.05$; ns, not significant (Student's *t* test). Right: Example of ROIs to measure Aurora B fluorescence intensity at the equatorial cortex and in the midzone. Note that images are the same as shown in Fig. 3 B. **(D and E)** HeLa cells stably expressing the Tubby-Aurora B FRET sensor and H2B-mCherry were synchronized in G2 using RO3306 and imaged live after release from the Cdk1 inhibitor. 35 min after RO3306 release, DMSO or the Aurora B inhibitor ZM447439 was added. **(D)** The emission ratio at the equatorial cortex was calculated in prometaphase (~17 min before furrow ingression) and in anaphase (~3 min after furrow ingression). Data were derived from experiment presented in E. **(E)** The emission ratio at the equatorial cortex was calculated for each time point (interval, 3 min). Mean ± SD of 11 (DMSO) or 10 (ZM447439) cells is shown. **(F)** HeLa cells stably expressing the Tubby-Aurora B FRET sensor and H2B-mCherry were treated with control (siLuc) or a MKLP2 siRNA and were synchronized in G2 using RO3306 and imaged live after release from the Cdk1 inhibitor. The emission ratio at the equatorial cortex was calculated for each time point (interval, 3 min). Mean ± SD of 15 (siLuc) and 14 (siMKLP2) cells is shown. **(G)** HeLa cells stably expressing the Tubby-Aurora B FRET sensor and H2B-mCherry were transfected with siLuc or siPRC1, synchronized in G2 using RO3306, and imaged live after release from the CDK1 inhibitor. The emission ratio at the equatorial cortex was calculated for each time point (interval, 3 min). Mean ± SD of six (siLuc) and nine (siPRC1) cells is shown. Bars: 5 µm (A); 10 µm (C).

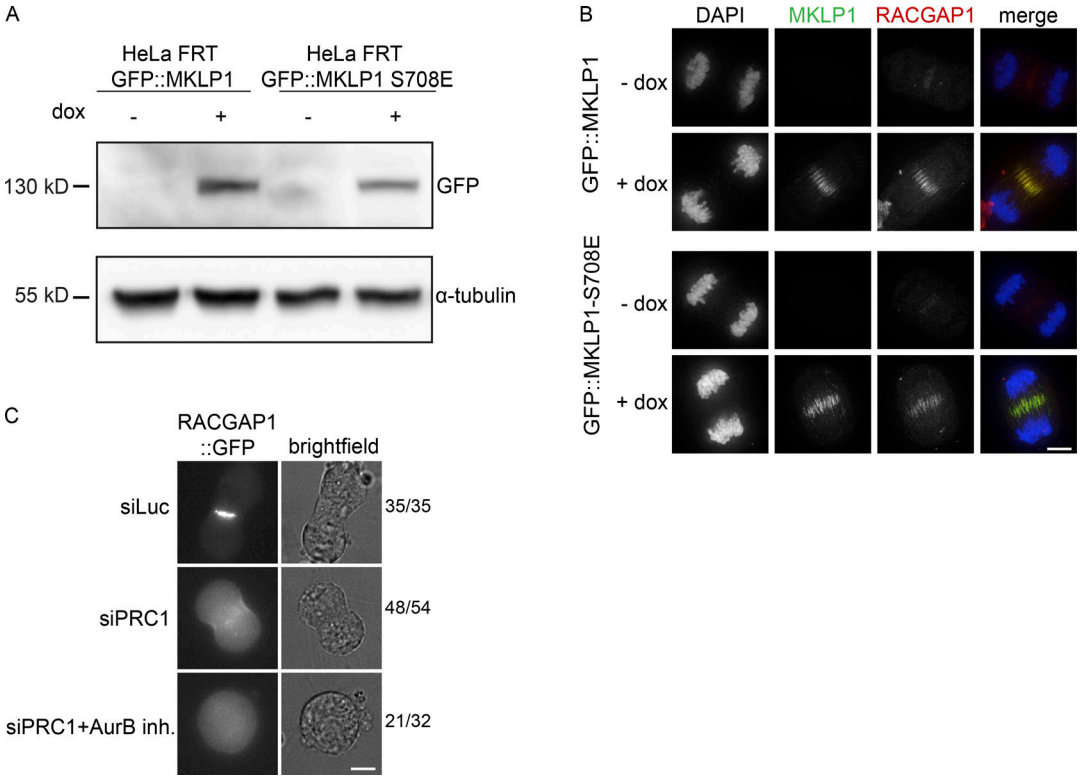


Figure S4. **Expression and localization of GFP-tagged MKLP1 and RACGAP1. (A)** Western blot of HeLa cells with doxycyclin-inducible expression of GFP::MKLP1 or GFP::MKLP1-S708E and treated with or without doxycyclin. The Western blot was probed with an anti-GFP antibody, and α-tubulin is shown as loading control. **(B)** HeLa cell lines with stable doxycycline-inducible expression of the indicated GFP-tagged (siRNA insensitive) MKLP1 proteins were transfected with an siRNA for MKLP1 and processed for IF to visualize GFP-tagged MKLP1 and endogenous RACGAP1. For GFP::MKLP1-expressing cells, 49/50 cells, and for the GFP::MKLP1-S708E–expressing cells, 47/51 cells, showed GFP midzone localization. DNA was visualized using DAPI. **(C)** Representative stills of HeLa cells with doxycycline-inducible expression of RACGAP1::GFP in anaphase and transfected with the indicated siRNAs and treated with (AurB inh.) or without ZM447439 (2 µM). Numbers indicate the number of times the depicted localization was observed/total number of cells that was imaged live. Dox, doxycycline. Bars: 10 µm (B and C).

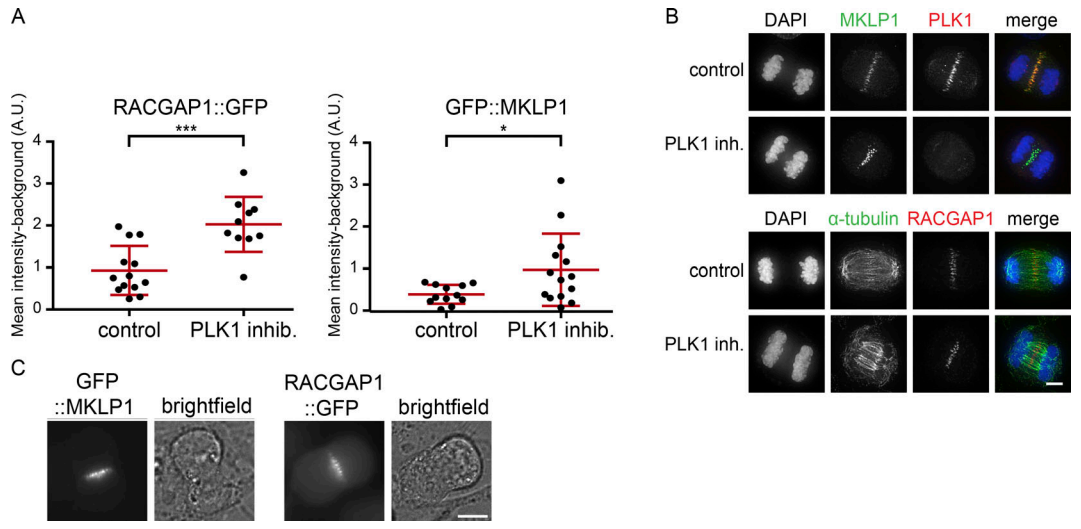
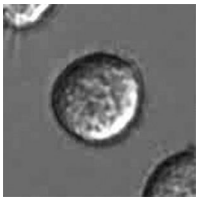


Figure S5. **Spindle midzone levels of MKLP1 and RACGAP1. (A)** Quantification of RACGAP1::GFP and GFP::MKLP1 levels on the spindle midzone in anaphases of living cells. Cells were treated with or without BI2536 (100 nM) before anaphase onset. One image per cell was captured, and each dot represents an individual cell. For the control cells, fluorescence quantifications were performed on anaphases with visible but not fully ingressed furrows. In case of PLK1 inhibition, no furrow ingression was observed (see also Fig. 6 C). A.U., arbitrary units. **(B)** IF for MKLP1, PLK1, RACGAP1, and α-tubulin in cells with or without BI2536. DNA was visualized using DAPI. **(C)** Representative stills of live HeLa cells infected with lentiviruses expressing GFP::MKLP1 or RACGAP1::GFP. Bars: 5 µm (B); 10 µm (C).

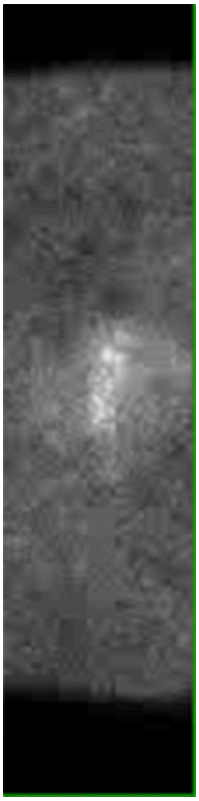
Video 1. **HeLa cells transfected with siLuc were filmed (brightfield) starting at metaphase.** Relevant stills are shown in Fig. 4 A, first row. Note that for the stills in Fig. 4 A, the best focus z plane is shown for each time point. For the video, the best focus z plane was selected for furrow ingression. Frame interval time, 5 min.

Video 2. **HeLa cells transfected with siLuc and treated with the Aurora B inhibitor ZM447439 (2 µM) were filmed (brightfield).** Relevant stills are shown in Fig. 4 A, second row. Note that for the stills in Fig. 4 A, the best focus z plane is shown for each time point. For the video, the best focus z plane was selected for furrow ingression. Frame interval time, 5 min.

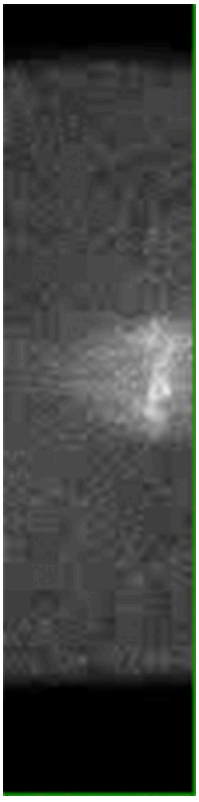
Video 3. **HeLa cells transfected with siPRC1 were filmed (brightfield) starting at prometaphase.** Relevant stills are shown in Fig. 4 A, third row. Note that for the stills in Fig. 4 A, the best focus z plane is shown for each time point. For the video, the best focus z plane was selected for furrow ingression. Frame interval time, 5 min.



Video 4. **HeLa cells transfected with siPRC1 and treated with the Aurora B inhibitor ZM447439 (2 µM) were filmed starting at metaphase (brightfield).** Relevant stills are shown in Fig. 4 A, fourth row. Note that for the stills in Fig. 4 A, the best focus z plane is shown for each time point. For the video, the best focus z plane was selected for furrow ingression. Frame interval time, 5 min.



Video 5. ***C. elegans* embryos expressing mCherry::PH membrane marker and CYK4::GFP were filmed starting at metaphase in the first division cycle.** Only CYK4::GFP is shown in the movie. Montages with mCherry::PH are shown in Fig. 5 E.



Video 6. ***C. elegans* embryos expressing mCherry::PH membrane marker and CYK4::GFP were depleted of endogenous Aurora B (AIR-2) by RNAi and filmed starting at metaphase in the first division cycle.** Only CYK4::GFP is shown in the movie. Montages with mCherry::PH are shown in Fig. 5 E.