Supplemental material

JCB

Rankin and Wordeman, http://www.jcb.org/cgi/content/full/jcb.201004017/DC1

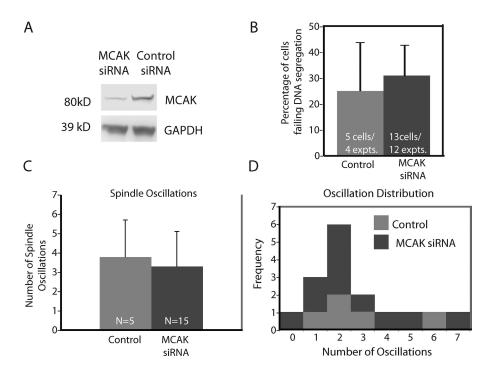


Figure S1. When severe spindle rocking appears in MCAK-depleted or control cells, it exhibits similar characteristics. (A) Depletion of MCAK protein in lysates from cells transfected with siRNA directed against MCAK (left) versus control siRNA (right). (B) Comparison of the percentage of cells that failed DNA segregation in control and MCAK-depleted cells with severe spindle oscillations. (C) Quantification of the number of oscillations by spindles in control and MCAK-depleted cells with severe spindle oscillations. (D) Distribution of the number of oscillations that occur before the cytokinetic furrow closes in cells with severe rocking for control and MCAK-depleted cells.

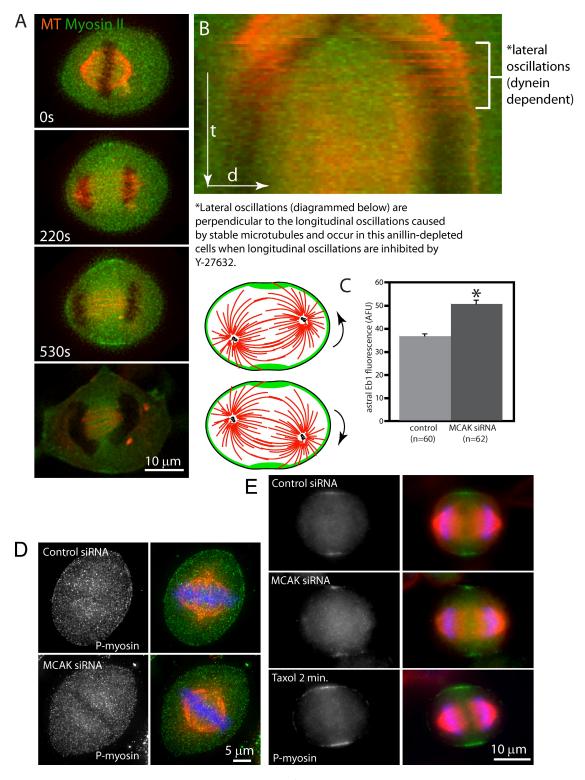


Figure S2. Spindle rocking is driven by myosin, but the overall distribution of phosphomyosin is not changed in MCAK-depleted cells. (A) Anillin-dependent rocking is inhibited with a concentration of Y-27632 that normally will not prevent cytokinesis (Rosenblatt et al., 2004). This cell is anillin depleted and will not complete cytokinesis. (B) Kymograph of live cell in A showing that lateral dynein-dependent oscillations (Pecreaux et al., 2006; Nguyen-Ngoc et al., 2007) of the spindle are still present even though longitudinal, myosin-dependent rocking is inhibited. d, distance; t, time. (C) Quantification of astral EB1 fluorescence in HeLa cells (*, P < 0.0001). (D) MCAK depletion does not increase phosphomyosin staining in metaphase. (E) Neither MCAK depletion nor taxol treatment significantly alters phosphomyosin staining at anaphase. Phosphomyosin is restricted to the midzone, and the label distribution is correlated with the progress through anaphase. Error bars indicate SEM.

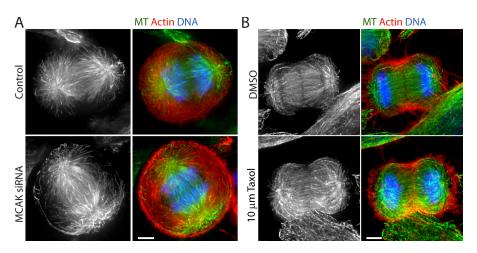
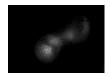


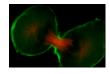
Figure S3. Astral MT lengths are increased in anaphase with MCAK depletion and taxol treatment. (A) In contrast to control cells, MCAK-depleted cells have long astral MTs that wrap around the cortex. (B) DMSO-treated cells have astral MTs that do not wrap as much around the polar cortex as taxol-treated cells. Bars, $5 \mu m$.



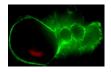
Video 1. An MCAK-depleted cell stably expressing EGFP-α-tubulin completes cytokinesis with severe anaphase spindle rocking. This video was acquired at 1 frame/20 s and played back at 7 frames/s.



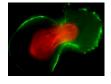
Video 2. A cell stably expressing EGFP- α -tubulin and treated with control siRNA successfully completes cytokinesis without severe spindle rocking. This video was acquired at 1 frame/20s and played back at 7 frames/s.



Video 3. A HeLa cell transfected with control siRNA and expressing GFP-myosin IIA and mCherry- α -tubulin with normal cytokinesis. This video was acquired at 1 frame/20 s and played back at 7 frames/s.



Video 4. An MCAK-depleted HeLa cell expressing GFP-myosin IIA and mCherry- α -tubulin exhibiting severe spindle rocking. This video was acquired at 1 frame/20 s and played back at 7 frames/s.



Video 5. A HeLa cell expressing GFP-myosin IIA and mCherry- α -tubulin. Severe spindle rocking caused by addition of 10 μ M taxol just after anaphase initiation is shown. This video was acquired at 1 frame/20 s and played back at 7 frames/s.



Video 6. An MCAK-depleted HeLa cell expressing GFP-Utr and mRFP-EB3 with severe spindle rocking. This video was acquired at 1 frame/5 s and played back at 7 frames/s.

References

- Nguyen-Ngoc, T., K. Afshar, and P. Gönczy. 2007. Coupling of cortical dynein and G alpha proteins mediates spindle positioning in *Caenorhabditis elegans. Nat. Cell Biol.* 9:1294–1302. doi:10.1038/ncb1649
- Pecreaux, J., J.C. Röper, K. Kruse, F. Jülicher, A.A. Hyman, S.W. Grill, and J. Howard. 2006. Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators. *Curr. Biol.* 16:2111–2122. doi:10.1016/j.cub.2006.09.030
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