SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: The distance from the spindle center to the cortex is not significantly changed by depletion of TPXL-1 or HCP-4. The distance between the center of the spindle midzone and the cortex was measured 200s after NEBD in single, central plane images of embryos expressing GFP:PH domain and GFP:tubulin. As illustrated in the schematic, 2 measurements, from the center of the spindle to the cortex on each side were made for each embryo (a and a'; see schematic). The plot shows all of the measurements made for control, *hcp-4(RNAi)*, and *tpxl-1(RNAi)* embryos. The pair of measurements (a and a') made in an embryo are shown in the same color in the distribution plot. The mean and standard deviation are indicated for each condition.

Figure S2: Aster separation is required for the equatorial enrichment of myosin II^{NMY-2} following anaphase onset. (**A**) Confocal optics were used to image the cortex in embryos expressing the heavy chain of myosin II, NMY-2, fused to GFP (NMY-2:GFP). Images are maximum intensity projections of 4 z-sections collected at 1μm intervals. During cytokinesis, NMY-2:GFP accumulates at the cell equator in a manner similar to GFP:Anillin. Delaying aster separation leads to the ectopic accumulation NMY-2:GFP on the polar corticies. A normal pattern of NMY-2:GFP accumulation is restored by co-depletion of HCP-4 along with TPXL-1. One difference between the localization patterns of NMY-2:GFP and GFP:Anillin is that prior to anaphase onset, cortical NMY-2 is present in an anterior cap that contributes to the polarity maintenance (Guo and Kemphues, 1996; Shelton *et al.*, 1999; Severson and Bowerman, 2003;

Munro *et al.*, 2004), whereas Anillin is not. In the early post-anaphase interval, this anterior cap localization is super-imposed with its accumulation at the cell equator. Times are in seconds after NEBD. **(B)** The mean post-anaphase accumulation of cortical NMY-2:GFP is plotted as a function of embryo length. The mean NMY-2:GFP in each segment, after subtraction of a background measurement made prior to anaphase onset, is plotted for each timepoint. Values were normalized by dividing by the average maximum value for controls. We note that although NMY-2:GFP accumulates with similar timing, the equatorial band of NMY-2:GFP remains detectable for slightly longer in embryos depleted of HCP-4 or co-depleted of TPXL-1 & HCP-4 than in controls due to the ~1 minute delay in furrow involution caused by HCP-4 depletion. Error bars are SEM. **(C)** Examples of cortical NMY-2:GFP accumulation in control and *tpxl-1(RNAi)* embryos 240 seconds after NEBD. Boxed regions are magnified 2x. Scale bars are 10 μm.

Figure S3: Localization of Centralspindlin and the CPC to microtubules in the midzone region in SPD-1 depleted embryos. Fixed control (n>30) and *spd-1(RNAi)* (n=20) embryos were stained for DNA, α-tubulin, ZEN-4, and AuroraB^{AIR-2}. 3D widefield data stacks were collected and computationally deconvolved. Equivalently scaled maximum intensity projections of the central region of representative early (***top two rows***) and late (***bottom two rows***) anaphase embryos are shown. Embryos were staged based on spindle pole morphology, which changes in a reproducible way during this transition. Scale bar is 5 μm.

Figure S4: The equatorial accumulation of GFP:Anillin occurs over a substantially broader region when aster separation is prevented by co-depletion of TPXL-1 & GPR-1/2.

Central plane (***left***) and cortical (***right***) images of a *tpxl-1 & gpr-1/2(RNAi)* embryo expressing GFP:Anillin. After furrow formation, GFP:Anillin is enriched at the tips of the ingressing furrows. Times are in seconds after NEBD. Scale bar is 10 μm.

SUPPLEMENTAL MOVIE LEGENDS

Movie S1: TPXL-1 depletion introduces a delay between anaphase onset and the point when the asters reach a normal extent of separation. Aster separation can be rescued by co-depletion of the essential kinetochore component HCP-4. Single central confocal sections of embryos expressing GFP-plasma membrane probe and GFP:β-tubulin were acquired every 20 seconds beginning at NEBD. Sequences end with furrow involution (***light purple arrowheads***). The playback rate is 120x real time.

Movie S2: Delaying aster separation leads to a corresponding delay in furrow formation. Single central confocal sections of control and *tpxl-1(RNAi)* embryos expressing the GFP-plasma membrane probe were acquired every 20 seconds beginning at NEBD. Playback rate is 120x real time.

Movie S3: Co-depletion of HCP-4 rescues the delay in the equatorial accumulation of GFP:Anillin resulting from TPXL-1 depletion. Cortical imaging of embryos expressing GFP:Anillin. Movies begin 140s after NEBD. Playback rate is 120x real time.

Movie S4: Aurora B^{AIR-2} localizes to the midzone region in embryos depleted of TPXL-1 alone or in combination with GPR-1/2. Single plane imaging of control, *tpxl-1(RNAi),* and *tpxl-1 & gpr-1/2(RNAi)* embryos expressing GFP:AIR-2 and mCherry:Histone H2B. Images were acquired every 10 seconds starting at NEBD. Playback rate is 60x real time.

Movie S5: Midzone-localized signaling complexes become essential for furrow formation when aster separation is delayed. Single central confocal sections of embryos expressing the GFP-plasma membrane probe were acquired every 20 seconds beginning at NEBD. Playback rate is 120x real time.

Movie S6: Multiple furrows form simultaneously and ingress towards the spindle center when aster separation is prevented. Four examples of *tpxl-1 & gpr-1/2(RNAi)* embryos expressing GFP-plasma membrane probe and GFP:β-tubulin. Images were acquired every 20 seconds beginning at NEBD. The playback rate is 120x real time.

SUPPLEMENTAL REFERENCES

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Munro, E., Nance, J., and Priess, J.R. (2004). Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early *C. elegans* embryo. *Dev Cell* 7, 413-424.

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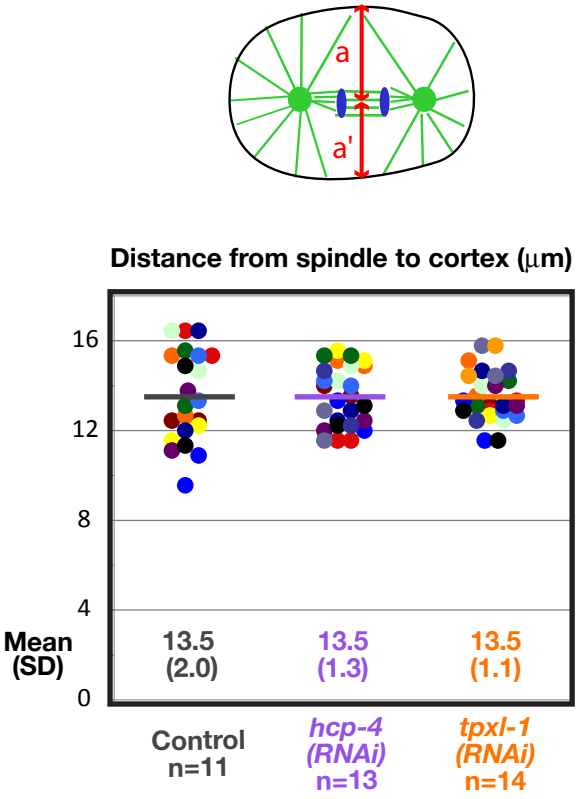
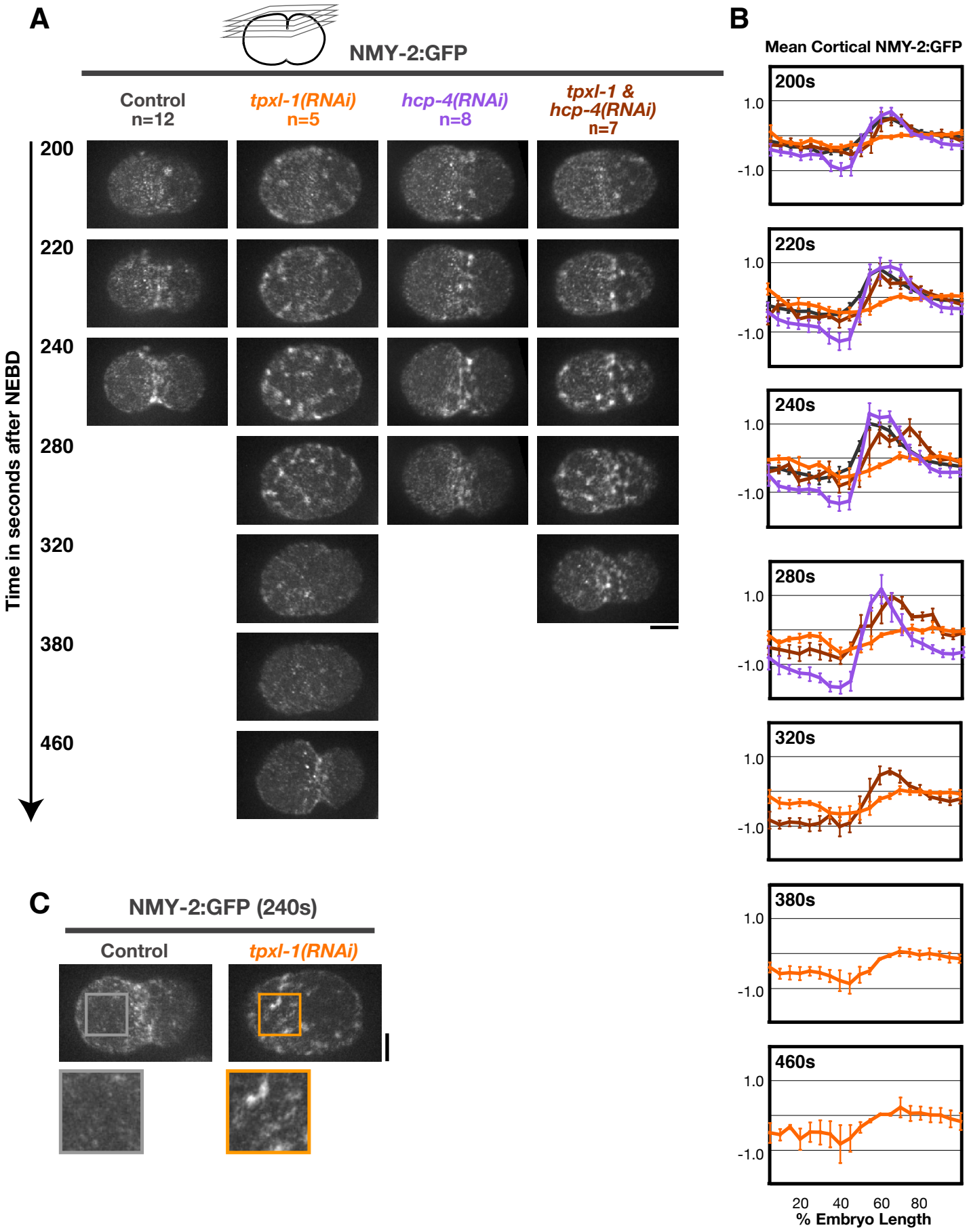
Shelton, C.A., Carter, J.C., Ellis, G.C., and Bowerman, B. (1999). The nonmuscle myosin regulatory light chain gene mlc-4 is required for cytokinesis, anterior-posterior polarity, and body morphology during *Caenorhabditis elegans* embryogenesis. *J Cell Biol* 146, 439-45.

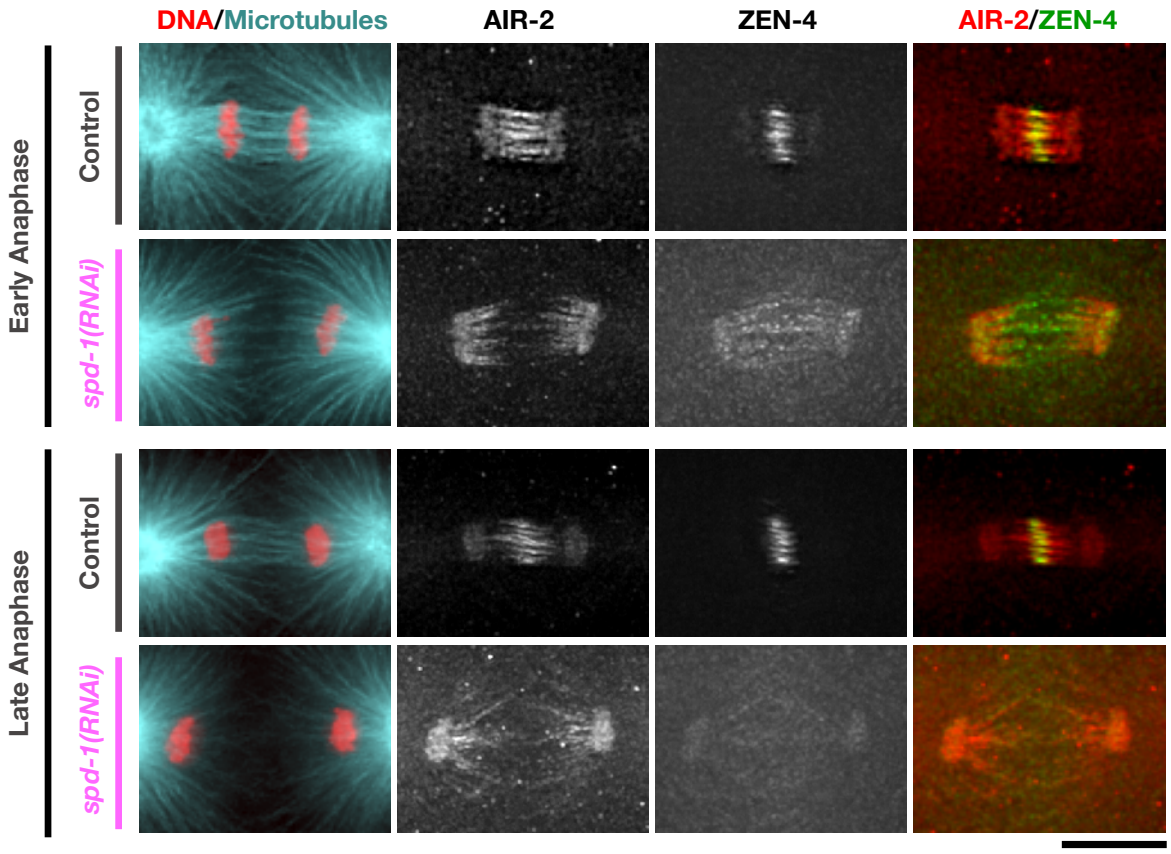
Lewellyn et al. Figure S1



Lewellyn et al. Figure S2



Lewellyn et al. Figure S3



Lewellyn et al. Figure S4